The Ludwig Institute For Cancer Research

Annual Research Report
2003

Publication date: June, 2004

Table of Contents

The Ludwig Institute for Cancer Research (LICR) is the largest international academic Institute dedicated to the fight against cancer. With ten Branches in seven countries across Australasia, Europe, and North and South America, and numerous Affiliates across Asia, Australasia, Europe, and North and South America, the scientific network that is the Ludwig Institute for Cancer Research quite literally covers the globe. The Institute is not only associated with established and world-renowned academic institutions, but has also, through the James R. Kerr Program, expanded its support into countries such as Brazil, China, and Russia from which advanced cancer research is beginning to emerge.

The uniqueness of the Ludwig Institute for Cancer Research lies not only in its size and scale, but also in its philosophy and ability to drive its results from the world of academia into the world of the clinic. The Institute has accumulated a staggering portfolio of reagents, knowledge, expertise, and intellectual property, and, unusually for an academic institution, has also actively assembled the personnel, facilities, and practices necessary to patent, license, and thus fully develop the most promising aspects of that portfolio into cancer therapies. The Institute believes that the same science-driven investigative rigor that yielded the academic discoveries should, indeed must, be applied in the clinic to fully develop the therapeutic potential of any discovery.

The goal of the LICR is to make a real contribution to the clinical management of cancer. This report summarizes the progress that LICR investigators made to that end in 2003.

In the 32 years since the Ludwig Institute for Cancer Research (LICR) was founded, the Institute has grown to become the largest international academic institute dedicated to the fight against cancer. The research of the Institute is carried out at ten Branches in seven countries around the world, and also in cooperation with an international network of affiliated scientists and clinicians. This model allows the Institute to interact with a number of different research and clinical environments, and facilitates the worldwide recruitment of outstanding scientists.

The research orientation of each Branch of the LICR is defined by the Branch Director in relation to the overall objectives of the Institute. The number of staff at each Branch is intended to be sufficiently large for them to collectively address complex biological problems related to cancer, and provide a critical mass of scientists with expertise in various scientific disciplines. Branches also have formal associations with University Hospitals, which allows not only a close interaction between laboratory and clinical scientists, but also the provision of clinical resources required for basic research, and the Institute's Clinical Trials Program.

The quality of research conducted by LICR scientists is monitored on an ongoing basis by the Institute's Administration and the Scientific Committee, and is independently assessed through an external peer-review process. The introduction of programmatic-based research has resulted in the establishment of numerous collaborations between and among Branches, and LICR Affiliates, in both basic research and clinical areas. The LICR Affiliates are individual investigators who are experts in fields that complement the research objectives of the Institute's Branches. The LICR also has affiliations, through the James R. Kerr Program, to investigators in Brazil, China, Russia, South Africa, Turkey, and Ukraine that are scientifically talented but have had little opportunity for international collaboration in cancer research. All of these activities extend the Institute's scientific global reach, in accordance with the wishes of our founder, Mr. Daniel K. Ludwig.

The following scientific progress report is organized around several of LICR's research Programs, which can be classified into four broad scientific disciplines: Genetics, Biochemistry, Cell Biology, and Immunology. Whilst much of the scientific research carried out by LICR investigators is conducted under the existing Programs, individual groups in Branches also continue to discover and pursue many new and exciting avenues of research. Examples of research highlights in each discipline, taken from studies published in 2003 by LICR Branch staff members and LICR Affiliates are used below to represent the science being conducted in LICR Programs. Details of the science conducted at each research center may be found in the Annual Research Report.

Genetics Discipline

Cancer arises originally from genetic alterations in normal cells. Although many single genes have been identified as oncogenic (i.e. cancer causing), their numbers are relatively few, and it appears that specific interactions between and among many genes may be responsible for the number and variety of human cancers. Programs in the discipline of Genetics include: Gene Discovery, Function, and Expression; Mutation and Epigenetic Screening; Cancer Genomics (Gene Expression Profiling, and Genome Annotation); and Cancer Epidemiology.

Functional Genomics Program

Key genes that are involved in oncogenesis fall into two major groups. Tumor suppressor genes protect against cancer, and thus need to be inactivated or eliminated for cancer to occur. Oncogenes, on the other hand, promote tumor development and are often found to be hyperactive in cancer cells. Also affected are changes in the function and expression of genes necessary for important cell processes such as growth, proliferation, differentiation, signaling, and apoptosis (programmed cell suicide).

There are concerted efforts in several LICR Branches to identify and characterize particular tumor suppressor genes and oncogenes, and genes involved in cellular processes that are corrupted in cancer. Defining, and determining the function/s of oncogenes and tumor suppressor genes have allowed LICR investigators to unravel many of the cellular signaling pathways, and identify potential therapeutic targets.

An excellent example of LICR work in this area is the analysis of the p53 transcription factor, a tumor suppressor gene that has been shown to have a central role in preventing oncogenesis by teams at the London St Mary's Branch and the San Diego Branch. In a normal cell, p53 expression levels increase in response to stress and DNA-damaging agents, and cause cell cycle arrest and apoptosis before the damage to the DNA of their nuclei can cause oncogenesis. However, it is estimated that as many as 50% of cancers have lost p53 function. LICR scientists have therefore been analyzing the expression and accumulation of p53 in response to DNA-damaging and stress-inducing agents to understand which pathways are controlled by p53 signaling, and to identify potential targets for therapeutic (re)activation of p53. The LICR team used caffeine, which abrogates the accumulation of p53, in combination with several forms of DNA-damaging and stress-inducing agents to investigate the induction of p53 in human tumor cell lines. Several of these p53-inducing agents are commonly used chemotherapies. The LICR team found several pathways may be involved in p53 accumulation and degradation.

The presence of functional p53 has been shown previously to determine the patient response to chemotherapeutic agents. Many chemotherapies are in fact DNA-damaging agents that, in cells with intact p53, induce the apoptosis of tumor cells. However, p53-dependent apoptosis does not entirely determine cellular sensitivity to chemotherapeutic agents. Therefore, the LICR team investigated the role of another transcription factor, called p73, which is part of the p53 family. The team found that p73 was also induced by some chemotherapies, and that inhibiting p73 activity resulted in the abrogation of apoptosis and the loss of cellular sensitivity to the chemotherapeutic agents. Even more interestingly, the team showed that certain p53 mutants were able to inhibit p73 activity, thus conferring resistance to chemotherapies through two modes of action: loss of p53 activity, and inhibition of p73 activity.

Investigating the relationship between p73 inhibition and the p53 gene sequence further, the LICR scientists also showed that polymorphisms (gene sequence variations that do not result in a dysfunctional protein) in the p53 sequence may affect tumor progression, as for example in head and neck cancer. Taken together, these data suggest that mutations and polymorphisms in both p53 and p73 may have prognostic significance in some cancers, and may have future utility in determining which chemotherapeutic agents will work best for certain patients. This finding complements work from the San Diego Branch in which polymorphisms in the MSH6 gene in the DNA mismatch repair system were found to affect the expression of MSH6. Defects in the DNA mismatch repair system have been reported to underlie a variety

of hereditary and sporadic cancers. Meanwhile, scientists at the Sâo Paulo Branch reported on a new microarray-based technique for identifying gene expression changes in gastric cancers, and a technique such as this could theoretically be applied to identifying the polymorphism-mediated gene expression changes identified by the San Diego and London St Mary's Branches for prognostic and/or diagnostic purposes.

In 2002, scientists at the London St Mary's Branch identified the ASPP proteins, which are activators of p53 function, and in so doing, recognized the possibility that inhibitors of p53 similar to the ASPP proteins might also exist. The hypothesis was correct, and a key finding in 2003 was the identification of the oncoprotein, iASPP, an inhibitor of p53. The team found that the levels of iASPP were found to be inversely correlated with p53's ability to cause cell apoptosis, and that increased levels of iASPP reduced the efficacy of chemotherapeutic agents. Importantly, the expression and function of iASPP appeared to explain, for the first time, how apoptosis and cell cycle controls may be over-ridden even when functional p53 is present. The targeting of iASPP is an attractive candidate for the development of future treatment strategies, and the team is now further investigating these strategies.

Biochemistry Discipline

Cancerous changes resulting from genetic mutations subvert the biochemical signals that drive normal cell biology. As a result, the regulation of cellular processes such as growth, division, migration, and energy metabolism is disrupted, and tumors grow and spread. Programs in the discipline of Biochemistry include: Signal Transduction (Receptor Kinases and Phosphatases, Non-Receptor Kinases and Phosphatases, Nuclear Receptors, and Cytokines); and Protein Chemistry (Mass Spectrometry, Proteomics, and Structural Modeling).

Signal Transduction Program - Nuclear Receptors

Cells interact with their environment by sending and receiving signals that initiate and terminate cellular processes such as cell division, growth, differentiation, migration and survival. Signaling, or signal transduction, also occurs within the cell, with external signals being passed along a chain of molecules from the cell surface receptor to initiate cellular processes such as cytoskeletal reorganization, gene transcription, and protein modification. Cancer cells have abnormal signal generation and reception, which allow them to grow out of control, escape apoptosis, and invade other tissues.

Since LICR's inception, understanding signaling in normal cells, and elucidating what goes wrong with signaling in cancer cells has constituted a major focus of research. LICR scientists have made significant and substantial contributions to the discovery of signaling molecules, and the dissection of the complex interactions between multiple signaling pathways. This has led to the identification of a number of potential targets for therapeutic intervention, and several of these targets are being pursued. LICR staff and Affiliates continue to identify additional members of key pathways, their interactions with other signaling molecules, and their involvement/s in cellular processes.

The analyses of the nuclear receptor (NR), Nurr1, by a team at the Stockholm Branch exemplify research on several different receptors that is ongoing within LICR. Nurr1 is an 'orphan receptor', meaning that as yet no ligands have been identified as binding to and activating the receptor. Identifying receptor ligands is crucial to understanding how and when receptors are activated, and therefore the LICR investigators set out to identify the Nurr1 ligand. The team used X-ray crystallography to determine the protein's three-dimensional structure, in order to infer the function and binding capacity of each domain (subunit structure) in the Nurr1 protein. This same technique was also used to great effect to determine the structure of the ErbB-2 receptor at the Melbourne Branch. Surprisingly, the team found that the structure of the ligand-binding domain (LBD) of Nurr1 was missing the cavity found in other receptors,

and in which ligand binds. Thus Nurr1 may be the first receptor identified as functioning independently of classic ligand-receptor interactions. By comparing the protein sequences of other orphan nuclear receptors, the investigators concluded that all members of Nurr1's subfamily may also function as ligand-independent nuclear receptors.

Even after altering the sequence of Nurr1's putative LBD, the investigators were unable to induce the binding of activator or repressor proteins. The results of the structural studies posed important questions about how the transcriptional activity of Nurr1 is regulated, if not by ligand-binding or the action of activator or repressor proteins. The team investigated further to find that there was a 'stabilization' of the LBD when fused to domains from other proteins, and that this stabilization correlated with transcriptional activity. This stabilization of the LBD was, in turn, regulated through intracellular cross-signaling from the Ret tyrosine kinase receptor.

Nurr1 was also found to dimerize with the retinoid X receptor, RXR, and mediate RXR-ligand induced signaling in the central nervous system (CNS). The research demonstrated not only the existence of exogenous ligands specific for RXR, but also that RXR-mediated signaling is a novel Nurr1-dependent model for neuronal survival. Nurr1 has also been shown to be essential for dopamine (DA) neuron development during mouse embryogenesis. However, mutations in the Nurr1 protein have also been implicated in familial Parkinson's disease in (adult) humans; a disease that is due to the degeneration of those same DA cells. Therefore, the Stockholm Branch team set out to determine the function of Nurr1, and its role in DA production and storage in mature DA cells. The results indicated that Nurr1 and retinoids both induced cell cycle arrest, and further investigation showed that Nurr1 induced the up-regulation of, and direct interaction with, the cell cycle protein $p57^{Kip2}$. Additionally, $p57^{Kip2}$ and Nurr1 appeared to cooperate to induce DA cell differentiation following the cell cycle arrest. Nurr1 (but not retinoids) also increased DA content and the expression of several DA markers, suggesting that Nurr1 (but not RXR-Nurr1 heterodimers) regulates certain dopaminergic functions. Work is continuing to elucidate if and how the regulatory function of Nurr1 can be exploited to improve current treatments for Parkinson's disease or stroke.

Cell Biology Discipline

Tumor formation and growth, and the spread of cancer (metastasis) result from the disruption of a number of different cellular processes, such as growth, division, migration, and programmed cell death (apoptosis). Programs in the discipline of Cell Biology include: Angiogenesis; Cell Migration and Metastasis; and Cell Cycle and Apoptosis (Cell Cycle, and Mitosis and The Centromere).

Angiogenesis Program

Angiogenesis is the process of forming new blood vessels (from endothelial cells). Although part of the body's natural processes (in wound healing and reproduction), angiogenesis is also stimulated by the release of angiogenic growth factors from tumor cells. Without the blood and nutrients that the newly-generated blood vessels supply, it is thought that no tumor would grow to be more than a few millimeters in size. Lymphangiogenesis is a related process, in which new lymphatic vessels are formed. Lymphatic vessels provide one of the principle routes by which cancer, particularly breast cancers and skin cancers (melanoma), spread throughout the body (metastasize) from the single tumor site of origin.

Work in the Angiogenesis Program in 2003 continued the analysis of the expression and effects of the angiogenic ligands and their receptors. Scientists at the London University College Branch have also analyzed endothelial cells, in which these ligands and receptors are expressed. Several studies from the Stockholm Branch reported on the effects of the PDGF receptor (PDGFR) and the PDGF ligands, in particular the two new forms of PDGF-C and PDGF-D. PDGF-D was found to be a potent transforming

and angiogenic factor that may promote tumor growth by accelerating the tumor cell proliferation and stimulating tumor neovascularization. The other new ligand, PDGF-C, was found to be constitutively expressed in human kidney, and had elevated expression following injury to, or activation of, some kidney cell types. Another study showed that transgenic overexpression of PDGF-C in the mouse heart induced cardiac fibroblast proliferation resulting in several cardiac defects.

Studies on the original angiogenic ligands, at the Stockholm Branch and the Helsinki Affiliate Center, showed that vascular endothelial growth factor (VEGF)-A and VEGF-C induce invasion and tube formation in bovine endothelial cells via activation of the VEGF receptor-2 (VEGFR-2). Additionally, a study from the Melbourne Branch and the Helsinki and Kuopio Affiliate Centers showed that VEGFR-2 is constitutively expressed in human heart arteries, in which VEGF-D also has a high expression, suggesting that VEGFR-2 activation by VEGF-D may have a role in the maintenance of vascular homeostasis. In fact through the action of the serine protease plasmin, VEGF-D is cleaved and thus has enhanced binding to VEGFR-2, making VEGF-D a potent new angiogenic factor, in addition to its previously known lymphangiogenic effects. Collaborative studies between the Melbourne Branch and the Helsinki and Kuopio Affiliate Centers used adenovirus-based gene therapy strategies to conclude that VEGF-A and the processed form of VEGF-D are the best candidates for therapeutic angiogenesis when delivered around large arteries, and that VEGF-D appears to be the strongest angiogenic and lymphangiogenic effector among VEGFs, at least when delivered into skeletal muscle.

Results from functional analyses have rapidly progressed the pre-clinical development of pro-and anti-angiogenic and pro- and anti-lymphangiogenic strategies. A study from the Uppsala Branch showed that the introduction of PDGFβR into cultured human keratinocytes, using a retrovirus-mediated gene therapy, produced an epidermis that proliferated in response to PDGF. This system presents itself as a useful model for the study of RTK-mediated skin repair and vascularization.

Immunology Discipline

The immune system has a remarkable capacity for fending off infectious diseases, and it has become clear that these same defenses can recognize and destroy cancer cells. LICR is working on treatment strategies, such as targeted antibodies and cancer vaccines, which harness the body's immune system to more effectively and specifically fight cancer cells. Programs in the discipline of Immunology include: Cancer Vaccine (Characterization of Human Immune Response, and Vaccine Constitution and Development); Cellular and Molecular Immunology (Adaptive Immune Response, and Innate Immune Response); Cancer Antigen Discovery (Cancer Antigen Identification, and Cancer Antigen Characterization); and Antibody Targeting (Antibody Characterization, and Antibody Engineering).

Cancer Vaccine Program - Characterization Of Human Immune Response

A successful cancer vaccine needs to first make the immune system recognize the tumor cells as abnormal, and then induce or strengthen an appropriate immune response that is strong enough to eliminate the tumor. There are two main components to an immune response: the innate response and the adaptive response. The adaptive response can be further divided into the 'humoral' arm of antibodies produced by white blood cells, called B lymphocytes, or B cells, or the 'cellular' arm of CD4+ and CD8+ T lymphocytes, or T cells. Once the mechanisms for inducing appropriate, consistent and robust human immune responses are understood, the effects of such responses on human tumors and, hence, the potential of cancer vaccines can be rationally evaluated.

An understanding of the role of the immune system in cancer development and the development of cancer therapies based on immunologic principles continue to be major objectives of the Institute. Indeed

the LICR has become, arguably, the largest academic center in the world for cancer immunology studies. Laboratory research into what constitutes a strong anti-tumor immune response, and how it can be monitored, is being conducted at the Branches in Brussels, Lausanne, Melbourne, and New York, and by a number of LICR Affiliates.

Characterization of Human Immune Response

The ability to monitor an immune response is critical for the development of maximally immunogenic vaccines. A prime objective of the program is, therefore, to develop standardized methodologies capable of monitoring T cell and antibody responses to cancer antigens following immunization.

An important laboratory methodology for immunological monitoring, which is crucial for vaccine assessment and optimization, utilizes major histocompatibility complex (MHC)-peptide complexes called 'tetramers'. These fluorescently-labeled tetramers can detect, and be used to isolate, individual CD8+ T cells that are specific for particular peptide antigens presented at the surface of tumor cells. CD8+ T cells constitute one half of the cellular component of the immune response, and activation of their 'cell-killing' function is vital for vaccine efficacy. The team at the LICR Tetramer Production Facility at the Lausanne Branch has been working to optimize the reagents that they produce, to maximize and refine their utilization and specificity for monitoring LICR clinical trials. The team found that the existing techniques for fluorescently labeling the tetramer produce a heterogeneous mix of MCH-peptide complexes of different sizes and constitution, which precludes their use in precise binding studies. Therefore, the team focused on developing new techniques to improve tetramer production, which resulted in the production of well-defined, homogenous tetramers capable of detecting and isolating target CD8+ T cells. The superior method discovered by the Lausanne Branch team has several advantages over conventional methods, and represents an important advance in tetramer methodology.

The CD4+ effector cell response constitutes the second half of the adaptive immune response's cellular component; enhancing the cytotoxic effect of CD8+ cells and maintaining the immune response over a sustained period of time. Existing techniques to measure CD4+ responses were of limited applicability and were unable to quantify increases and decreases in CD4+-mediated immunity at a single cell level. Thus the New York Branch, together with Affiliates in Frankfurt, Zurich, and Oxford set out to develop new methodologies that would allow standardized monitoring for LICR Clinical Trials. This collaboration resulted, in 2002, in the development of a quantitative, sensitive, and reproducible method for expanding the number of CD8+ cells in vivo. The team then applied a similar method to expand CD4+ effector cells. The investigators then trialed the ability of different types of cells to act as antigen presenting cells (APC) and isolate the expanded, antigen-specific CD4+ cells. Surprisingly, the team found that CD4+ T cells could themselves act as novel target APC (T-APC) using MHC class II presentation, and shown to be able to detect influenza peptides in healthy donors and in cancer patients.

A growing number of cancer vaccines under investigation are based on full-length proteins. Therefore the team also investigated the ability of the T-APC to detect different peptide antigens derived from a full-length protein. Remarkably, they found that the T-APC were not only able to detect different antigens, they also produced the antigens by cleaving the protein into peptides. This is the first report of functional processing by T cells for MHC class II presentation. This new methodology thus allows the monitoring of vaccination protocols designed to elicit CD4+ effector cell responses induced by viral and tumor peptide and full-length antigens.

James R Kerr Program

Named after Mr. James R. Kerr, the late Chairman of the Institute, the Program is developing LICR activities in countries that are scientifically talented but have had limited opportunities for international collaboration in areas of cancer research. LICR has established collaborative research projects at leading academic centers in China, Ukraine, Russia, South Africa, and Turkey.

Some of the research being conducted within the James R. Kerr Program relates to the identification of cancer antigens. A report from Russia describes a novel method that speeds the identification of cancer antigens using the technique of SEREX (serological expression of recombinant cDNA libraries), which was developed by LICR Affiliates and is used extensively within the Cancer Antigen Discovery Program. The method, serological mini-arrays of recombinant tumor antigens (SMARTA), essentially allows the simultaneous, semi-quantitative evaluation of multiple SEREX-derived antigens, and offers significant savings in reagent cost and time of screening. A rapid and simple method to screen individual cancer patients and detect immune responses against cancer antigens might have prognostic and diagnostic value for the treating oncologist.

Clinical Trials Program

The importance of a new discovery in cancer cannot be assessed until its clinical impact has been established. Accordingly, the Institute is committed to taking its discoveries from the laboratory into the clinic within a collaborative academic environment, and following the principles of strict academic rigor. All LICR clinical activities are centrally coordinated by the Office of Clinical Trials Management in New York. The trials are conducted by a network of Clinical Affiliates and LICR staff, and are generally carried out in hospitals affiliated with the Branches.

LICR currently sponsors over 30 early-phase clinical trials primarily using the expertise and reagents generated through the Antibody Targeting and Cancer Vaccine Programs. These early-phase clinical trials are designed to assess the safety of the investigational agent, as well as its ability to induce an immunological response. The clinical trials also gather initial data on tumor response efficacy.

Targeted Antibodies

The monoclonal antibody (mAb) A33, which detects an antigen, first identified by LICR scientists, is expressed in 95% of colorectal tumors. A mouse A33 mAb against the human A33 antigen has undergone extensive preclinical and radio-labeled localization studies through the LICR's Antibody Targeting (Antibody Characterization) Program. These preliminary studies showed that the antibody had very specific targeting and was able to induce cytolytic anti-tumor activity, but was also immunogenic resulting in patients only being able to receive one dose. The mAb thus passed into the Antibody Engineering component of the Antibody Targeting Program, and a humanized A33 (huA33) mAb was generated.

The team from the New York Branch and Affiliates at Memorial Sloan-Kettering Cancer Center found that this re-engineered huA33 had very little toxicity from its targeting to normal tissue, and preliminary data indicated that there was a limited anti-tumor effect. However, the majority of the patients developed human anti-human antibodies (HAHA) against huA33 indicating that immunogenicity was not fully overcome by the humanization of the mouse A33 mAb.

A second trial investigated the huA33 mAb in combination with chemotherapy, as an observation made in earlier studies indicated that A33 targeting might sensitize tumors to chemotherapeutic agents. The trial involved patients with advanced colorectal disease that was resistant to other chemotherapeutic drugs. The combination was found to be safe, although HAHA remained an obstacle. Several of the patients had

mixed or partial responses and one patient had disease stabilization for some months following the therapy regime. These encouraging results warrant the further investigation of a non-immunogenic A33 mAb in combination with chemotherapeutic agents.

Clinical Trials Centers

The LICR conducts a centrally managed, coordinated, global network of Phase I and II clinical trials that utilize standardized monitoring and technologies to compare single trial variables in parallel. This model allows the Institute to examine the clinical potential of new treatment strategies with maximum efficiency and flexibility, whilst ensuring patient safety, regulatory compliance, and the control of its intellectual property.

The following LICR Clinical Trials Centers had active trials in 2003:

Asia

- Gunma University School of Medicine, Gunma
- University School of Medicine, Mie
- Okayama University Medical School, Okayama
- Nagasaki University Graduate School of Biomedical Sciences, Nagasaki

Australasia

- LICR Melbourne Branch / Austin Hospital, Melbourne, Australia

Europe

- LICR Brussels Branch / Clinique Universitaires Saint-Luc, Brussels, Belgium
- Krankenhaus Nordwest, Frankfurt, Germany
- University of Saarland Medical School, Homburg, Germany
- LICR Lausanne Branch / Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland
- University Hospital Nijmegen, Nijmegen, Netherlands
- John Radcliffe Hospital, Oxford, United Kingdom

North America

- LICR New York Branch / Memorial Sloan-Kettering Cancer Center, New York
- Weill Medical College of Cornell University, New York
- Columbia-Presbyterian Medical Center, New York
- Mount Sinai School of Medicine, New York
- Roswell Park Cancer Institute, Buffalo
- Mayo Clinic, Rochester

Biological Production Facilities

There are complex production requirements for standardization and quality control that must be met when producing clinical reagents for use in human clinical trials. These "current Good Manufacturing Practices" (cGMP) ensure the safety of the patients receiving the products, and that the products comply with legal and regulatory approval, and licensing requirements. To rapidly and effectively translate discoveries into reagents for investigation in clinical trials, a bioproduction facility (BPF) is required to produce regulatory-standard material.

The BPF in Melbourne, which uses eukaryotic cell production, supplies many of the cGMP-grade antibody and peptide reagents used in LICR clinical trials. In 2003, the facility vialed and dispensed several antibodies and vaccine reagents for LICR clinical trials, whilst the production focused on the mAb 806. This mAb is scheduled to enter early-phase clinical trials in 2004, and will be investigated initially for its application to head and neck cancers.

The Cornell University/LICR BPF at Ithaca, New York, which uses microbial systems to produce reagents, began its first production run in 2003. The team at the Bioprocess Laboratory generated a 'single-chain' A33 construct that is expected to overcome immunogenicity problems with the huA33, and developed a system in which the construct is expressed in a particular type of yeast. This system was scaled up, and subsequently entered the BPF in December 2003. The Bioprocess Laboratory at the Ithaca facility has also generated other single-chain constructs for early-phase clinical trials, and BPF production of these is scheduled for 2004.

Intellectual Property Program

To ensure that the Institute is able to capitalize on its discoveries, a vigorous patent protection policy has been pursued. In 2003, 94 patents were issued to the Institute in the United States of America with corresponding issued and published world-wide patents, and a further 51 new priority patents were filed. Most of these patents are related to growth factors, cytokines, signaling molecules, antibodies and human tumor antigens.

The research efforts of the LICR have resulted in a series of unique scientific discoveries, leading to the establishment of a significant, intellectual property portfolio. As an example, the Institute's Antigen Discovery Program underlies several other LICR Programs, including the Antibody Targeting, Bioinformatics, Genomics, and Cancer Vaccine Programs. Intense endeavors are now underway to command the attention of the pharmaceutical and biotechnology industries to these discoveries as a source of licensing acquisitions for the development of future cancer therapies.

To date, certain antigens, such as those of the MAGE family and the NY-ESO-1 antigen discovered as part of the Antigen Discovery Program, have been licensed to pharmaceutical or biotechnology partners for development as cancer vaccines. In most cases, only specific utilities for these antigens have been licensed as appropriate to the partner's expertise and proprietary technology.

Mr. R. Palmer Baker, Jr.
Chairman

Mr. Georges-Andre Cuendet

Mr. Olivier Dunant

Dr. Adolf E. Kammerer

Mr. Pierre Languetin

Mr. Edward A. McDermott, Jr.

Dr. Lloyd J. Old

Sir Derek Roberts

Dr. Jane Royston (from June)

Mr. Alfred Berger (from June)

Mr. John D. Gordan III (from June)

Mr. Richard D.J. Walker
Secretary To The Board

Dr. Lloyd J. Old
Chairman

Dr. Douglas T. Fearon	*University of Cambridge, Cambridge, England*
Dr. Samuel Hellman	*University of Chicago, Chicago, U.S.A.*
Dr. George Klein	*Karolinska Institute, Stockholm, Sweden*
Dr. Ira Mellman	*Yale University, New Haven, U.S.A.*
Dr. Lucille Shapiro	*Stanford University, Stanford, U.S.A.*
Dr. Phillip Sharp	*Massachusetts Institute of Technology, Cambridge, U.S.A.*
Dr. Harald Zur Hausen	*Deutsches Krebschforschungszentrum, Heidelberg, Germany*

Dr. A. Munro Neville
Secretary To The Committee

Ludwig Institut für Krebsforschung
Stadelhoferstrasse 22
8001 Zurich, Switzerland

Registered Address

Postfach 8024 Zurich, Switzerland

Postal Address

Telephone: [41] (0)1-267 6262
Fax: [41] (0)1-267 6200

Mr. R. Palmer Baker Jr.
Chairman

Dr. Lloyd J. Old
Institute Director, and Chief Executive Officer

Mr. Edward A. McDermott, Jr.
President

Dr. A. Munro Neville
Institute Associate Director

Mr. Richard D.J. Walker
Chief Financial Officer

Milbank, Tweed, Hadley & McCloy, New York
Niederer, Kraft & Frey, Zurich

Counsels

KPMG Fides Peat, Zurich

Auditors

Credit Suisse, Zurich
National Westminster Bank PLC, London
HSBC Bank USA, New York

Principal Bankers

Australia: A.R.B.N. 001 379 344
Belgium: 415.420
Switzerland: CH-020.3.916.330-2
United Kingdom: fc007198
United States of America: EIN 23-712-1131

Company Registration Numbers

Ludwig Institute for Cancer Research *Address*
605 Third Avenue / 33rd Floor
New York, NY 10158 U.S.A.
Telephone: [1] 212 450 1500
Fax: [1] 212 450 1555

Mr. R. Palmer Baker, Jr. *Chairman*
Ms. Karen A. Hyland *Executive Assistant*
Fax: [1] 212 450 1545

Dr Lloyd J. Old *Institute Director and Chief Executive Officer*
Ms Nancy Leyrer *Assistant to Institute Director*
Fax: [1] 212 450 1515

Mr. Edward A. McDermott, Jr. *President*
Ms. Kathy Meaghan *Assistant to President*
Ms. Nadette Bulgin *Deputy Assistant to President*
Fax: [1] 212 450 1555

James R. Kerr Program

Dr. Andrew J.G. Simpson *Director*
Fax: [1] 212 450 1555

Office of Academic Review

Dr. Ellen Puré *Director*
Ms. Teresa Pappas *Assistant to Director*
Fax: [1] 212 450 1525

Office of Clinical Trials Management

Dr. Eric W. Hoffman *Director*
Dr. Gerd Ritter *Assistant Director*
Ms. Lisa Pugliese *Manager (until November)*
Dr. Ralph Venhaus *Head of Clinical Affairs*
Ms. MaryJane Rafii *Head of Regulatory Affairs and Safety*
Ms. Julie Andrew *Clinical Project Manager*
Ms. Kristin Iversen, *Clinical Project Manager*
Mr. Lawrence T. Persaud *IT Portfolio (until October)*
Mr. John C-K. Tang *Clinical Data Management*
Mr. Gary O'Donnell *Manager Drug Safety*
Dr. Linda Pan *Clinical Programs Associate*
Ms. June Swirz *Assistant to Director*
Ms. Elisa Ruiz *Executive Secretary*
Fax: [1] 212 450 1535

Office of Communications

Mr. Eric Sabo *External Relations Officer*
Dr. Sarah L. White *Communications Officer*
Mr. Joshua Carr *Electronic Communications Assistant*

Office of Intellectual Property

Dr. Jonathan C. Skipper *Manager*
Dr. Pär Olsson *Scientific Administrative Officer*
Dr. Mona Jhaveri *Scientific Administrative Officer*

Office of Program Development

Dr. Jonathan C. Skipper *Director*

Office of the Protocol Review Committee

Dr. Herbert F. Oettgen *Chairman*
Ms. Nancy Leyrer *Secretary*

Ludwig Institute for Cancer Research *Address*
Horatio House
77-85 Fulham Palace Road / 5th Floor South
London, W6 8JC, United Kingdom
Telephone: [44] (0)20 8735 9240
Fax / Main: [44] (0)20 8741 4990
Fax / Second: [44] (0)20 8741 5884

Office of Intellectual Property

Dr. A. Munro Neville *Director*
(Associate Director of Institute)

Dr. Deborah Carter *Associate Director*
Dr. Pauline Stasiak *Senior Scientific Administrative Officer*
Dr. Amy (Lam) Jeffs *Scientific Administrative Officer*
Mr. Ben Williams *Scientific Administrative Assistant (from September)*
Ms. Linda A. Lenthall *Assistant to Director*
Ms. Brenda Goulder *Secretary*

Office of Technology Licensing

Dr. Robert Burns *Director*

Ludwig Institut für Krebsforschung *Address*
Stadelhoferstrasse 22
8001 Zürich, Switzerland

Postfach: 8024 Zürich, Switzerland *Postal Address*

Telephone: [41] (0)1 267 62 62
Fax / Main: [41] (0)1 267 62 00

Mr. Richard D.J. Walker *Chief Financial Officer*
Ms. Andrea Keller *Assistant to Chief Financial Officer*
Mr. Odd Aardalsbakke *Deputy Chief Financial Officer*

Mr. Martin Born *Financial Controller*
Ms. Patricia McCormick *Assistant Controller IP*
Mr. Darren Shearer *Assistant Controller IP*
Ms. Haruko Hartmann *Assistant IP (until March)*

Ms. Paola Maio *Accountant*
Mr. Reto Schläfle *Accountant*

Mr. Steve Birnbaum *Manager IT Finance*

Mr. Richard McCleary *Budget and Planning Manager/Internal Auditor*

Ms. Karin Genhart *Secretary*

Ludwig Institute for Cancer Research
Chemin des Boveresses 155
1066 Epalinges
Switzerland
Telephone: [41] (0)21 692 59 91
Fax / Main: [41] (0)21 692 59 45

Address

Office of Information Technology

Dr. C. Victor Jongeneel *Director*
Ms. Jocelyne Muller *Secretary/Administrator*
Dr. Christian Iseli *Systems Manager*
Dr. Dmitri Kuznetsov *Biocomputing Specialist*
Dr. Brian Stevenson *Bioinformatics Specialist*
Dr. Yves Stauffer *Postdoctoral Fellow*
Mr. Gregory Theiler *Graduate Student*

"Cancer Immunity" Journal

Dr. Monique Zahn *Editorial Assistant*

Brussels Branch of Human Cancer Cell Genetics
Avenue Hippocrate 74, UCL 7459
1200 Brussels, Belgium
Tel: [32] (0)2 764 74 59
Fax: [32] (0)2 762 94 05

Dr. Thierry Boon *Branch Director*

Lausanne Branch of Immunology
Chemin des Boveresses 155
1066 Epalinges s/Lausanne, Switzerland
Tel: [41] (0)21 692 59 66
Fax: [41] (0)21 653 44 74

Dr. Jean-Charles Cerottini *Branch Director*

London/St Mary's Branch of Molecular Virology
Imperial College of Science Technology and Medicine/St Mary's Campus
Norfolk Place, W2 1PG, London, UK
Tel: [44] (0)20 7724 55 22
Fax: [44] (0)20 7724 85 86

Dr. Paul J. Farrell *Branch Director*

London/University College Branch of Cell and Molecular Biology
Courtauld Building, 91 Riding House Street
W1W 7BS, London UK
Tel: [44] (0)20 7878 4000
Fax: [44] (0)20 7878 4040

Dr. Michael D. Waterfield *Branch Director*

Melbourne Branch of Tumour Biology
PO Box 2008 Royal Melbourne Hospital
Parkville, Victoria 3050, Australia
Tel: [61] (0)3 9341 31 55
Fax: [61] (0)3 9341 31 04

Dr Antony W. Burgess *Branch Director*

New York Branch of Human Cancer Immunology
Memorial Sloan-Kettering Cancer Center
1275 York Avenue, Box 32
New York, NY 10021-6007 USA
Tel: [1] 212 639 8600
Fax: [1] 212 717 3100

Dr Lloyd J. Old *Branch Director*

San Diego Branch of Cancer Genetics
University of California San Diego
9500 Gilman Drive
La Jolla, CA 92093-0660 USA
Tel: [1] 858 534 7802
Fax: [1] 619 534 7750

Dr Webster K. Cavenee *Branch Director*

São Paulo Branch of Cancer Genetics
Rua Prof. Antonio Prudente
109-4 Andar, Liberdade 01509-010
São Paolo, SP, Brazil
Tel: [55] (0xx) 11 33 88 32 00
Fax: [55] (0xx) 11 32 07 70 01

Dr Ricardo R. Brentani *Branch Director*

Stockholm Branch of Molecular and Cell Biology
Karolinska Institute, Box 240
S-17177 Stockholm, Sweden
Tel: [46] (0)8 728 71 00
Fax: [46] (0)8 33 28 12

Dr Ralf E. Pettersson *Branch Director*

Uppsala Branch of Growth Regulation
Biomedical Center, Box 595
S-751 24 Uppsala, Sweden
Tel: [46] (0)18 16 04 00
Fax: [46] (0)18 16 04 20

Dr. Carl-Henrik Heldin *Branch Director*

LUDWIG INSTITUTE FOR CANCER RESEARCH

Brussels Branch
of Human Cancer Cell Genetics

Brussels, Belgium

Staff List

Boon, Thierry *Director, Member*

Tumor Immunology Group

Van den Eynde, Benoît *Member*
Uyttenhove, Catherine *Senior Investigator*
Chapiro, Jacques *Postdoctoral Fellow*
Ma, Wenbin *Postdoctoral Fellow*
Morel, Sandra *Postdoctoral Fellow (until July)*
Théate, Ivan *Postdoctoral Fellow, part time*
Pilotte, Luc *Research Associate*
Donckers, Dominique *Technician*
Piette, Fanny *Technician*
Huijbers, Ivo *Graduate Student*
Guillaume, Benoit *Graduate Student*
Naslund, Tanja *Graduate Student (from April)*
Parmentier, Nicolas *Graduate Student (from October)*
Vigneron, Nathalie *Graduate Student*
Warren, Edus *Visiting Scholar (from July)*

Tumor Genetics Group

De Plaen, Etienne *Assistant Member*
De Smet, Charles *Assistant Member*
Blondiaux, Claudine *Technician*
Kholmanskikh, Olga *Graduate Student (from October)*
Loriot, Axelle *Graduate Student*

Human Tumor Antigens Group

van der Bruggen, Pierre *Member*
Stroobant, Vincent *Postdoctoral Fellow*
Wildmann, Claude *Research Associate*
Zhang, Yi *Postdoctoral Fellow*
Renkvist, Nicolina *Postdoctoral Fellow*
Sun, Zhaojun *Visiting scientist*
Authom, Anne *Technician (until June)*
Loeuille, Sébastien *Technician (until March)*
Demotte, Nathalie *Technician*
Ottaviani, Sabrina *Technician*
Tollet, Bénédicte *Technician*
Nicolay, Hugues *Graduate Student*

Immunotherapy Analysis Group

Van Pel, Aline *Associate Member*
Colau, Didier *Senior Investigator*
Godelaine, Danièle *Senior Investigator*
Brasseur, Francis *Associate Investigator*
Lethé, Bernard *Associate Investigator*
Lurquin, Christophe *Associate Investigator*

Bastin, David *Technician (from November)*
Ha Thi, Vinh *Technician*
Letellier, Marie-Claire *Technician*
Panagiotakopoulos, Maria *Technician*
Swinarska, Magdalena *Technician*
Van Vooren, Alice *Technician (until August)*
Carrasco, Javier *Graduate Student*

Therapeutic Vaccination Group

Marchand, Marchand *Senior Clinical Investigator*
van Baren, Nicolas *Clinical Investigator*
Bousmans, Nathalie *Clinical Research Associate*
Degueldre, Jérôme *Clinical Research Associate*
Verfaille, Christian, M.D. *Clinical Research Associate*
Gueur, Marie-Christine *Secretary, part-time (until June)*

Cytokine Group

Renauld, Jean-Christophe *Member*
Van Snick, Jacques *Member*
Dumoutier, Laure *Postdoctoral Fellow*
Louahed, Jamila *Postdoctoral Fellow*
Cormont, Françoise *Technician*
de Lestré, Brigitte *Technician*
Stevens, Monique *Technician*
Van Roost, Emiel *Technician*
Darcaigne, David *Graduate Student (until August)*
Knoops, Laurent *Graduate Student*
Lejeune, Diane *Graduate Student (until September)*
Steenwinckel, Valérie *Graduate Student*

Signal Transduction Group

Constantinescu, Stefan *Assistant Member*
Moucadel, Virginie *Postdoctoral fellow*
Kubatzky, Katharina *Postdoctoral fellow*
Li, Mingli *Technician (from November)*
Menu, Catherine *Technician*
El-Najjar, Nicole *Graduate Student (until April)*
Royer, Johan *Graduate Student*
Staerck, Judith *Graduate Student*

Pre-clinical Research Resources Group

Warnier, Guy *Veterinarian and Group Head*
Matthews, Jean-Pierre *Technical Assistant, part time*
Sirichanto, Phone Praseuth *Technical Assistant*
Souvannaraj, Keota *Technical Assistant*
Souvannaraj, Keoprasith *Technical Assistant, part time*

Technical Support

Lejeune, Joseph *Head of Computer Systems*
Wauters, Paul *Laboratory Manager*

Tonon, André *FACS Operator*
Daubresse, Pierre *Computer Analyst (until February)*
Oaks, Lee *Computer Analyst (April to June, part time)*
Germis, Jean-Luc *Technician*
Orviz, Manuel *Technical Assistant*
Van Goetsenhoven, Antoinette *Technical Assistant*
Ahloumou, Mohamed *Laboratory Helper (from December)*
Hamed, Mustafa *Laboratory Helper*
Jebari, Samira *Laboratory Helper*
Mahieu-Gaudy, Pascale *Laboratory Helper*
Marianini, Encarnacion *Laboratory Helper*
Reyes Prieto, Rafaela *Laboratory Helper*
Calleja, Michel *Office Clerk*

Administration & Secretariat

Florean, Dario *Administrator*
Alsteens, Matthieu *Accounting Assistant (February to April)*
Bien, Aline *Accounting and Administrative Assistant*
Hoppe, Sylvie *Scientific Secretary*
Krack, Nathalie *Librarian*
Lemoine, Carine *Scientific and Administrative Assistant, part time*
Roks, Arnaud *Accounting Assistant (from April)*
Schoonheydt, Geneviève *Administrative Assistant*
Stavaux, Pierren *Accounting Assistant (until March)*
Winand, Valérie *Scientific Secretary (from August)*

Director's Report

The Brussels Branch specializes in cancer immunology and cancer genetics. The notion that the immune system might be enlisted to rid the body of cancer draws on past work at the Branch that revealed that most human tumors bear antigens that can be recognized by cytotoxic T lymphocytes (CTLs). Some of these antigens are highly tumor-specific, others are expressed on certain normal cells. A number of antigens have been found on many different types of tumors, suggesting that a therapeutic strategy targeting such antigens could be used to treat a wide range of cancers. The Branch continues the search for tumor antigens, and evaluates their therapeutic potential in vaccine trials of cancer patients.

The Brussels Branch is also involved in research on the immunological functions of several cytokines, particularly IL-9 and IL-22, which were discovered at the Branch. Signal transduction by certain cytokine receptors is also under intense study.

Thierry Boon

Research Report

Tumor Immunology Group

The central research theme of the group of Dr. Benoît Van den Eynde is the study of tumor antigens recognized by T lymphocytes. Besides a continued effort to identify additional antigens of interest, the group addresses a number of fundamental or mechanistic issues that have a direct impact on the utilization of such antigens as cancer vaccines in human patients. These antigens consist of peptides that are presented by MHC molecules at the cell surface and derive from intracellular proteins that are degraded by the proteasome. The intracellular pathway leading from the protein to the peptide/MHC complex is known as "antigen processing". The group is currently studying the processing of several human tumor antigens by the proteasome, and is particularly interested in the processing differences that have been observed between the standard proteasome, which is present in most cells, and the immunoproteasome which is found in some dendritic cells and in cells exposed to interferon-gamma. The group is also investigating a novel aspect of the proteasome function in antigen processing, which is its ability to produce antigenic peptides by a mechanism of "peptide splicing".

The group is also studying a mouse preclinical model of cancer immunotherapy, to define the optimal conditions to induce effective anti-tumor responses by various vaccination approaches against defined antigens. This has led to the discovery of a powerful mechanism of tumor resistance which is based on tryptophan catabolism by indoleamine-2,3 dioxygenase, an enzyme that was found frequently expressed in tumors. The resulting local tryptophan shortage appears to prevent the proliferation of lymphocytes at the tumor site. Inhibitors of indoleamine-2,3 dioxygenase can be used in vivo to counteract this tumor resistance mechanism. To obtain the most relevant information from such preclinical models, the group is also attempting to generate a new mouse melanoma model in which tumors expressing a given antigen could be induced, using a transgenic system based on Cre-lox recombination. This should recapitulate the long-term host/tumor relationship that occurs in humans when a tumor slowly develops within a normal tissue

Tumor Genetics Group

Human tumors express specific antigens arising from the activation of genes, such as MAGE, BAGE, GAGE and LAGE/NY-ESO1, which are normally expressed only in germ cells. As germ cells are not subject to scrutiny by the immune system, antigens encoded by these genes are strictly tumor-specific. The group of Drs. Etienne De Plaen and Charles De Smet is trying to identify new genes that are specifically expressed in tumors and germ cells. Screening procedures based on differential expression profiling allowed the isolation of several genes with cancer and germline specific expression. Most of these genes are normally expressed in spermatogonia, the pre-meiotic stage of sperm development, and are located on the X chromosome

Additionally, efforts are devoted to determining the function of these "cancer-testis" (CT) genes. To analyze the functions of a MAGE protein, MAGE-A1, the group has searched for binding partners of this protein. Using yeast two-hybrid screening, they found interaction between MAGE-A1 and transcriptional regulator SKIP. SKIP is an adaptor protein that connects DNA-binding proteins to proteins that activate or repress transcription. The results suggest that by binding to SKIP and by recruiting the histone deacetylase 1, protein MAGE-A1, when present in the nucleus, represses transcription. The group is now trying to identify the genes that are regulated by MAGE-A1 using an inducible transfected MAGE-A1 gene and microarray technology.

Finally, the group is also studying the mechanisms leading to the activation of CT genes in tumors.

It has been previously shown by the group that DNA methylation is an essential component of their repression in normal somatic tissues. Demethylation and therefore activation of CT genes in tumors was found to be coincident with overall genome demethylation, a process known to occur in many cancers. The group is now focusing on the mechanisms of demethylation of these genes in tumors. Activation of cancer-germline genes in tumors appears to rely on a historical event of demethylation, and on the presence of specific transcription factors that maintain the promoter region unmethylated. The transcription factors, DNA methyltransferases and chromatin modifying enzymes involved in regulating the methylation status of these genes are currently being characterized.

Human Tumor Antigens Group

The group led by Dr. Pierre van der Bruggen is defining antigenic peptides encoded by CT genes like those of the MAGE family, which are expressed by cells from many different cancer types. Therapeutic vaccination of cancer patients with MAGE peptides is now in progress, and the identification of additional antigenic peptides is important to increase the range of patients eligible for therapy, and to provide tools for a reliable monitoring of the immune response. Stimulation of CD8+ T lymphocytes with antigen-presenting dendritic cells (DC) infected with viruses carrying MAGE genes has led to the identification of a large number of new antigenic peptides presented by HLA class I molecules. Similarly, stimulation of CD4+ T cells with DC pulsed with a MAGE protein has revealed new antigenic peptides presented by HLA class II molecules. The finding that almost every cancer patient whose tumor expresses a MAGE gene has at least one HLA molecule presenting a MAGE antigenic peptide suggests numerous possibilities for therapeutic vaccination. Efforts are currently devoted to set up assays that accurately monitor CD4+ T cell responses to cancer vaccines. In collaboration with Dr. Didier Colau (Institute of Cellular Pathology, Brussels), the group recently validated the use of the first HLA-DP4 tetramer, which was folded with a MAGE-3 peptide. More versatile approaches to isolate anti-vaccine T cells from patients injected with proteins are now being developed.

Functional defects of T cells are also being studied. The group has observed that human CTL clones lose their specific cytolytic activity and cytokine production under certain stimulation conditions. These inactive CTL simultaneously lose their labeling by an HLA-peptide tetramer, even though the amount of TCR-CD3 at their surface is not reduced. Our results suggest the existence of a new type of functional defect of CTL.

Immunotherapy Analysis Group

After vaccination with tumor-specific MAGE antigens, either as peptide alone, as recombinant Alvac canarypox or with autologous DC pulsed with antigenic peptides, no more than 20% of patients display tumor regression. In order to search for a correlation between the clinical responses and the anti-vaccine cytotoxic CD8+ T cell responses, the group of Drs. Aline Van Pel and Danièle Godelaine has pursued its collaboration with Drs. Pierre Coulie (Institute of Cellular Pathology, Brussels), Gerold Schuler (University Hospital, Erlangen, Germany), and Kris Thielemans at the Vrije Universiteit Brussel (Brussels). The anti-vaccine T cell response was first followed by using the well established HLA-peptide tetramer approach that allows the isolation of antigen-specific T cells, the obtention of clones and the analysis of their TCR sequences. Some patients were also studied for their immune reactivity against their tumor. For this purpose, metastases were removed by surgery and tumor cell lines were established in culture. T lymphocytes infiltrating metastases were isolated by mixed lymphocyte-tumor culture, cloned and characterized. Similarly, T cell clones recognizing the tumor were derived from the blood and analyzed, and novel T cell epitopes were identified. Importantly, from the knowledge of their TCR and by using a genetic approach, it will be possible to evaluate whether some T cell clones were amplified after the vaccination, either at the site of a metastasis or in the blood.

Another approach was followed to understand why a minority of patients experienced regression

of metastatic lesions following vaccination. Pre-vaccine tumor samples were tested for the quantitative expression of several CT genes, of genes encoding melanoma differentiation antigens, and genes that may be involved in tumor resistance to CTL attack. However, no difference in the expression profiles of these genes was observed between patients with (n=20) or without (n=27) regression.

Therapeutic Vaccination Group

Because of their tumor specificity, MAGE antigens are promising candidates for cancer vaccine development. Led by Drs. Marie Marchand and Nicolas Van Baren, the group designs and carries out such clinical trials. While it is possible to make CTL recognize and kill autologous tumor cells in vitro, the precise way to induce an effective CTL response against a MAGE antigen in cancer patients is not yet known. Clinical vaccination trials have two main objectives. First, the effectiveness of various vaccination modalities can be assessed by following the clinical evolution of the tumor, by analyzing whether a specific CTL response to the vaccine antigen occurred, and by determining whether immunological and clinical responses are correlated. Secondly, T lymphocytes and tumor samples collected at different time points during vaccination can be analyzed in detail, which improves the understanding on what happens in patients who experience regression of metastatic lesions, and which may explain why this does not happen in the majority of patients with overall disease progression. This knowledge can then be used to design new vaccination modalities.

Trials investigating either MAGE peptides, recombinant MAGE proteins, and a recombinant ALVAC virus encoding a MAGE minigene have been completed or are ongoing. All of these cancer vaccines are very well tolerated by the patients. Tumor regressions are observed in about 20% of the vaccinated patients with metastatic melanoma, with complete or partial clinical responses being observed in about 10% of the patients.

Cytokine Group

Led by Dr. Jean-Christophe Renauld, the group studies the biology of interleukin-9 (IL-9) and IL-22, two cytokines discovered at the Branch. IL-9 is a Th2 cytokine that plays a role in immune responses against intestinal parasites and asthma. IL-22, originally identified as a gene induced by IL-9 in T lymphocytes, upregulates the production of acute phase reagents in liver. Its activity in inflammatory responses is modulated by a specific antagonist, the IL-22 binding protein. The role of IL-9 and IL-22 in inflammation is currently investigated using transgenic and gene-targeted mice for these cytokines and their receptors.

First, IL-9 transgenic mice that have a high level of this cytokine in all tissues, are characterized by a high susceptibility to the development of T cell lymphomas. Another major aspect of IL-9 biology is its effect on growth and differentiation activity of mast cells. IL-9 transgenic mice show increased numbers of mast cells in the gut and airways. Finally, a puzzling activity of IL-9 is a selective increase in the peritoneal B1b cell subpopulation. Although the specificity of these cells is far from clear, they might be related to some auto-immune processes. In line with the oncogenic activity of IL-9 in transgenic mice, this cytokine was shown to be a potent anti-apoptotic factor for T cell lymphomas. The anti-apoptotic effect of IL-9 does not involve MAP-kinases but is mediated by the JAK/STAT pathway. Therefore, the group is now focusing on the characterization of genes whose expression is regulated by IL-9 through the activation of STAT transcription factors.

Signal Transduction Group

Cytokines and their receptors are critical for the formation of blood cells and for the function of the immune system. The group led by Dr. Stefan Constantinescu studies the mechanisms by which Janus

kinases (JAKs) act as chaperones for the intracellular traffic of cytokine receptors, and attempts to identify novel proteins that bind to JAKs and receptors during trafficking. A major effort is dedicated to the determination of the structure and orientation of the transmembrane (TM) and juxtamembrane (JM) domains of the receptors for erythropoietin, thrombopoietin, G-CSF and IL-9. Orientation and sequence-specific interactions between TM domains were shown to be crucial for productive signaling through the use of a novel approach in which fusion proteins are engineered between a dimeric coiled-coil, replacing the extracellular domain of cytokine receptors, of the TM and cytosolic domains of receptors. These fusion proteins are engineered so that all seven possible relative orientations of the dimeric receptors are obtained. Using this system the group has recently identified the active dimeric conformation of the erythropoietin receptor (EpoR), as well as a partially active conformation which preferentially activates MAP-kinase signaling and cell-survival, but cannot support cell proliferation and STAT activation. The group has applied this approach to other receptors and has identified different active dimeric conformations of the thrombopoietin receptor (TpoR), which appear to act at different stages of hematopoietic development via distinct signaling pathways. In vivo experiments are in progress in order to ascribe different signaling modes and biologic effects to the differently oriented EpoR and TpoR dimers. The group also studies the mechanisms by which STAT proteins become constitutively activated and how they function in the nucleus of transformed hematopoietic or patient-derived leukemia cells.

Publications

Primary Research Articles

1. Bonehill A., Heirman C., Tuyaerts S., Michiels A., Yi Zhang, van der Bruggen P., Thielemans K. Efficient presentation of known HLA class II restricted MAGE-3 epitopes by dendritic cells electroporated with mRNA encoding an invariant chain with genetic exchange of CLIP. Cancer Research (2003) 63:5587–5594.
2. Bouzin C., Clotman F., Renauld J-C., Lemaigre F.P., Rousseau G.G. The Onecut Transcription Factor Hepatocyte Nuclear Factor-6 Controls B Lymphopoiesis in Fetal Liver. Journal of Immunology (2003) 171:1297-1303.
3. Constantinescu S. Stemness, fusion and renewal of hematopoietic and embryonic stem cells. Journal of Cellular and Molecular Medicine (2003) 7:103-112.
4. Constantinescu S., Keren T., Russ W., Ubarretxena-Belandia I., Malka Y., Kubatzky K., Engelman D., Lodish H., and Henis Y. The Epo receptor transmembrane domain mediates complex formation with viral anemic and polycythemic gp55 proteins. Journal of Biological Chemistry (2003) 278:43755-43763.
5. Dadaglio G., Morel S., Bauche C., Moukrim Z., Lemonnier F.A., Van den Eynde B.J., Ladant D., Leclerc C. Recombinant adenylate cyclase toxin of Bordetella pertussis induces cytotoxic T lymphocyte responses against HLA*0201-restricted melanoma epitopes. International Immunology (2003) 15:1423-1430.
6. Demoulin J-B., Louahed J., Dumoutier L., Stevens M., Renauld J-C. MAP kinase activation by IL-9 in lymphoid and mast cell lines. Oncogene (2003) 22:1763-1770.
7. Dogusan Z., Martens N., Stinissen P., Hellings N., Demotte N., Hooghe R., Hooghe-Peters E. Effects of prolactin on cloned human T-lymphocytes. Endocrine (2003) 20:171-176.
8. Dumoutier, L., Lejeune D., Hor S., Fickenscher H., Renauld J-C. Cloning of a new type II cytokine receptor activating signal transducer and activator of transcription (STAT)1, STAT2 and STAT3. Biochemical Journal (2003) 370:391-396.
9. Godelaine D., Carrasco J., Lucas S., Karanikas V., Schuler-Thurner B., Coulie P., Schuler G., Boon T., Van Pel A. Polyclonal cytolytic T lymphocyte responses observed in melanoma patients vaccinated with dendritic cells pulsed with a MAGE-3.A1 peptide. Journal of Immunology (2003) 171:4893-4897.
10. Karanikas V., Lurquin C., Colau D., van Baren N., De Smet C., Lethé B., Connerotte T., Corbière V., Demoitié M-A., Liénard D., Dréno B., Velu T., Boon T., Coulie P. Monoclonal anti-MAGE-3 CTL responses in melanoma patients displaying tumor regression after vaccination with a recombinant canarypox virus. Journal of Immunology (2003) 171:4898-4904.
11. Jacquemin M., Vantomme V., Buhot C., Lavend'homme R., Burny W., Demotte N., Chaux P., Peerlinck K., Vermylen J., Maillère B., van der Bruggen P., Saint-Remy J-M. CD4+ T-cell clones specific for wild-type factor VIII : a molecular mechanism responsible for a higher incidence of inhibitor formation in mild/moderate hemophilia A. Blood (2003) 101:1351-1358.
12. Khan W.I., Richard M., Akiho H., Blennerhasset P.A., Humphreys N.E., Grencis R.K., Van Snick J., Collins S.M. Modulation of Intestinal Muscle Contraction by Interleukin-9 (IL-9) or IL-9 Neutralization: Correlation with Worm Expulsion in Murine Nematode Infections. Infection and Immunity (2003) 71:2430-2438.

13. Kobayashi T., Lonchay C., Colau D., Demotte N., Boon T., van der Bruggen P. New MAGE-4 antigenic peptide recognized by cytolytic T lymphocytes on HLA-A1 tumor cells. Tissue Antigens (2003) 62:426-432.

14. Lange K., Uckert W., Blankenstein T., Nadrowitz R., Bittner C., Renauld J-C., Van Snick J., Feller A., Merz H. Overexpression of NPM-ALK induces different types of malignant lymphomas in IL-9 transgenic mice. Oncogene (2003) 22:517-527.

15. Loriot A., Boon T., De Smet C. Five new human cancer-germline genes identified among 12 genes expressed in spermatogonia. International Journal of Cancer (2003) 105:371-376.

16. Louahed J., Struyf S., Demoulin J.-B., Parmentier M., Van Snick J., Van Damme J., Renauld J.-C. CCR8-dependent activation of RAS/MAKP pathway mediates anti-apoptotic activity of I309/CCL1 and vMIP-I. European Journal of Immunology (2003) 33:494-501.

17. Nguyen T.D., Bigaignon G., Markine-Goriaynoff D., Heremans H., Nguyen T.N., Warnier G., Delmee M., Wolf S., Uyttenhove C., Van Snick J., Coutelier J-P. Virulent toxoplasma gondii strain RH promotes T-cell-independent overproduction of proinflammatory cytokines IL12 and gamma-interferon. Journal of Medical Microbiology (2003) 52:869-876.

18. Peri S., Navarro D., Amanchy R., Kristiansen T., Jonnalagadda C, Surendranath V., Niranjan V., Muthusamy B., Gandhi T., Gronborg M., Ibarrola N., Deshpande N., Shanker K., Shanker S., Rashmi P., Ramya M. Chandrika K., Padma N., Harsha H., Yatish A., Poovaiah K., Menezes M., Choudhury D., Suersh S., Ghosh N., Saravana R., Chandran S., Krishna S., Joy M., Anand S., Madavan V., Ansamma J., Wong G., Schiemann W., Constantinescu S., Huang L., Khosravi-Far R., Steen H., Tewari M., Ghaffari S., Blobe G., Dang C., Garcia J., Pevsner J., Jensen O., Roepstorff P., Deshpande K., Chinnaiyan A., Hamosh A., Chakravarti A., Pandy A. Development of human protein reference database as an initial platform for approaching systems biology in humans. Genome Research (2003) 13:2363-2371.

19. Poulin, L.F., Richard, M., Le Moine, A., Kiss, R., McKenzie, A.N., Goldman, M., Renauld, J.-C., Van Snick, J., Braun, M.Y. IL-9 promotes eosinophilic rejection of mouse heart allografts. Transplantation (2003) 76:572-577.

20. Ramesh R., Mhashilkar A.M., Tanaka F., Saito Y., Branch C.D., Sieger K., Mumm J.B., Stewart A.L., Boquio A., Dumoutier L., Grimm E.A., Renauld J-C., Kotenko S., Chada S. Melanoma Differentiation-associated Gene 7/Interleukin (IL)-24 Is a Novel Ligand That Regulates Angiogenesis via the IL-22 Receptor. Cancer Research (2003) 63:5105-5113.

21. Seubert N., Royer Y., Staerk J., Kubatzky K., Moucadel V., Krishnakumar S., Smith S., and Constantinescu S. Active and inactive orientations of the transmembrane and cytosolic domains of the erythropoietin receptor dimer. Molecular Cell (2003) 12:1239-1250.

22. Turner J., Faulkner H., Kamgno J., Cormont F., Van Snick J., Else K., Grencis R., Behnke J., Boussinesq M., Brandley J. Th-2 like cytokine responses correlate with age-dependent reduction in worm burdens in a human intestinal nematode infection. Journal of Infectious Diseases (2003) 188:1768-1775.

23. Uyttenhove C., Pilotte L., Théate I., Stroobant V., Colau D., Parmentier N., Boon T., Van den Eynde B. Evidence for a tumoral resistance mechanism based on tryptophan degradation by indoleamine 2,3-dioxygenase. Nature Medicine (2003) 9:1269-1274.

24. Vantomme V., Boël P., De Plaen E., Boon T., van der Bruggen P. A new tumor-specific antigenic peptide encoded by MAGE-6 is presented to cytolytic T lymphocytes by HLA-Cw16. Cancer Immunity (2003) 3:17.

25. Wilke M., Dolstra H., Maas F., Pool J., Brouwer R., Falkenburg J.H., Rebello A., Lamers F., Schuuring E., Kluin P., Brasseur F., Goulmy E. Quantification of the HA-1 gene product at the RNA level; relevance for immunotherapy of hematological malignancies. Hematology Journal (2003) 4:315-320.

26. Zhang Y., Chaux P., Stroobant V., Eggermont A., Corthals J., Maillère B., Thielemans K., Marchand M., Boon T., van der Bruggen P. A MAGE-3 peptide presented by HLA-DR1 to CD4+ T cells that were isolated from a melanoma patient vaccinated with a MAGE-3 protein. Journal of Immunology (2003) 171 : 219-225.

Reviews / Book Chapters / Commentaries

1. Boon T, van Baren N. Immunosurveillance against cancer and immunotherapy - synergy or antagonism? New England Journal of Medicine (2003) 348:252-254, 2003 (editorial).

2. Boon T., Van den Eynde B. Tumor Immunology. Editorial Overview. Current Opinion in Immunology (2003) 15:129-130.

3. Coulie P., van der Bruggen P. T-cell responses of vaccinated cancer patients. Current Opinion in Immunology (2003) 15:131-137.

4. Marchand M., Punt C., Aamdal S., Escudier B., Kruit X., Keilholz U., Håkansson L., van Baren N., Humblet Y., Mulders P., Avril M-F., Eggermont A.M.M., Scheibenbogen C., Uiters J., Wanders J., Delire M., Boon T., Stoter G. Immunisation of metastatic cancer patients with MAGE-3 protein combined with adjuvant SBAS-2: a clinical report. European Journal of Cancer (2003) 39:70-77.

5. Renauld J-C. Interleukin 9. The Encyclopedia of Hormones & Related Cell Regulators, H.L. Henry and A.W. Norman (Eds), Academic press, pp. 446-453, 2003.

6. Renauld J-C. Class II cytokine receptors and their ligands : key antiviral and inflammatory modulators. Nature Review Immunology (2003) 3:667-676.

7. Renauld J-C. IL-22 inhibitors to treat inflammatory disorders such as arthritis. Expert Opinions on Therapeutic Patents (2003) 13:373-374.

8. Van den Eynde B., Probst-Kepper M. Antigens recognized by T lymphocytes on renal cell carcinoma. In : Renal and Adrenal tumours - Renal Cell Carcinoma : molecular genetics and immunobiology. Editors : A. Belldegrun, A.W.S. Ritchie, R.A. Figlin, R.T.D. Oliver, E. Darracott Vaughan, Jr. Oxford University Press, New York, pp. 111-117, 2003.

9. Constantinescu S., Moucadel V. STAT signaling by erythropoietin. In Signal transducers and activators of transcription (STATs). Activation and Biology. Eds. Sehgal P., Levy D., Hirano T., Kluwer Academic Publishers, Chapter 36, 2003.

10. Renauld J-C., Van Snick J. Interleukin-9. In The Cytokine Handbook. A. Thomson & M. Lotze (Eds.), 4th Edition, Elsevier Science Ltd., Chapter 14, pp.347-362, 2003.

LUDWIG INSTITUTE FOR CANCER RESEARCH

Lausanne Branch of Immunology

Lausanne, Switzerland

Staff List

Cerottini, Jean-Charles *Director, Member*

Cell Fate Determination Group

Radtke, Freddy *Assistant Member*
Mancini, Stéphane *Postdoctoral Fellow*
Lathion, Stéphanie *Postdoctoral Fellow (from October)*
Besseyrias, Valérie *Research Associate*
Cobas, Monica *Ph.D. Student*
Nicolas, Michael *Ph.D. Student (until June)*
Riccio, Orbicia *Ph.D. Student (from April)*
Vauclair, Sophie *Ph.D. Student*

Developmental Immunology Group

MacDonald, H. Robson *Associate Director, Member*
Wilson, Anne *Associate Investigator*
Ernst, Bettina *Postdoctoral Fellow (until July)*
Schümann, Jens *Postdoctoral Fellow*
Voyle, Roger B. *Postdoctoral Fellow (until September)*
Ferrero, Isabel *Research Associate*
Lees, Rosemary K., *Research Associate*
Bianchi, Teresa *Ph.D. Student (from June)*

Innate Immunity Group

Held, Werner *Associate Member*
Coudert, Jérôme *Postdoctoral Fellow*
Zimmer, Jacques R. *Postdoctoral Fellow (until September)*
Goux, Delphine *Ph.D. Student*
Scarpellino, Leonardo *Senior Technical Assistant*

Molecular Immunology Group

Luescher, Immanuel F. *Member*
Guillaume, Philippe *Assistant Investigator (Tetramer Production Facility)*
Cebecauer, Marek *Postdoctoral Fellow*
Goffin, Laurence *Postdoctoral Fellow*
Mark, Silke *Postdoctoral Fellow*
Neff, Laurence *Postdoctoral Fellow (from October)*
Baumgärtner, Meier Petra *Research Associate*
Cornaz, Rolf *Technical Assistant (Tetramer Production Facility)*

Viral Immunology Group

Acha-Orbea, Hans *Associate Member (until August)*
Otten, Luc *Postdoctoral Fellow (until August)*
Rybtsov, Stanislav *Visiting Scientist (until August)*
Steiner, Quynh-Giao *Ph.D. Student (until August)*
Wagner, Julian *Ph.D. Student (until August)*
Bochatay, Maria *Technical Assistant (until August)*

Antigen Processing Group

Lévy, Frédéric *Associate Member*
Lindholm, Cecilia *Postdoctoral Fellow*
Chapatte, Laurence *Ph.D. Student*
Viatte, Sébastien *M.D.-Ph.D. Student*
Lévy, Nicole *Senior Technical Assistant*
Peitrequin, Anne-Lise *Senior Technical Assistant*

Molecular Tumor Immunology Group

Cerottini, Jean-Charles *Director, Member*
Ayyoub, Maha *Assistant Member*
Valmari, Danila *Assistant Member*
Rimoldi, Donata *Associate Investigator*
Michielin, Olivier *Postdoctoral Fellow*
Fagerberg, Theres *Ph.D. Student*
Mühlethaler, Katja *Senior Technical Assistant*
Salvi, Susanne *Senior Technical Assistant*

Clinical Tumor Immunology Group

Romero, Pedro *Member*
Speiser, Daniel E. *Associate Member*
Alves, Pedro *Postdoctoral Fellow (from May)*
Mahnke, Yolanda *Postdoctoral Fellow (from December)*
Pittet, Mikaël J. *Postdoctoral Fellow (until February)*
Tabiasco, Julie *Postdoctoral Fellow (from April)*
Zippelius, Alfred K.J. *Postdoctoral Fellow (until February)*
Meraldi, Verena *Research Associate (from July)*
Bioley, Gilles *Ph.D. Student*
Bricard, Gabriel *Ph.D. Student*
Dutoit, Valérie *Ph.D. Student (until March)*
Milesi, Renate *Senior Technical Assistant*
Montandon, Nicole *Senior Technical Assistant*
Porret, Andrée *Senior Technical Assistant (until April)*
Baroffio, Céline *Technical Assistant*

Clinical Trials Program

Speiser, Daniel E. *Associate Member*
Liénard, Danielle *Clinical Investigator*
Lejeune, Ferdy *Affiliate Clinical Investigator*
Geldhof, Christine *Clinical Research Associate*
Minaidis, Danielle *Research Nurse*

Technical Services

Zaech, Pierre *Technical Engineer*
Devêvre, Estelle *Technical Services (from June)*

Administration & Secretariat

Méan, Marie-Madeleine *Administrator*
Seravalli, Capucine *Accounting Chief*
Marouani, Iris *Accounting Assistant*
Nogaré, Nicole *Accounting Assistant*
Lopez, Celia *Secretary to Director*
Poncelet, Audrey *Secretary*
Van Overloop, Martine *Secretary*
Erismann-Zoppi, Anna *Secretary*

Branch Director's Report

It is well established that cancer development in a patient is not solely dependent on the intrinsic properties of tumor cells but is the net result of complex interactions between tumor cells and a variety of normal cells within a given tissue. Among host-tumor interactions, lymphocyte-mediated activities are thought to play a major role in the control of tumor cell proliferation and dissemination. The aim of the work performed at the Lausanne Branch is to delineate the various mechanisms used by different lymphoid cell types to inhibit tumor cell growth. The current research activities are focused on the molecular and cellular mechanisms that regulate immunity mediated by T cells, natural killer (NK) cells and NKT cells, and include a strong patient-oriented program devoted to the development of therapeutic cancer vaccines.

In 2003, Dr. Hans Acha-Orbea, head of the Viral Immunology group, who had a joint appointment as Associate Professor in the Department of Biochemistry, University of Lausanne, since 1994, left the Branch to take up a full-time position in the same Department. We thank him for providing leadership and scientific excellence to the Branch for the past fifteen years and look forward to continuing our scientific collaborations with him and benefiting from his expertise in the context of the immunology training and research program carried out at the University of Lausanne. The links with the latter will be strengthened by the recent appointment of three of our group leaders, Dr. Pedro Romero, Dr. Frédéric Lévy and Dr. Freddy Radtke as Professor (P. R.) and Assistant Professors (F. L. and F. R.), respectively, in the newly created Faculty of Biology and Medicine.

In addition to its core research, the Branch is actively involved in the National Center for Competence in Research program entitled "Molecular Oncology : from basic research to therapeutic approaches", that has been established by the Swiss National Science Foundation and is carried out in partnership with the Swiss Institute for Experimental Cancer Research (ISREC) and the Multidisciplinary Oncology Center (CePO) at the Lausanne University Hospital. The NCCR program has opened up new collaborative links between scientists and clinicians in the area of translational cancer research and will serve as a model system for the implementation in Lausanne of an interdisciplinary consortium in cancer research in the near future.

Jean-Charles Cerottini

Research Report

Cell Fate Determination Group

The Cell Fate Determination Group led by Dr. Freddy Radtke is interested in the molecular mechanisms controlling stem cell maintenance, lineage commitment and differentiation in self-renewing systems such as the hematopoietic system, the skin and the gut. In addition this group investigates the potential role of these mechanisms in tumorigenesis.

Current attention is focused on the Notch signaling pathway, which is responsible for numerous binary cell fate decisions in diverse organisms. Previous studies by the group using conditional gene targeting strategy have established an essential role for Notch1 in specifying the T cell lineage. In addition a novel function for Notch1 has been uncovered at a later stage of T cell development, where it is involved in the control of VDJ rearrangement at the T cell receptor (TCR) beta locus. In the skin an unexpected function of Notch1 as a tumor suppressor gene has been discovered.

Developmental Immunology Group

The Developmental Immunology Group led by Dr. Rob MacDonald continues to be interested in the development of T cells and in particular a subset of nonconventional T cells known as NKT cells. NKT cells utilize a semi-invariant αβTCR to recognize glycolipids presented by the monomorphic CD1d molecule. This semi-invariant TCR is comprised of an invariant Vα14-Jα18 chain paired preferentially with Vβ8.2 and Vβ7 chains. Using novel dimeric CD1d-IgG molecules complexed with the artificial agonist glycolipid α-galactosyl-ceramide, the group has found that the Vβ domain of the semi-invariant TCR strongly influences TCR avidity for CD1d: glycolipid complexes. This result is currently being exploited to investigate the role of TCR avidity in positive and negative selection of NKT cells during thymus development.

In contrast to conventional T cells NKT cells express markers normally associated with NK cells including members of the Ly-49 receptor family. However whereas NK cells express both activating and inhibitory Ly-49 receptors, NKT cells only express inhibitory family members. The group has constructed transgenic mice expressing either activating or inhibitory Ly-49 receptors in NKT cells to investigate the role of Ly-49 receptor signaling in NKT cell development. It was found that forced ligation of activating Ly-49 receptors blocks NKT cell development at an early stage, while co-expression of an inhibitory Ly-49 receptor with the same ligand specificity rescues NKT cell development. Since Ly-49 receptors utilize similar positive (ITAM) and negative (ITIM) signaling motifs as TCR, these data indicate that appropriate TCR signaling thresholds are essential for NKT cell development.

Innate Immunity Group

The Innate Immunity Group led by Dr. Werner Held studies the development and function of natural killer (NK) cells. NK cells play important roles for innate immunity to infection and tumor cells. During the last year, the group reported that development of NK cells is dependent on the transcription factor TCF-1 (T cell factor 1). One of the targets of TCF-1 is Ly-49A , an inhibitory NK cell receptor specific for Major Histocompatibility Complex (MHC) class I molecules. This receptor normally prevents NK cell auto-aggression and enables NK cell reactions to aberrant cells lacking MHC class I molecules. Inhibitory NK cell receptors are also expressed on T cells, in particular on CD8+ memory T cells. In contrast to the constitutive expression on NK cells, the group found that Ly-49A expression was induced after the stimulation of auto-reactive TCRαβ T cells with their cognate self-antigen. Ly-49A expression substantially reduced the activation of auto-reactive T cells. Thus induction of inhibitory NK cell receptors on T cells contributes to self-tolerance.

Molecular Immunology Group

The Molecular Immunology Group led by Dr. Immanuel Luescher continues to investigate the molecular mechanisms involved in CD8 cytolytic T lymphocyte (CTL) recognition of, and activation by, peptide-MHC (pMHC) complexes. The current research effort deals with the preparation of well-defined soluble murine and human MHC class I (H2-Kd and HLA-A2) and their testing on cloned CTL of appropriate specificity. Using soluble Kd-PbCS(ABA) complexes, the group tested a family of dimers containing spacers of different length. Interestingly, dimers containing short linkers (3-30 Å) efficiently bound to and activated cloned CTL, whereas those containing long spacers (> 80 Å) did not. Based on fluorescence resonance energy transfer (FRET) experiments and computer assisted docking experiments, the group has described a high avidity binary antigen recognition mode, in which two pMHC complexes engage two TCR and CD8 molecules in an anti-parallel fashion. In addition, short dimers (as well as tetramers and octamers) induced rapid and vigorous apoptosis of antigen-specific CTL. This apoptosis was not perforin/granzyme, Fas or caspase dependent, but seemed to be mediated by pro-apoptotic molecules of the Bcl2 family, most likely BNIP3. Finally, long dimers (and tetramers) not only failed to activate antigen-specific CTL, but effectively inhibited their activation by agonists. The mechanism for this new type of antagonism seems to be interference with TCR dimerization/aggregation.

Viral Immunology Group

The Viral Immunology Group led by Dr. Hans Acha-Orbea has continued the study of the development and function of germinal centers in response to chronic viral infections. An important role of the lymph node draining the site of mouse mammary tumor virus (MMTV) injection in the maintenance of a chronic neutralizing antibody response was found. Surgically removing the draining lymph node after establishment of the germinal center reaction led to complete loss of neutralizing antibodies despite comparable systemic spread of MMTV infected lymphocytes. Importantly, in the absence of neutralization, only the exocrine organs (mammary gland, salivary gland and pancreas) and skin showed strikingly increased infection resulting in accelerated mammary tumor development. Therefore, the antigen deposits in the draining lymph node and the chronic immune response in this site appear to be crucial for the maintenance of a strong neutralizing immune response.

The group is now investigating the co-stimulation requirements for the maintenance of chronic germinal center reactions. For this purpose the group produced antibodies and recombinant molecules as well as generated several recombinant viruses that encode either stimulating or inhibiting co-stimulatory molecules. In parallel, genes specifically expressed in germinal centers are being cloned. For this purpose the group has isolated different germinal center subsets such as centrocytes, centroblasts or follicular dendritic cells and analyzed their gene expression program.

Antigen Processing Group

The Antigen Processing Group led by Dr. Frédéric Lévy is involved in the analysis of the intracellular events that contribute to the production of CTL-defined tumor antigens. The group has shown previously that the HLA-A*0201-restricted immunodominant peptide derived from Melan-A is inefficiently processed in cells expressing the immunoproteasome, a type of proteasome that is constitutively expressed in dendritic cells and induced in many other cells upon exposure to IFN-γ. Immunization of HLA-A2 transgenic mice with recombinant vectors expressing the minimal antigenic sequence of Melan-A induced a potent CTL response. In contrast, a modest response was detected after immunization with vectors expressing the full length Melan-A protein. We are currently evaluating whether the immunoproteasome contributes to this phenomenon.

Although the proteasomes produce the C-termini of most antigenic peptides, they do not always generate the appropriate N-termini. Those intermediates carry N-terminal extensions of varying length. However, for the HLA-A*0201-restricted Melan-A peptide, the proteasomes generate both N-terminally

extended intermediates and fully processed peptides. In such cases, the group found that the fully processed proteasomal product was preferentially selected for presentation by HLA-A*0201 molecules. This result implies that immunization with vectors coding for minimal Melan-A peptides should be favored.

Molecular Tumor Immunology Group

The current research efforts of the Molecular Tumor Immunology Group, led by Dr. Jean-Charles Cerottini, are devoted to the characterization of human tumor antigens that are recognized by CD8+ T cells from melanoma patients. An ongoing project is the delineation of the function of the melanocyte lineage specific protein Melan-A, a small transmembrane protein that is the target of vaccine trials in the Lausanne Branch. Previous studies by the group have revealed that its subcellular localization is distinct from typical melanosomal proteins, such as tyrosinase, as Melan-A accumulates in the trans-Golgi network and in early stage melanosomes. Furthermore, the group found that Melan-A is palmitoylated, suggesting the association with particular membrane subdomains. To understand the role of Melan-A in melanocytic cells, in vitro knock-down cells have been generated using lentivirus-delivered siRNA. The phenotype of the cells thus generated points to a possible negative role of Melan-A in pigmentation. In addition, the group has found that Melan-A is mono-ubiquitylated, a modification that can act as a sorting signal for the endocytic pathway. The implications of this modification on the protein function, localization and stability are currently under investigation.

A major breakthrough in melanoma research has been the recent discovery of a somatic point mutation in the BRAF gene in over 65% of tumors. BRAF is a serine/threonine kinase in the MAPK pathway mediating cellular responses to growth signals. The mutated BRAF protein possessed constitutively elevated kinase activity. The presence of the mutation may also have immunological consequences, as it may lead to the generation of novel or more antigenic CTL epitopes. As a background study to explore this hypothesis, a series of melanomas has been screened for the common BRAF mutation. The group could confirm that the majority of cutaneous melanoma metastases harbour the mutation. Surprisingly, all of the primary and metastatic uveal melanoma tumors tested carried a wild-type sequence, indicating a different etiology for cutaneous and uveal melanocytic tumors. Despite the absence of BRAF mutations, uveal melanomas, similar to their cutaneous counterparts, displayed an activated MAPK pathway, suggesting that deregulation of this pathway may be a hallmark of melanocytic transformation.

A distinct project, carried out in collaboration with the LICR Office of Information Technology, led by Dr. Victor Jongeneel, and the Swiss Institute of Bioinformatics, has used and developed molecular modeling techniques, such as homology modeling, molecular dynamics and free energy simulations, to design optimized peptides for cancer vaccines and construct models of T cell receptor-peptide-MHC complexes. This approach has been applied to the well defined Melan-A peptide presented by HLA-A2. Modified peptide candidates have been identified that exhibit increased HLA-A2 binding and protease resistance without alteration of the conformation adopted by the native peptide in the HLA-A2 groove. The best candidates have been synthesized and are being tested for efficient recognition by bona fide Melan-A specific CTL clones derived from melanoma patients.

Clinical Tumor Immunology Group

The Clinical Tumor Immunology Group led by Dr. Pedro Romero is focused on the design of molecularly defined therapeutic cancer vaccines. The melanocyte/melanoma associated antigen Melan-A/MART-1 is a well defined model system for these studies. An immunodominant peptide binding to the HLA-A2 molecule is recognized by tumor reactive T cells present at high numbers in the metastatic lesions of the majority of melanoma patients. An additional unique feature of this antigen is the presence of a high frequency of functionally naïve antigen specific CD8+ T cells in the peripheral blood of the majority HLA-A2 individuals, independent of the presence of a malignant melanoma lesion. This provides a convenient

baseline to monitor the frequency as well as the functional differentiation of Melan-A-specific T cells. As part of the Institute Clinical Trials program, a series of closely related phase I clinical trials with Melan-A peptide based vaccines have been carried out in collaboration with the Multidisciplinary Oncology Center (CePO, CHUV, Lausanne), the Division of Oncology of the University Hospital in Geneva (HUG) and the Brussels Branch. Forty nine patients with Melan-A+ high risk stage III and IV melanoma were vaccinated with Melan-A peptide +/- adjuvant +/- low dose recombinant IL-2. The peptide-specific T cell response in blood was measured directly ex vivo by both multiparameter flow cytometry combining HLA-A2/Melan-A peptide multimers and cell surface markers and direct IFN-γ ELISPOT. These studies indicated that repeated vaccination with Melan-A peptide and an incomplete Freund's adjuvant equivalent approved for human use frequently leads to sustained ex vivo detectable CD8 T cell responses. Two new, but related, phase I clinical trials were initiated this year. The first is designed to test the immunogenicity of the same peptide vaccine + Montanide combined with synthetic oligodeoxynucleotides containing bacterial DNA CpG motifs, a new adjuvant that activates plasmacytoid dendritic cells, NK and B cells via Toll-like receptor-9. Preliminary results indicate that addition of the CpG-ODN to the vaccine greatly enhances its ability to induce rapid A2/Melan-A peptide multimer+ CD8+ T cell responses. The second trial will determine the immunogenicity of the same Melan-A peptide admixed with the outer surface membrane protein of Gram negative bacteria OMPA-40, a new adjuvant that activates dendritic cells via the Toll-like receptor-2. The goal of these trials is to make stepwise progress towards the optimization of synthetic cancer vaccines.

The group is also involved in the analysis of the naturally acquired CTL responses directed against well defined tumor antigens. In addition to the response to Melan-A, the responses to other melanoma associated tumor antigens such as tyrosinase, gp100, NY-ESO-1/LAGE-1, MAGE-A10 and SSX-2 are investigated. The aim of these studies is to obtain a complete quantitative and functional assessment of antigen-specific T cells freshly isolated from human tissues. In this regard, the group demonstrated that HLA-A2 multimers bearing two mutations in the α3 domain, which abolish their interaction with the CD8 coreceptor on T cells, selectively bind to high avidity T cells. Thus, these reagents may be well suited to directly visualize T cells expressing high avidity T cell receptors, a critical parameter in vaccination. The group has also initiated the analysis of human CD4 T cell responses to well defined tumor and virus-derived antigens. To increase the analytical power, new PCR-based techniques are now routinely applied to the study of single antigen-specific T cells. These include T cell receptor CDR3-spectratyping and sequencing and single cell PCR. The latter is used to assess the expression of transcripts encoding molecules associated with T cell functions. In a separate project, collaborative work with the groups of Drs. MacDonald and Held investigates the role of human NKT cell responses to CD1/α-galactosylceramide in tumor immunity. Altogether, the results will be of great help for the accurate monitoring and development of new cancer vaccines.

Publications

Primary Research Articles

1. Finke, D., Luther, S.A., Acha-Orbea, H. The role of neutralizing antibodies for mouse mammary tumor virus transmission and mammary cancer development. Proceedings of the National Academy of Sciences USA (2003) 100:199-204.
2. Doucey, M.-A., Goffin, L., Naeher, D., Michielin, O., Baumgärtner, P., Guillaume, P., Palmer, E., Luescher, I.F. CD3δ establishes a functional link between the T cell receptor and CD8. Journal of Biological Chemistry (2003) 278:3257-3264.
3. Otten, L.A., Tacchini-Cottier, F., Lohoff, M., Annunziato, F., Cosmi, L., Scarpellino, L., Louis, J., Steimle, V., Reith, W., Acha-Orbea, H. Deregulated MHC class II transactivator expression leads to a strong Th2 bias in CD4+ T lymphocytes. Journal of Immunology (2003) 170:1150-1157.
4. Ayyoub, M., Zippelius, A., Pittet, M.J., Rimoldi, D., Valmori, D., Cerottini, J.-C., Romero, P., Lejeune, F., Liénard, D., Speiser, D.E. Activation of human melanoma reactive CD8+ T cells by vaccination with an immunogenic peptide analog derived from Melan-A/melanoma antigen recognized by T cells-1. Clinical Cancer Research (2003) 9:669-677.
5. Guillaume, P., Legler, D.F., Boucheron, N., Doucey, M.-A., Cerottini, J.-C., Luescher, I.F. Soluble major histocompatibility complex-peptide octamers with impaired CD8 binding selectively induce Fas-dependent apoptosis. Journal of Biological Chemistry (2003) 278:4500-4509.
6. Nicolas, M., Wolfer, A., Raj, K., Kummer, J.A., Mill, P., van Noort, M., Hui, C.-C., Clevers, H., Dotto, G.P., Radtke, F. Notch1 functions as a tumor suppressor in mouse skin. Nature Genetics (2003) 33:416-421.
7. Capone, M., Cantarella, D., Schümann, J., Naidenko, O.V., Garavaglia, C., Beermann, F., Kronenberg, M., Dellabona, P., MacDonald, H.R. and Casorati, G. 2003. Human invariant Vα24-JαQ TCR supports the development of CD1d-dependent NK1.1+ and NK1.1- T cells in transgenic mice. Journal of Immunology (2003) 170:2390-2398.
8. Held, W., Coudert, J.D., Zimmer, J. The NK cell receptor repertoire: formation, adaptation and exploitation. Current Opinion in Immunology (2003) 15:233-237.
9. Voyle, R.B., Beermann, F., Lees, R.K., Schümann, J., Zimmer, J., Held, W., MacDonald, H.R. Ligand-dependent inhibition of CD1d-restricted NKT cell development in mice transgenic for the activating receptor Ly49D. Journal of Experimental Medicine (2003) 197:919-925.
10. Smith, J.W., Walker, E.B., Fox, B.A., Haley, D., Wisner, K.P., Doran, T., Fisher, B., Justice, L., Wood, W., Vetto, J., Maecker, H., Dols, A., Meijer, S., Hu, H.-M., Romero, P., Alvord, W.G., Urba, W.J. Adjuvant immunization of HLA-A2-positive melanoma patients with a modified gp100 peptide induces peptide-specific CD8+ T-cell responses. Journal of Clinical Oncology (2003) 21:1562-1573.
11. Capone, M., Lees, R.K., Finke, D., Ernst, B., Van Meerwijk, J.P.M. and MacDonald, H.R. 2003. Selective absence of CD8+ TCRαβ+ intestinal epithelial cells in transgenic mice expressing β2-microglobulin-associated ligands exclusively on thymic cortical epithelium. European Journal of Immunology (2003) 33:1471-1477.
12. Held, W., Clevers, H., Grosschedl, R. Redundant functions of TCF-1 and LEF-1 during T and NK cell development, but unique role of TCF-1 for Ly49 NK cell receptor acquisition. European Journal of Immunology (2003) 33:1393-1398.

13. Dietrich, P.-Y., Le Gal, F.-A., Dutoit, V., Pittet, M.J., Trautman, L., Zippelius, A., Cognet, I., Widmer, V., Walker, P.R., Michielin, O., Guillaume, P., Connerotte, T., Jotereau, F., Coulie, P.G., Romero, P., Cerottini, J.-C., Bonneville, M., Valmori, D. Prevalent role of TCR α-chain in the selection of the preimmune repertoire specific for a human tumor-associated self-antigen. Journal of Immunology (2003) 170:5103-5109.

14. Ramirez, J.C., Finke, D., Esteban, M., Kraehenbuhl, J.P., Acha-Orbea, H. Tissue distribution of the Ankara strain of vaccinia virus (MVA) after mucosal or systemic administration. Archives of Virology (2003) 148:827-839.

15. Waldburger, J.-M., Rossi, S., Hollander, G.A., Rodewald, H.-R., Reith, W., Acha-Orbea, H. Promoter IV of the Class II transactivator gene is essential for positive selection of CD4+ T cells. Blood (2003) 101:3550-3559.

16. Matter, M., Lejeune, F., Antonescu, C., Bischof Delaloye, A., Gugerli, O., Lobrinus, J.A., Cerottini, J.-P., Guggisberg, D., Liénard, D. Revue du traitement chirurgical du mélanome malin. Medicine and Hygiene (2003) 2438:1088-1097.

17. Dutoit, V., Guillaume, P., Ayyoub, M., Hesdorffer, C.S., Luescher, I.F., Valmori, D. Decreased binding of peptides-MHC class I (pMHC) multimeric complexes to CD8 affects their binding avidity for the TCR but does not significantly impact on pMHC/TCR dissociation rate. Journal of Immunology (2003) 170:5103-5109.

18. Esslinger, C., Chapatte, L., Finke, D., Miconnet, I., Guillaume, P., Lévy, F., MacDonald, H.R. In vivo administration of a lentiviral vaccine targets DCs and induces efficient CD8+ T cell responses. Journal of Clinical Investigation (2003) 111:1673-1681.

19. Pear, W. S., Radtke, F. Notch signaling in lymphopoiesis. Seminars in Immunology (2003) 15:69-79.

20. Corcoran, L., Ferrero, I., Vremec, D., Lucas, K., Waithman, J., O'Keeffe, M., Wu, L., Wilson, A., Shortman, K. The lymphoid past of mouse plasmacytoid cells and thymic dendritic cells. Journal of Immunology (2003) 170:4926-4932.

21. Schümann, J., Voyle, R.B., Wei, B.-Y., MacDonald, H.R. Cutting Edge: Influence of the TCR Vβ domain on the avidity of CD1d:α-galactosylceramide binding by invariant Vα14 NKT cells. Journal of Immunology (2003) 170:5815-5819.

22. Doucey, M.-A., Legler, D.F., Faroudi, M., Boucheron, N., Baumgärtner, P., Naeher, D., Cebecauer, M., Hudrisier, D., Rüegg, C., Palmer, E., Valitutti, S., Bron, C., Luescher, I.F. The β1 and β3 integrins promote T cell receptor-mediated cytotoxic T lymphocyte activation. Journal of Biological Chemistry (2003) 278:26983-26991.

23. Ioannidis, V., Kunz, B., Tanamachi, D.M., Scarpellino, L., Held, W. Initiation and limitation of Ly-49A NK cell receptor acquisition by T cell factor-1. Journal of Immunology (2003) 171:769-775.

24. Mpandi, M., Otten, L.A., Lavanchy, C., Acha-Orbea, H., Finke, D. Passive immunization with neutraliz ing antibodies interrupts the mouse mammary tumor virus life circle. Journal of Virology (2003) 77: 9369-9377.

25. Rufer, N., Zippelius, A., Batard, P., Pittet, M.J., Kurth, I., Corthesy, P., Cerottini, J.-C., Leyvraz, S., Roosnek, E., Nabholz, M., Romero, P. Ex vivo characterization of human CD8+ T subsets with distinct replicative history and partial effector functions. Blood (2003) 102:1779-1787.

26. Pittet, M.J., Rubio-Godoy, V., Bioley, G., Guillaume, P., Batard, P., Speiser, D., Luescher, I., Cerottini, J.-C., Romero, P., Zippelius, A. α3 domain mutants of peptide/MHC class I multimers allow the selective isolation of high avidity tumor-reactive CD8 T cells. Journal of Immunology (2003)

171:1844-1849.

27. Speiser, D.E., Rimoldi, D., Batard, P., Liénard, D., Lejeune, F., Cerottini, J.-C. and Romero, P. Disease-driven T cell activation predicts immune responses to vaccination against melanoma. Cancer Immunity (2003) 3:12.
28. Ayyoub, M., Rimoldi, D., Guillaume, P., Romero, P., Cerottini, J.-C., Valmori, D., Speiser, D. Tumor-reactive, SSX-2-specific CD8+ T cells are selectively expanded during immune responses to antigen-expressing tumors in melanoma patients. Cancer Research (2003) 63:5601-5606.
29. Rimoldi, D., Lemoine, R., Kurt, A.-M., Salvi, S., Berset, M., Matter, M., Roche, B., Cerottini, J.-P., Guggisberg, D., Krischer, J., Braun, R., Willi, J.-P., Antonescu, C., Slosman, D., Lejeune, F.J., and Liénard, D. Detection of micrometastases in sentinel lymph nodes from melanoma patients: direct comparison of multimarker molecular and immunopathological methods. Melanoma Research (2003) 13:511-520.
30. Rimoldi, D., Salvi, S., Liénard, D., Lejeune, F.J., Speiser, D., Zografos, L. and Cerottini, J.-C. Lack of BRAF mutations in uveal melanoma. Cancer Research (2003) 63:5712-5715.
31. Valmori, D., Dutoit, V., Ayyoub, M., Rimoldi, D., Guillaume, P., Liénard, D., Lejeune, F., Cerottini, J.-C., Romero, P., Speiser, D.E. Simultaneous CD8+ T cell responses to multiple tumor antigen epitopes in a multipeptide melanoma vaccine. Cancer Immunity (2003) 3:15.
32. Saurer, L., Seibold, I., Vallan, C., Held, W., Mueller, C. Cutting Edge: Stimulation with the cognate self-antigen induces expression of the Ly49A receptor on self-reactive T cells which modulates their responsiveness. Journal of Immunology (2003) 171: 6334-6338.
33. Speiser, D.E., Pittet, M.J., Rimoldi, D., Guillaume, P., Luescher, I.F., Liénard, D., Lejeune, F., Cerottini, J.-C., Romero, P. Evaluation of melanoma vaccines with molecularly defined antigens by ex vivo monitoring of tumor-specific T cells. Seminars in Cancer Biology (2003) 13:461-472.
34. Krug, A., Rothenfusser, S., Selinger, S., Bock, C., Kerkmann, M., Battiany, J., Sarris, A., Giese, T., Speiser, D., Endres, S. and Hartmann, G. CpG-A oligonucleotides induce a monocyte-derived dendritic cell-like phenothype that preferentially activates CD8 T cells. Journal of Immunology (2003) 170:3468-3477.
35. Karanikas, V., Lurquin, C., Colau, D., van Baren, N., De Smet, C., Lethé, B., Connerotte, T., Corbière, V., Demoitié, M.-A., Liénard, D., Dréno, B., Velu, T., Boon, T., Coulie, P.G. Monoclonal anti-MAGE-3 CTL responses in melanoma patients displaying tumor regression after vaccination with a recombinant canarypox virus. Journal of Immunology (2003) 171:4898-4904.

Reviews / Book Chapters / Commentaries

1. Radtke, F., Raj, K. The role of Notch in tumorigenesis: Oncogene or tumour suppressor? Nature Reviews Cancer (2003) 3:756-767.
2. MacDonald, H.R. Introduction: notch and lymphopoiesis. Seminars in Immunology (2003) 15:67-68.
3. Rimoldi, D., Cerottini, J.-C. and Romero, P. 2003. Fas/Fas ligand and tumor immunity. In: Mechanisms of tumor escape from the immune response. A.C. Ochoa, ed. Taylor & Francis, London and New York. pp. 213-235.

LUDWIG INSTITUTE FOR CANCER RESEARCH

London St. Mary's Branch of Molecular Virology

London, England

Staff List

Farrell, Paul J *Director, Member*

Epstein-Barr Virus and Cell Growth Control Group

Farrell, Paul J *Director, Member*
Crook, Timothy *Associate Clinical Investigator*
Spender, Lindsay *Assistant Investigator*
Bryant, Helen *Postdoctoral Fellow (until February)*
White, Robert *Postdoctoral Fellow (from September)*
Sullivan, Alex *Assistant Laboratory Manager/Research Assistant*
Attard, Marlene *Technician*
Elgueta, Claudio *Technician*
Syed, Nelofer *Technician*
Al-Mozaini, Maha *Student*
Amon, Wolfgang *Student*
Pattle, Samuel *Student*
Whiteman, Hannah *Student*

Tumour Suppressor Gene Group

Lu, Xin *Member*
Bergamaschi, Daniele *Postdoctoral Fellow*
Llanos, Susannah *Postdoctoral Fellow*
Samuels-Lev, Yardena *Postdoctoral Fellow (until May)*
Vives, Virginie *Postdoctoral Fellow*
Zhong, Shan *Senior Technician*
Ali, Sofia *Technician (until August)*
Ratnayaka, Indrikah *Technician*
Slee, Elizabeth *Technician*
Fogal, Valentina *Student*
Gillotin, Sebastien *Student*
Godin, Nadia *Student*
Su, Jian *Student*

Oncogene Group

Watson, Roger *Reader, Imperial College (BBSRC)*
Tavner, Fiona *Postdoctoral Fellow*
Catchpole, Steven *Technician*

B Lymphocyte Biology and Epstein-Barr Virus Group

Allday, Martin *Professor, Imperial College, (Wellcome Trust, MRC)*
Heaney, Jude *Postdoctoral Fellow (until May)*
O'Nions, Jenny *Postdoctoral Fellow (from July)*
Sourisseau, Tony *Postdoctoral Fellow*
Spyer, Moira *Postdoctoral Fellow*
Gillian Parker *Research Assistant*
Emma Anderton *Student (from October)*
Mark Hickabottom *Student (until December)*
Maria Leao *Student*
Paul Young *Student (from October)*

Technical Support

Coulson, Lee *Laboratory Aide*
Zlobecki, Martin *Laboratory Aide*

Administration and Secretariat

Curtis, Julie *Branch Administrator*
De Felice, John *Laboratory Manager*
Sabina Jankowska *Secretary*

Branch Director's Report

Specific treatments for cancer will depend on exploiting differences between cancer cells and normal cells or on reversing some of the molecular changes that have caused the cells to form the cancer. Most of our scientific research program is directed towards these objectives. For example, loss of p53 tumour suppressor gene function is very frequent in cancers but the p53 protein often continues to be expressed. The p53 activity is sometimes lost because of mutation of the p53 and sometimes because of changes in its regulation. We are therefore investigating the detailed mechanisms by which the activity of p53 and the related p63 and p73 proteins are altered in cancers with the hope that the function of this pathway might be restored. There is a complex network of regulatory interactions between these proteins and the ASPP proteins (discovered last year in our lab) as well as E2F1 and some other cell cycle regulatory proteins. E2F activity links these functions with cell proliferation through genes such as the Rb family of proteins and B-Myb, which we are also studying.

Usually the distinction between normal cells and cancer cells is very subtle and this is why it is difficult to make cancer treatments very specific. Some virus associated cancers offer an attractive possibility of designing specificity around viral functions. In the cancers associated with Epstein-Barr virus, every tumour cell carries the virus but very few normal cells in the patient are infected. We are therefore trying to understand the mechanisms by which EBV alters cell growth control, causing cell proliferation, and also hoping to devise ways in which the presence of the virus in cancer cells could make them sensitive to novel therapies. New insights into the mechanisms by which EBV causes cells to overcome normal cell cycle checkpoints also suggest a possible mechanism by which the virus may contribute to cancer.

It is clear that cancer arises because of accumulated alterations in controls that normally operate in cells on proliferation and programmed cell death. Much of the circuitry involved has now been identified in model systems but the physiological significance of various aspects of this in human cancers can only be deduced by studying genetic and epigenetic changes in real cancer samples and then working out which of the many pathways were the important ones. This is one of the purposes of our work on molecular phenotyping human cancers. The other exciting aspect of this work is the identification of molecular markers that can predict the outcome of current therapy regimes. If subgroups of patients who will not respond can be identified in advance, they could be spared inappropriate treatment and be offered other treatments, treatments that were perhaps not the best for all patients but might be superior for that subgroup.

This year has seen further progress in our close association with Imperial College Faculty of Medicine. The refurbishment of the building is now progressing well and some of us have moved into additional new labs, creating improved conditions for all. Further new facilities will come on stream soon and we look forward to the completion of most of the building work in 2004. We value our close association with the Imperial College Department of Virology and have now also formed a Section of Cancer Biology. This will help our interactions with other cancer researchers within the College and take advantage of the expansion of Oncology at St Mary's Hospital.

Paul J. Farrell

Research Report

Epstein-Barr Virus and Cell Growth Control Group

The studies of EBV biology by Dr Paul Farrell's group are designed to identify the basis for novel approaches to eliminate cells infected by EBV, particularly the tumour cells of EBV associated cancers. EBV infection of human B lymphocytes causes cell proliferation and the cell lines that can easily be grown out are very similar cells to those present in EBV driven immunoblastic lymphomas. In these cells the viral EBNA2 transcription factor activates cell gene expression and this is essential for continued cell proliferation. Some important target cell genes for EBNA2, such as c-myc, Runx3 and several others have been identified. The group focussed on Runx3 and identified a new mechanism of regulatory interaction of Runx3 on Runx1. To test the role of these EBNA2 target genes in cell proliferation, conditional siRNA systems have been created in which the individual gene expression can be knocked down and the consequences for the cell determined.

The fact that most of the EBV genes that cause proliferation of infected B lymphocytes are not expressed in EBV associated human cancers suggests that the virus has a different role in the cancer cells. The group is studying the viral genes that are expressed in cancers such as the EBER RNAs and BART genes, trying to determine their function. The latent viral infection present in the lymphocytes and EBV associated cancers can reactivate to the productive virus cycle in which infectious virus particles are secreted. The mechanism by which reactivation occurs is a major topic in the herpesvirus family (of which EBV is a member) because several human diseases are caused by such reactivation. The group has analysed the molecular mechanism by which induction of the Zp promoter is caused by signal transduction activated by cross linking the BCR so as to mimic the binding of antigen, thought to be a physiological route for EBV reactivation. The regulation of early and late genes and their relative timing of expression have been quantitatively reconstituted on small plasmids to allow the study of these processes.

Dr Tim Crook has led the studies on linking molecular changes detectable in human cancer samples to clinical outcome. Many changes can be detected, sometimes mutations but more frequently changes in gene expression, for example loss of tumour suppressor gene expression. This can be measured as less mRNA for the tumour suppressor gene in well preserved tumour biopsy samples but can also often be inferred from methylation of CpG sequences in the promoter of the gene, a process involved in permanent inactivation of genes. Molecular genetic markers that would predict the outcome of cancer therapy would be very valuable in targeting treatment to those patients who will respond. They might also suggest trials of treatments that would be better for subgroups of patients but presently cannot be used because they are not the best for all patients. A relationship between polymorphism at codon 72 of the p53 tumour suppressor gene and outcome of chemotherapy in head and neck cancer has been found. If there is a p53 mutation in an allele with Arg at codon 72, the patient has a much lower chance of responding to standard therapy than if the same mutation is in p53 with Pro at codon 72. This effect occurs partly through modulation of p73 dependent apoptosis by the p53, which is affected by the codon 72 status.

B lymphocyte Biology and Epstein-Barr Virus Group

Dr Martin Allday's group continued to investigate the deregulation of cell cycle checkpoints by latent EBV infection. A comparison of EBV-infected primary B cells with an isogenic population induced to proliferate by CD40-ligand (CD40L) and IL4 has revealed that EBV can override the p53/pRb-mediated G1 checkpoint activated in normal B cells by genotoxic stress. In cells responding to cisplatin, although p53 is stabilized and activated, EBV latent gene expression inhibits the accumulation of newly synthesised cdki p21WAF1/CIP1 and the down-regulation of cyclin D2 that occur in the normal cells. Consequently in EBV-infected cells, CDK2 remains active, hyperphosphorylation of pRb is maintained and replication of damaged DNA can occur. EBV acts downstream of p53 and appears to prevent the inactivation of CDK2

by p21WAF1/CIP1, perhaps by targeting the latter for degradation by the proteasome system or modifying CDK2. Under conditions of severe genomic stress, this absence of p21WAF1/CIP1 function can result in apoptosis, however, when damage is less sustained, genomic instability may arise and this in turn could contribute to the development of EBV-associated B cell malignancies. A surprising discovery was that suppression of this checkpoint by EBV is dependent on the type of DNA damage sustained by the B cell. The group is currently exploring the nature of this discrimination and molecular basis of the checkpoint suppression. This study using explanted normal B cells also revealed that in vitro the response of human B cells to chronic stimulation by T cell-derived mitogens is plasmacytoid differentiation associated with an increase in the levels of cdkis p18 and p21WAF1/CIP1. The fact that EBV prevents this suggests some latent EBV protein(s) might interfere with B cell differentiation. In addition to targeting a G1 checkpoint by acting downstream of p53 but upstream of pRb, recent preliminary data suggests that EBV can also disrupt a mitotic checkpoint – the molecular basis of this is currently being determined.

Tumour Suppressor Gene Group

The objective of this research led by Dr. Xin Lu is to develop new strategies to reactivate wild type p53 functions in the 50% of human tumours where p53 is still present but its tumour suppression function is lost. In the past year, studies on the regulation of p53 function by cellular proteins including E2F1 and the ASPP family of proteins have continued. To reactivate the tumour suppression function of p53 in cancer cells, an understanding of how p53 is regulated in normal cells is required. The group showed that the cell cycle dependent DNA binding and transcriptional activity of p53 correlates with E2F expression in human primary fibroblasts. E2F-1 binds and stimulates DNA binding, transactivation and apoptotic functions of p53 but not p63 and p73. E2F-1 binds residues 347-370 of p53 and enhances nuclear retention of Ser315 phosphorylated p53. This regulation of p53 by E2F-1 is cell cycle dependent as the cellular distribution of Ser315 phosphorylated p53 is associated with the periodic expression of E2F and cyclin A throughout the cell cycle. This is the first demonstration that the activities of p53 are regulated during the cell cycle by E2F/p53 interactions and that phosphorylation of p53 at Ser315 is required for this regulation.

In addition to functioning via p53, the group has shown that ASPP1 and ASPP2 also induce apoptosis independent of p53. By binding to p63 and p73 in vitro and in vivo, ASPP1 and ASPP2 stimulate the transactivation function of p63 and p73 on promoters of Bax, PIG3, PUMA but not mdm2 or p21WAF-1/CIP1. The expression of ASPP1 and ASPP2 also enhances the apoptotic function of p63 and p73 by selectively inducing the expression of endogenous p53 target genes such as PIG3 and PUMA but not mdm2 or p21WAF-1/CIP1. Removal of endogenous p63 or p73 with RNAi demonstrated that the p53 independent apoptotic function of ASPP1 and ASPP2 is mediated mainly by p63 and p73. Hence ASPP1 and ASPP2 are the first two identified common activators of all p53 family members. All these results suggest that ASPP1 and ASPP2 could suppress tumour growth even in tumours expressing mutant p53.

Oncogene Group

Dr. Roger Watson's group continued their study of cell cycle transcriptional regulation. One focus of this work is the B-Myb transcription factor, which is required for early embryonic development and is implicated in regulating cell proliferation and differentiation. Previously the group showed that B-Myb interacted with the cyclin-binding domain of the p107 retinoblastoma (Rb)-related protein, and it was investigated whether B-Myb could also interact with similar cyclin/cdk-binding motifs in related cyclin-dependent kinase inhibitors. No in vivo interaction was found with either p21Waf1/Cip1 or p27Kip1, however, binding was readily detectable to the cyclin-binding domain of p57Kip2. B-Myb was found to compete with cyclin A2 for binding to p57Kip2 and thereby release active cyclin/Cdk2 kinase, moreover, B-Myb overcame G1 arrest in Saos-2 cells induced by p57Kip2. B-Myb may therefore promote cell proliferation by a non-transcriptional mechanism involving release of active cyclin/Cdk2 from p57Kip2 as well as p107. In common with many cell cycle-regulatory genes, B-myb transcription is temporally controlled by E2F transcription factors acting in concert with members of the Rb family. Using in vivo

promoter assays coupled with chromatin immunoprecipitation (ChIP), the group has now shown that transcription of survivin, which is typically deregulated in cancer cells, involves E2F repression through atypical E2F-binding sites. These studies are aimed at extending the knowledge of defects in cell cycle gene expression present in cancer cells.

Publications

Primary Research Articles

1. Bergamaschi D., Gasco M., Hiller L., Sullivan A., Syed N., Trigiante G., Yulug I., Merlano M., Numico G., Comino A., Attard M., Gusterson B., Bell A.K., Heath V., Tavassoli M., Farrell P.J., Smith P., Lu X., Crook T. p53 polymorphism influences response in cancer chemotherapy via modulation of p73-dependent apoptosis. Cancer Cell (2003) 3:387-402.
2. Bergamaschi D., Samuels Y., O'Neil N.J., Trigiante G., Crook T., Hsieh J.K., O'Connor D.J., Zhong S., Campargue I., Tomlinson M.L., Kuwabara P.E., Lu X. iASPP oncoprotein is a key inhibitor of p53 conserved from worm to human. Nature Genetics (2003) 33:162-167.
3. de Jesus O., Smith P.R., Spender L., Elgueta Karstegl C., Niller H.H., Huang D., Farrell PJ. Updated Epstein-Barr virus DNA sequence and analysis of a promoter for the BART (CST, BARF0) RNAs of Epstein-Barr virus. Journal of General Virology (2003) 84:1443-1450.
4. Joaquin M., Watson R.J. The cell cycle-regulated B-Myb transcription factor overcomes cyclin-dependent kinase inhibitory activity of p57Kip2 by interacting with its cyclin-binding domain. Journal of Biological Chemistry (2003) 278:44255-44264.
5. O'Nions J., Allday M.J. Epstein-Barr virus can inhibit genotoxin-induced G1 arrest downstream of p53 by preventing the inactivation of CDK2. Oncogene (2003) 22: 7181-7191.
6. Osin P., Lu Y-J., Stone J., Crook T., Houlston R.S., Gasco M., Gusterson B.A., Shipley J. Distinct genetic and epigenetic changes in medullary breast cancer. International Journal of Surgical Pathology (2003) 11: 153-158.
7. Renton A., Llanos S., Lu X. Hypoxia induces p53 through a pathway distinct from most DNA-damaging and stress-inducing agents. Carcinogenesis (2003) 24:1177-1182.
8. Slee E.A., Lu X. The ASPP family: deciding between life and death after DNA damage. Toxicology Letters (2003) 139:81-87.

Reviews / Book Chapters / Commentaries

1. Farrell P.J. Mechanisms of viral carcinogenesis. In: Introduction to the Cellular and Molecular Biology of Cancer, 4th Edition. Eds M. Knowles and P. Selby, Oxford University Press, 2003.
2. Gasco M., Crook T. The p53 network in head and neck cancer. Oral Oncology (2003) 39: 222-231.
3. Gasco M., Crook T. p53 family members and chemoresistance in cancer: what we know and what we need to know. Drug Resistance Updates (2003) 6:323-328, 2003
4. Gasco M., Yulug I.G., Crook T. TP53 mutations in familial breast cancer: functional aspects. Human Mutation (2003) 21:301-306.
5. Joaquin M., Watson R.J. Cell cycle regulation by the B-Myb transcription factor. Cell and Molecular Life Sciences (2003) 60:2389-2401.
6. Melino G., Lu X., Gasco M., Crook T., Knight R.A. Complexities in the functional regulation of p63 and p73: from development to cancer? Trends in Biological Sciences (2003) 28: 663-670.

London University College Branch
of Cell and Molecular Biology

London, England

Staff List

Waterfield, Michael *Director, Member*

Proteomics Section

Waterfield, Michael *Head of Proteomics Section*
Akunna Akpan *Laboratory Manager*
Barnouin, Karin *Postdoctoral Fellow*
Gharbi, Severine *Postdoctoral Fellow (from May)*

Proteomics Section - Bioinformatics

Zvelebil, Marketa *Senior Investigator*
Lykostratis, Kostas *Graduate Student*
Hurwitz, Naama *Higher Scientific Officer (until March)*
Qiong, Alice *GAO, Scientific Officer (from April)*
Yang, Alice *Scientific Officer*

Proteomics Section - Bioanalytical Chemistry

Cramer, Rainer *Assistant Member*
Thompson, Andrew *Postdoctoral Fellow*
Gerrits, Albert *Graduate Student*
Hart, Sarah *Graduate Student (until April)*
Jacob, Richard *Graduate Student*
Rodriguez-Cutillas, Pedro *Graduate Student (until October)*
Rodriguez-Cutillas, Pedro *Postdoctoral Fellow (from October)*
Vilasi, Annalisa *Graduate Student (from October)*
Calnan, Denis *Higher Scientific Officer*
Corless, Steve *Higher Scientific Officer*
Saxton, Malcolm *Scientific Officer*

Proteomics Section - Cancer Biochemistry Group

Timms, John *Assistant Member*
Weeks, Mark *Postdoctoral Fellow*
Bertani, Mariana *Graduate Student*
Chan, Hong-Lin *Graduate Student*
Gharbi, Severine *Graduate Student (until May)*
Jovceva, Eleonora *Graduate Student (from July)*
Sinclair, John *Scientific Officer (from March)*

Breast Cancer Laboratory

O'Hare, Mike *Group Leader*
Fulford, Laura *Clinical Fellow (from August)*
Kim, Jong-Bin *Graduate Student*
Burt, Avril *Tissue Collector*
Mackay, Alan *Higher Scientific Officer (until April)*
Grigoriadis, Anita *Scientific Officer*

Cell Regulation Laboratory

Gout, Ivan *Assistant Member*
Zhyvoloup, Alexander *Postdoctoral Fellow (until September)*
Fenton, Timothy *Graduate Student*
Rebholz, Heike *Graduate Student*
Valovka, Taras *Graduate Student (until February)*
Wang, Mong-Lien *Graduate Student*
Nemezanyi, Ivan *Visiting Student (until November)*
Panasyuk, Ganna *Visiting Student (until November)*
Foxon, Richard *Higher Scientific Officer*

Cell Shape and Polarity Laboratory

Baum, Buzz *Assistant Member*
Craig, Gavin *Postdoctoral Fellow*
Duchek, Peter *Postdoctoral Fellow*
Kunda, Patricia *Postdoctoral Fellow*
Sims, David *Graduate Student*
Dominguez, Veronica *Research Assistant*

Cell Signaling Laboratory

Vanhaesebroeck, Bart *Assistant Member*
Bilancio, Antonio *Postdoctoral Fellow*
Box (Sawyer), Carol *Postdoctoral Fellow (until May)*
Foukas, Lazaros *Postdoctoral Fellow*
Graupera Garcia-Mila, Maria *Postdoctoral Fellow (from October)*
Salpekar, Ashreena *Postdoctoral Fellow*
Ali, Khaled *Graduate Student*
Geering, Barbara *Graduate Student*
Woods, Lizzie *Graduate Student*
Pearce, Wayne *Scientific Officer*
Peskett, Emma *Scientific Officer*

Cellular and Molecular Biology Laboratory

Ridley, Anne *Member*
Datta, Arpita *Research Associate (until October)*
Entwistle, Alan *Research Associate*
Cernuda Morollan, Eva *Postdoctoral Fellow (from October)*
Cory, Giles *Postdoctoral Fellow (until June)*
De Villalonga Smith, Priam *Postdoctoral Fellow*
Ivetic, Aleksandar *Postdoctoral Fellow*
Millan Martinez, Jaime *Postdoctoral Fellow*
Riento, Kirsi *Postdoctoral Fellow*
Wells, Claire *Postdoctoral Fellow (until June)*
Wojciak-Stothard, Beata *Postdoctoral Fellow (until April)*
Bhavsar, Parag *Graduate Student (from October)*
Christoloulou, Maria *Graduate Student (from October)*
Franz, Clemens *Graduate Student (until June)*
McKenzie, Jenny *Graduate Student*
Smith, Steven *Graduate Student*

Wheeler, Ann *Graduate Student*
Bigarella Carolina, *Visiting Student (August to December)*
Garg, Ritu *Higher Scientific Officer*

Clinical Studies Laboratory

Stein, Robert *Clinical Fellow*

Drosophila Morphogenesis Laboratory

Barrett, Katherine *Assistant Member*
Loh, Samantha Hui Yong *Postdoctoral Fellow*
Nikolaidou, Kelly [Kyriaki] *Postdoctoral Fellow*
Escott, Gareth *Graduate Student*
Hiley, Charlotte *Graduate Student*
Kitsou, Efstathia *Graduate Student*
Batchelor, Emily *Scientific Officer*

Proteomics Laboratory

Naaby-Hansen, Soren *Assistant Member*
Warnasuriya, Gayathri *Postdoctoral Fellow*
Hastie, Claire *Graduate Student*
Wood, Chris *Assistant Scientific Officer (until September)*

Structural Biology Laboratory

Driscoll, Paul *Postdoctoral Fellow*
Vines, David *Postdoctoral Fellow*
Sankar, Andrew *Higher Scientific Officer*

Transformation Studies Laboratory

Jat, Parmjit *Associate Member (until September)*
Hardy, Kristine *Postdoctoral Fellow*
Relph (Boast), Kate *Postdoctoral Fellow*
Tarunina, Marina *Research Associate*
Martin, Rowena *Graduate Student*
Mansfield, Louise *Graduate Student*
Arora, Parineeta *Scientific Officer*
Nikitopoulou, Athena *Scientific Officer*

General Support Services

Scrace, Geoff *Research Associate*
Mistry, Kay *Laboratory Manager*
Williams, Simon *Assistant Scientific Officer*
Garady, Adam *Security*
McCreadie, James *Security*

Administration & Secretariat

Anning, Richard *Administrator*
Emakpose, Loretta *Administrative Assistant (from March)*
Targett, Rebecca *Administrative Assistant (until March)*
Ward, Alex *Administrative Assistant*
Stone, Deborah *Director's Assistant*
Ahmadi, Khatereh *Scientific Administrator (until July)*
Barnsley, Sarah *Scientific Administrator (from November)*

Branch Director's Report

It is now almost a decade since the UCL Branch groups of Drs. Mike Waterfield and Peter Parker (now at Cancer Research UK, CRUK) purified, cloned, expressed and characterized a family of phosphatidylinositol 3-kinase enzymes (PI3Ks), which control the production of lipid second messengers that are triggered by growth factor signaling. From the work of many laboratories, including our own, it has become clear that persistent activation of PI3Ks contributes to the loss of control over cellular processes that accompany cancer. In fact the subversion of the PI3K enzymes may occur in more than half of all human cancers.

Given that one of the major objectives of the LICR is to translate its research studies into patient benefit, it became clear some years ago that we should initiate a drug program targeted at making inhibitors of the PI3Ks. This program became a unique tripartite effort with CR UK through collaborations with Drs. Peter Parker and Paul Workman (Institute of Cancer Research, London). Through a long collaboration with industry, the tripartite group was able to generate potential lead compounds that targeted the PI3Ks. During 2003, the biotechnology company Piramed was created, which will enable the next stages of the industrial scale work of drug structure refinement to be carried out to deliver candidates for Phase I clinical trials. The creation of Piramed was a remarkable achievement given the difficulties in the biotechnology industry at the time, and it is to the credit of the LICR Office of Intellectual Property and scientists that this was a unique accomplishment in the sector in the fall of 2003.

Dr. Bart Vanhaesebroeck's work on PI3K animal models continues to be critical in defining the role of the different PI3Ks in cancer and other diseases. In addition, Dr. Anne Ridley's work on understanding cell migration pathways has shown that PI3Ks impact on some of these pathways. Dr. Ivan Gout is deciphering more about downstream targets in the PI3K pathway, and has identified excellent markers for the clinical monitoring of new signal transduction inhibitors. It is clear that drugs with unique PI3K specificity may offer special strategies for cancer and inflammation treatment – a remarkable, growing treasure of potential diagnostics and treatments that could come from our PI3K research. Linked to these programs are the basic discovery studies of motility mechanisms that Dr. Buzz Baum is undertaking using cutting edge RNAi screening technique in Drosophila. The studies of Dr. Parmjit Jat confirm the importance of understanding the life span of normal cells; the mortalization and immortalization processes appear to be interlocked in controlling the fate of cancer cells. The proteomics team, Drs. Rainer Cramer, John Timms, Marketa Zvelebil and Mike Waterfield, has continued building its unique panel, of skills based on Mass Spectrometry and 2D-DIGE, a combination that has made possible quantitative expression analysis and protein discovery in cancer, which will be the central theme of future research.

Michael D. Waterfield

Research Report

Proteomics Section - Bioinformatics

The Bioinformatics Group, led by Dr Marketa Zvelebil, has been working closely with the Proteomics laboratories to generate integrated software (LIMS) that can be used to gather and interpret protein expression data from two dimensional- (2D)-gels, and correlate these with patient and cell data. This type of informatics analysis is being extended to include other data such as RNAi, gene expression and mathematical model descriptors. The group has moved into new areas of research by collaborating with the groups of Drs. Anne Ridley and Buzz Baum to launch programs that model complex signal transduction pathways. A very detailed and accurate model of the Rac/Rho pathway has been constructed and mathematical simulations are being generated. Meanwhile, the essential modelling of protein structures in the PI3K field is being continued through exploitation of the group's experience in computational approaches that decipher the basis of interactions between ligands and proteins.

Proteomics Section - Bioanalytical Chemistry

The strength of the research activity in the Bioanalytical Chemistry group, led by Dr Rainer Cramer, which uses mass spectrometry (MS) technology has been further complemented by several new programs focused on sample preparation and data analysis. Novel chemical derivatization methods have been designed and are currently under development for the characterization of whole and sub-proteomes at the attomole level. These methods are specifically designed for large-scale high-throughput analyses. Affinity purification methods for the analysis of protein phosphorylation have also been further developed. Some of the emerging ideas in this field are being pursued through industrial collaborations. In addition, instrumentation and methods have been created that enable automated sample processing for almost every aspect of the processing of samples from cell lysates to MS analysis. The informatics analyses of MS data using steps such as data handling, processing, and mining to allow routine operation is still a field that has not been adequately addressed in the community. Thus, the group has continued to work with established, and new, commercial and academic partners on software solutions that will help us to deal with the future flux of proteomic data from projects investigating whole proteomes. From this work (see also Bioinformatics above), the informatics tools that will allow integration of all hardware components (robotics, mass spectrometers, etc.) are being developed, and a full working LIMS system will be tested in the next few months.

The continuation of our collaborative work has led to numerous new research projects. For example, together with Professor Ian Jacobs, we are exploiting the MRC bank of 200,000 human blood serum samples from postmenopausal women for the development of clinical screening tools and biomarker discovery for ovarian and other cancers.

Cancer Proteomics Group

Research within the Cancer Proteomics Group, jointly led by Drs. John Timms and Mike Waterfield, has continued to focus on elucidating mechanisms of cancer cell signalling using state-of-the-art proteomic approaches. The group mostly uses fluorescence 2D-difference gel electrophoresis (2D-DIGE) for sensitive and accurate quantification of differential protein expression between biological samples. This has been integrated with a global expression analysis approach utilizing high-throughput MS (with Dr. Rainer Cramer) to identify target proteins. This approach is currently being applied to a variety of cancer research projects.

On-going research is aimed at identifying downstream targets/markers of the oncogenic receptor tyrosine kinase ErbB-2 and the growth factor ligands that activate the receptor. This work includes a parallel differential mRNA expression analysis, and has allowed comparison of gene and protein

expression, thus revealing the modes of regulation and mechanisms by which ErbB-2 overexpression causes transformation. Selected targets are being further characterised as cancer markers using conventional biochemical and cell biological methods. Proteomic work is also focused on deciphering the cellular responses to stress in model systems, including luminal epithelial cells and the yeast *Saccharomyces pombe*, identification of disease markers of essential thrombocythaemia, and the development of novel cysteine-labelling 2D-DIGE reagents as functional proteomic probes. The group is also beginning to combine proteomic and RNAi technologies to assess the effects of knocking down the expression of specific cancer-associated genes. These studies in Drosophila (with the group of Dr. Baum) are aimed at elucidating the signalling networks and cellular mechanisms involved in the determining cell shape, adhesion and motility.

Cell Regulation Laboratory

The focus of research in the Cell Regulation Laboratory, led by Dr. Ivan Gout, has been to elucidate molecular mechanisms regulating cell growth via PI3-K and mTor signalling pathways. Ribosomal S6 kinase (S6K) is a key player in mediating cellular responses to various extracellular stimuli, including growth factors, hormones and nutrients. The activity of S6K is tightly regulated by multiple phosphorylation events. Numerous studies have placed the tumour suppressor proteins, PTEN and TSC1/2 (tuberous sclerosis complex 1/2) as an antagonist of S6K activation in normal and tumour cells, indicating the importance of S6K in malignant transformation and human cancer. Moreover, an indirect inhibitor of S6K, rapamycin and its derivatives, are currently in clinical trials as anti-cancer drugs. Therefore, an overwhelming amount of evidence supports S6K as an attractive target for the development of novel anti-cancer drugs.

The group has made the progress in the following areas of S6K signalling: a) the first demonstration of a direct association between S6K and receptor and non-receptor tyrosine kinases. S6K was found to be tyrosine phosphorylated, and the site of phosphorylation was identified by mass spectroscopy. The importance of this modification is currently under investigation; b) identification of a novel substrate for S6K. The group discovered that MDM2 is physically associated with S6K in vitro and in vivo, leading to the phosphorylation of MDM2 at serine 166 (S166). Phosphorylation of MDM2 at S166 closely correlates with the activation pattern of S6K, but not PKB/Akt, when cells are treated with various mitogenic stimuli. Moreover, S166 is phosphorylated in response to nutritional signals such as amino acid stimulation, which activates S6K, but not the PKB/Akt pathway. Consistent with these data, phosphorylation of MDM2 at S166 is highly sensitive to rapamycin and could be rescued by overexpression of rapamycin-resistant form of S6K. These results also provide an insight into how rapamycin may block G1/S transition of the cell cycle; and c) establishing a functional link between mTor/S6K signalling pathways and energy metabolism. The group found that S6K interacts specifically with CoA synthase, which mediates the final steps in CoA biosynthesis. These results indicate that S6K may regulate cell growth and size by augmenting ribosomal biogenesis, protein synthesis and energy metabolism.

Cell Shape And Polarity Laboratory

The Cell Shape and Polarity Laboratory, led by Dr. Buzz Baum, has been pioneering the use of RNAi in *Drosophila* cell culture as a functional genomic tool with which to identify the genes regulating animal cell morphogenesis. Research is currently focused on the roles of Cdc42, Rac, SCAR, Abi and c-Abl in the formation of actin-based protrusions. To identify new players in this pathway, the group recently undertook a genome-wide RNAi screen for genes controlling the formation of lamellipodia in *Drosophila* cells. The novel cytoskeletal regulators identified will now be functionally analysed in cell culture and in the fly. To complement the genetic and cell biological analysis, the group is also working with Dr. Timms to generate a fingerprint of the cytoskeletal proteome in dsRNA treated cells. The large phenotypic, microarray and proteomic data sets generated will be cross-referenced in a database built in collaboration with Dr. Zvelebil. Once the database is in place, the group will work with Drs. Ridley and Zvelebil to

generate pathway diagrams that summarize, as simply as possible, the network of actin cytoskeleton control. These models will then be used to stimulate the development and testing of new hypotheses. As cytoskeletal regulators are highly conserved through evolution, it is hoped that this research in *Drosophila* will throw light on the control of cell morphogenesis in humans and its deregulation in cancer.

Cell Signalling Laboratory

The aim of the Cell Signalling Laboratory, led by Dr. Bart Vanhaesebroeck, this year has been to uncover the function of the individual mammalian PI3K isoforms in the organism, with a focus on the processes involved in cancer. Mammals have multiple PI3K isoforms, the roles of which are largely unknown, that control numerous cellular activities such as proliferation, apoptosis and differentiation, and that play critical roles in inflammation, cancer and diabetes. The group has previously documented that PI3K isoforms have non-redundant roles in cell lines.

Work in the group combines cell biological and biochemical approaches with studies at the organism level, using the mouse as a model system. PI3K isoforms are inactivated in a constitutive or conditional manner by the introduction of point mutations into the mouse genome. This strategy more faithfully mimics the action of small molecule chemical inhibitors than classical gene knockout or transgenic approaches, and provides a strong translational aspect to our research. The group has validated this strategy in a pilot project on the p110δ isoform of PI3K, which has established p110δ as a prime target for therapeutic intervention in inflammation. Gene targeting of the other PI3K isoforms is now well under way, and other important physiological roles of the p110α isoform have been discovered, that have potential implications for cancer. Several mouse models of cancer for testing the PI3K gene-targeted mice have been established. This work will have significant repercussions in understanding basic mechanisms of cell signalling but will also provide a conceptual framework for the development and use of isoform-specific PI3K inhibitors as drugs for cancer treatment.

Cellular And Molecular Biology Laboratory

The Cellular and Molecular Biology Laboratory, headed by Dr. Anne Ridley, has been continuing its work on cell migration, which underlies the invasion and metastasis process of tumour cells, and is also vital for angiogenesis, and leukocyte recruitment to tumours.

Angiogenesis involves both endothelial proliferation and migration, and thus the group has been studying the Rho GTPase family proteins, which are known to coordinate morphological changes during cell migration. Endothelial cells are constantly exposed to shear stress, and this is likely to modulate their responses to angiogenic stimuli. Shear stress induces endothelial polarization in the direction of flow, and the group's recent results indicate that Rho and the Rho GTPase Rac act sequentially to induce this response. Current studies are aimed at characterizing the signalling processes leading to Rho and Rac activation by shear stress. Epithelial cell invasion is also contributed to by the loss of cell-cell junctions, and the group has shown that a junction-associated protein, p120ctn, acts as a signalling protein both to stimulate Rac and to increase microtubule stability. The group has also been investigating the molecular basis for its observation that the GTPases RhoA and RhoE have opposite effects on epithelial cell morphology and migration. RhoA activates the protein kinase ROCK I and inhibits migration, whereas the team's recent work demonstrated that RhoE binds to and inhibits the activity of ROCK I, explaining why RhoE stimulates migration.

Macrophages are often recruited to epithelial cancers, and the group's analysis has delineated the roles of each PI 3-kinase isoform in macrophage migration and phagocytosis, as well as providing the surprising result that Rac1 is not essential for macrophage migration. In addition, the team has analyzed the molecular regulation of WASP, an important mediator of actin polymerization during cell migration. In collaboration with the Dr. Rainer Cramer, novel phosphorylation sites were identified on WASP and found to enhance the protein's ability to stimulate Arp2/3 complex-mediated actin polymerization.

Drosophila Morphogenesis Laboratory

The Drosophila Morphogenesis Laboratory, led by Dr Kathy Barrett, has continued to focus its research on the control of cell shape and motility. The control of cell shape and motility is of prime importance in development, resistance to disease, and tumorigenesis. The mechanisms used in this control include tight regulation of the actin and microtubule cytoskeleton. There is increasing evidence that all aspects of cytoskeletal regulation involve Rho GTPase-based signal transduction pathways. In the last decade, research into these signalling pathways has progressed into *in vivo* models including mouse, *C. elegans*, and *Drosophila*. Using these models and recent advances in microscopy, it has become possible to visualise, in real time, changes in cell shape and migration. This has dramatically increased the understanding of the role of Rho GTPase signalling in these processes. The exact mechanisms for this type of control, however, are still unknown. The group is using the powerful genetics of *Drosophila* together with *in vivo* real time imaging to elucidate the role of Rho GTPase signalling in the regulation of cell shape and motility.

Novel genes that are linked to Rho GTPase signal transduction pathways have been identified, and the group is beginning to clarify the links. Of these novel genes, one contributes to the initiation of *Drosophila* gastrulation. This hitherto elusive gene has been the focus of much research over the past 10 years. In combination with the genes the group has previously identified in this process, this gene appears to control molecules required for cell contraction. It has previously been thought that morphological changes are closely linked in some cases to the process of differentiation, but the group has demonstrated that these processes are separate entities *in vivo*. The team has also initiated, in collaboration with Mark Miodownik, a physicist from King's College London, a pioneering study to model mathematically the cell shape changes involved in development. These studies will incorporate the effect of mutations in the genes that control morphogenesis and provide unique insights into morphogenetic mechanisms.

Proteomics Laboratory

The Proteomics Laboratory, headed by Dr. Soren Naaby-Hansen, has been using a variety of proteomic techniques to study the global effects of receptor signalling. By such combinatorial approaches the group has identified the molecular mechanisms underlying platelet-derived growth factor (PDGF)-specific reorganization of the actin cytoskeleton, which enables transformation of fibroblasts into a migratory phenotype. The group has also shown that IFNγ treatment reduces the density of surface bound Annexin II, without affecting the total cellular abundance of the protein. Consistent with specific down-regulation of surface exposed Annexin II, which captures and creates a reservoir of surface proteases such as plasmin and procathepsin B, the invasive potential of prostate cancer cells was found to be significantly reduced following treatment with IFNγ, but unaltered in Annexin II negative cells. This finding explains how IFNγ acts to reduce the spread of prostate tumours in metastatic mouse models, and may have therapeutic implications.

Structural Biology Laboratory

The Structural Biology Laboratory, which is led by Dr. Paul Driscoll and located in the University College London (UCL) Biochemistry Laboratory, has been following a program that involves the development and application of NMR methodology for the elucidation of structural, dynamic, and functional properties of proteins. This year the group has been working towards providing tractable samples for structural biology of a number of challenging targets, including the p85BD/interSH2 domain subunit combining centre of class IA phosphoinositide 3-kinase, the phox homology domain of class II phosphoinositide 3-kinases, and the complex formed between the death domains of the CD95/Fas receptor and the adapter protein FADD. In the latter project, the group has established, for the first time, NMR evidence for the formation of high molecular weight aggregate complexes between the two death domains, which may be relevant to the supposed intramembrane clustering mechanism of death receptor signalling. The group is in the process of establishing cell-based assays of mutant CD95/Fas signalling to probe the structure-function relationship

in an in vivo context, to compare with NMR and yeast 2-hybrid analyses of this core interaction that initiates the apopototic death-inducing signalling complex (DISC).

In other work, the group has established the full resonance assignments for a 29 kD protein involved in the metabolism of methylated arginines, which is a record for this laboratory and which relied on state-of-the-art methods in isotope labelling and heteronuclear NMR pulse sequences. This data will provide a basis for further studies in ligand binding, drug discovery and dynamical characterisation of the enzyme.

Transformation Studies Laboratory

Research in the Transformation Studies Laboratory, led by Dr. Parmjit Jat, has been aimed at identifying the underlying molecular basis for the finite proliferative life span of normal cells. Normal cells undergo a finite number of divisions and then cease dividing whereas cancer cells are able to proliferate indefinitely. The acquisition of an infinite proliferative potential is one of the six key events required for neoplastic transformation. However, this event is one the least understood since the underlying mechanism that limits the mitotic potential of normal cells, and how this is subverted in cancer cells, is not known. The group's aim is to determine if these key components are conserved across species and in different cell types. Such components should represent novel, important and direct targets for cancer and anti-ageing therapies.

Publications

Primary Research Articles

1. Adam P.J., Berry J., Loader J.A., Tyson K.L., Craggs G., Smith P., De Belin J., Steers G., Pezzella F., Sachsenmeir K.F., Stamps A.C., Herath A., Sim E., O'Hare M.J., Harris A.L., Terrett J.A. Arylamine N-acetyltransferase-1 is highly expressed in breast cancers and conveys enhanced growth and resistance to Etoposide in vitro. Molecular Cancer Research (2003) 1(11):826-35.
2. Bird M.M., Lopez-Lluch G., Ridley A.J., Segal A.W. Effects of microinjected small GTPases on the actin cytoskeleton of human neutrophils. Journal of Anatomy (2003) 203(4):379-89.
3. Brentani H., Caballero O.L., Camargo A.A., da Silva A.M., da Silva W.A., Jr., Dias Neto E., Grivet M., Gruber A., Guimaraes P.E., Hide W., Iseli C., Jongeneel C.V., Kelso J., Nagai M.A., Ojopi E.P., Osorio E.C., Reis E.M., Riggins G.J., Simpson A.J., de Souza S., Stevenson B.J., Strausberg R.L., Tajara E.H., Verjovski-Almeida S., Acencio M.L., Bengtson M.H., Bettoni F., Bodmer W.F., Briones M.R., Camargo L.P., Cavenee W., Cerutti J.M., Coelho Andrade L.E., Costa dos Santos P.C., Ramos Costa M.C., da Silva I.T., Estecio M.R., Sa Ferreira K., Furnari F.B., Faria M., Jr., Galante P.A., Guimaraes G.S., Holanda A.J., Kimura E.T., Leerkes M.R., Lu X., Maciel R.M., Martins E.A., Massirer K.B., Melo A.S., Mestriner C.A., Miracca E.C., Miranda L.L., Nobrega F.G., Oliveira P.S., Paquola A.C., Pandolfi J.R., Campos Pardini M.I., Passetti F., Quackenbush J., Schnabel B., Sogayar M.C., Souza J.E., Valentini S.R., Zaiats A.C., Amaral E.J., Arnaldi L.A., de Araujo A.G., de Bessa S.A., Bicknell D.C., Ribeiro de Camaro M.E., Carraro D.M., Carrer H., Carvalho A.F., Colin C., Costa F., Curcio C., Guerreiro da Silva I.D., Pereira da Silva N., Dellamano M., El-Dorry H., Espreafico E.M., Scattone Ferreira A.J., Ayres Ferreira C., Fortes M.A., Gama A.H., Giannella-Neto D., Giannella M.L., Giorgi R.R., Goldman G.H., Goldman M.H., Hackel C., Ho P.L., Kimura E.M., Kowalski L.P., Krieger J.E., Leite L.C., Lopes A., Luna A.M., Mackay A., Mari S.K., Marques A.A., Martins W.K., Montagnini A., Mourao Neto M., Nascimento A.L., Neville A.M., Nobrega M.P., O'Hare M.J., Otsuka A.Y., Ruas de Melo A.I., Paco-Larson M.L., Guimaraes Pereira G., Pesquero J.B., Pessoa J.G., Rahal P., Rainho C.A., Rodrigues V., Rogatto S.R., Romano C.M., Romeiro J.G., Rossi B.M., Rusticci M., Guerra de Sa R., Sant' Anna S.C., Sarmazo M.L., Silva T.C., Soares F.A., Sonati Mde F., de Freitas Sousa J., Queiroz D., Valente V., Vettore A.L., Villanova F.E., Zago M.A., Zalcberg H. The generation and utilization of a cancer-oriented representation of the human transcriptome by using expressed sequence tags. Proceedings of the National Academy of Sciences USA (2003) 100(23):13418-23.
4. Chalkley R.J., Burlingame A.L. Identification of Novel Sites of O-N-Acetylglucosamine Modification of Serum Response Factor Using Quadrupole Time-of-flight Mass Spectrometry. Molecular and Cellular Proteomics (2003) 2(3):182-90.
5. Corless S., Cramer R. On-target oxidation of methionine residues using hydrogen peroxide for composition-restricted matrix-assisted laser desorption/ionisation peptide mass mapping. Rapid Commununications in Mass Spectrometry (2003) 17(11):1212-5.
6. Cory G.O., Cramer R., Blanchoin L., Ridley A.J. Phosphorylation of the WASP-VCA domain increases its affinity for the Arp2/3 complex and enhances actin polymerization by WASP. Molecular Cell (2003) 11(5):1229-39.
7. Cutillas P.R., Norden A.G., Cramer R., Burlingame A.L., Unwin R.J. Detection and analysis of urinary peptides by on-line liquid chromatography and mass spectrometry: application to patients with renal Fanconi syndrome. Clinical Sciences (London) (2003) 104(5):483-90.
8. Davies B.R., Steele I.A., Edmondson R.J., Zwolinski S.A., Saretzki G., von Zglinicki T., and O'Hare M.J. Immortalisation of human ovarian surface epithelium with telomerase and temperature-

sensitive SV40 large T antigen. Exp Cell Res (2003) 288(2):390-402.

9. Dong R., Cwynarski K., Entwistle A., Marelli-Berg F., Dazzi F., Simpson E., Goldman J.M., Melo J.V., Lechler R.I., Bellantuono I., Ridley A., Lombardi G. Dendritic cells from CML patients have altered actin organization, reduced antigen processing, and impaired migration. Blood (2003) 101(9):3560-7.
10. Foukas L.C., Okkenhaug K. Gene-targeting reveals physiological roles and complex regulation of the phosphoinositide 3-kinases. Archives of Biochemistry and Biophysics (2003) 414(1):13-8.
11. Galbraith T.P., Harris R., Driscoll P.C., Wallace B.A. Solution NMR studies of antiamoebin, a membrane channel-forming polypeptide. Biophysical Journal (2003) 84(1):185-94.
12. Hanze J., Eul B.G., Savai R., Krick S., Goyal P., Grimminger F., Seeger W., and Rose F. RNA interference for HIF-1alpha inhibits its downstream signalling and affects cellular proliferation. Biochemical and Biophysical Research Communications (2003) 312(3):571-7.
13. Jacinto A., Baum B. Actin in development. Mechanisms of Development (2003) 120(11):1337-49.
14. Jones G.E., Prigmore E., Calvez R., Hogan C., Dunn G.A., Hirsch E., Wymann M.P., Ridley A.J. Requirement for PI 3-kinase gamma in macrophage migration to MCP-1 and CSF-1. Experimental Cell Research (2003) 290(1):120-31.
15. Kiger A., Baum B., Jones S., Jones M., Coulson A., Echeverri C., Perrimon N. A functional genomic analysis of cell morphology using RNA interference. Journal of Biology (2003) 2(4):27.
16. Kit Y.Y., Drel V.R., Petriv O.I., Kovalyova V.A., Shuvaeva G.Y., Palivoda O.Y., Vovk E.I., Bobak Y.P., Rzeszowska-Wolny J., Gout I.T., Buchman V.L., Drobot L.B. Adaptor protein Ruk1 forms protein-protein complexes with endonuclease activity in HEK293 cells. Biochemistry (Mosc) (2003) 68(7):810-5.
17. Kunda P., Craig G., Dominguez V., Baum B. Abi, Sra1, and Kette control the stability and localization of SCAR/WAVE to regulate the formation of actin-based protrusions. Current Biology (2003) 13(21):1867-75.
18. Leverrier Y., Okkenhaug K., Sawyer C., Bilancio A., Vanhaesebroeck B., Ridley A.J. Class I phosphoinositide 3-kinase p110beta is required for apoptotic cell and Fcgamma receptor-mediated phagocytosis by macrophages. Journal of Biological Chemistry (2003) 278(40):38437-42.
19. Mackay A., Jones C., Dexter T., Silva R.L., Bulmer K., Jones A., Simpson P., Harris R.A., Jat P.S., Neville A.M., Reis L.F., Lakhani S.R., O'Hare M.J. cDNA microarray analysis of genes associated with ERBB2 (HER2/neu) overexpression in human mammary luminal epithelial cells. Oncogene (2003) 22(17):2680-8.
20. Maffucci T., Brancaccio A., Piccolo E., Stein R.C., Falasca M. Insulin induces phosphatidylinositol-3-phosphate formation through TC10 activation. EMBO Journal (2003) 22(16):4178-89.
21. Mataraza J.M., Briggs M.W., Li Z., Entwistle A., Ridley A.J., Sacks D.B. IQGAP1 promotes cell motility and invasion. Journal of Biological Chemistry (2003) 278(42):41237-45.
22. Mitsopoulos C., Zihni C., Garg R., Ridley A.J., Morris J.D. The prostate-derived sterile 20-like kinase (PSK) regulates microtubule organization and stability. Journal of Biological Chemistry (2003) 278(20):18085-91.

23 Mohd Mustapa M.F., Harris R., Esposito D., Chubb N.A., Mould J., Schultz D., Driscoll P.C., Tabor A.B. Synthesis of a cyclic peptide containing norlanthionine: effect of the thioether bridge on peptide conformation. Journal of Organic Chemistry (2003) 68(21):8193-8.

24. Mohd Mustapa M.F., Harris R., Bulic-Subanovic N., Elliott S.L., Bregant S., Groussier M.F., Mould J.,

Schultz D., Chubb N.A., Gaffney P.R., Driscoll P.C., Tabor A.B. Synthesis of orthogonally protected lanthionines. Journal of Organic Chemistry (2003) 68(21):8185-92.

25. Naaby-Hansen S., Nagano K., Gaffney P., Masters J.R., Cramer R. Proteomics in the analysis of prostate cancer. Methods in Molecular Medicine (2003) 81:277-97.
26. Riento K., Guasch R.M., Garg R., Jin B., Ridley A.J. RhoE binds to ROCK I and inhibits downstream signaling. Molecular Cell Biology (2003) 23(12):4219-29.
27. Sawyer C., Sturge J., Bennett D.C., O'Hare M.J., Allen W.E., Bain J., Jones G.E., Vanhaesebroeck B. Regulation of breast cancer cell chemotaxis by the phosphoinositide 3-kinase p110delta. Cancer Research (2003) 63(7):1667-75.
28. Smith P.D., Crocker S.J., Jackson-Lewis V., Jordan-Sciutto K.L., Hayley S., Mount M.P., O'Hare M.J., Callaghan S., Slack R.S., Przedborski S., Anisman H., Park D.S. Cyclin-dependent kinase 5 is a mediator of dopaminergic neuron loss in a mouse model of Parkinson's disease. Proceedings of the National Academy of Sciences USA (2003) 100(23):13650-5.
29. Streets A.J., Newby L.J., O'Hare M.J., Bukanov N.O., Ibraghimov-Beskrovnaya O., Ong A.C. Functional analysis of PKD1 transgenic lines reveals a direct role for polycystin-1 in mediating cell-cell adhesion. Journal of the American Society of Nephrology (2003) 14(7):1804-15.
30. Thompson A.J., Hart S.R., Franz C., Barnouin K., Ridley A., Cramer R. Characterization of protein phosphorylation by mass spectrometry using immobilized metal ion affinity chromatography with on-resin beta-elimination and Michael addition. Analytical Chemistry (2003) 75(13):3232-43.
31. Valovka T., Verdier F., Cramer R., Zhyvoloup A., Fenton T., Rebholz H., Wang M.L., Gzhegotsky M., Lutsyk A., Matsuka G., Filonenko V., Wang L., Proud C.G., Parker P.J., Gout I.T. Protein kinase C phosphorylates ribosomal protein S6 kinase betaII and regulates its subcellular localization. Molecular Cell Biology (2003) 23(3):852-63.
32. Waugh M.G., Minogue S., Anderson J.S., Balinger A., Blumenkrantz D., Calnan D.P., Cramer R., Hsuan J.J. Localization of a highly active pool of type II phosphatidylinositol 4-kinase in a p97/valosin-containing-protein-rich fraction of the endoplasmic reticulum. Biochemistry Journal (2003) 373(Pt 1):57-63.
33. White S.L., Gharbi S., Bertani M.F., Chan H.L., Waterfield M.D., Timms J.F. Cellular responses to ErbB-2 overexpression in human mammary luminal epithelial cells: comparison of mRNA and protein expression. British Journal of Cancer (2004) 90(1):173-81.
34. Wojciak-Stothard B., Ridley A.J. Shear stress-induced endothelial cell polarization is mediated by Rho and Rac but not Cdc42 or PI 3-kinases. Journal of Cell Biology (2003) 161(2):429-39.
35. Zhyvoloup A., Nemazanyy I., Panasyuk G., Valovka T., Fenton T., Rebholz H., Wang M.L., Foxon R., Lyzogubov V., Usenko V., Kyyamova R., Gorbenko O., Matsuka G., Filonenko V., Gout I.T. Subcellular localization and regulation of coenzyme A synthase. Journal of Biological Chemistry (2003) 278(50):50316-21.

Reviews / Book Chapters / Commentaries

1. Wymann M.P., Zvelebil M., Laffargue M. Phosphoinositide 3-kinase signalling--which way to target? Trends in Pharmacological Sciences (2003) 24(7):366-76.
2. Entwistle A. Formats of image data files that can be used for routine digital light micrography. Part one. Biotechnic and Histochemistry (2003) 78(2):77-89.
3. Entwistle A. Formats of image data files that can be used in routine digital light micrography. Part

two. Biotechnic and Histochemistry (2003) 78(2):91-9.

4. Okkenhaug K., Vanhaesebroeck B. PI3K-signalling in B- and T-cells: insights from gene-targeted mice. Biochemical Society Transactions (2003) 31(Pt 1):270-4.
5. Okkenhaug K., Vanhaesebroeck B. PI3K in lymphocyte development, differentiation and activation. Nature Reviews Immunology (2003) 3(4):317-30.
6. Ridley A.J., Schwartz M.A., Burridge K., Firtel R.A., Ginsberg M.H., Borisy G., Parsons J.T., Horwitz A.R. Cell migration: integrating signals from front to back. Science (2003) 302(5651):1704-9.
7. Riento K., Ridley A.J. Rocks: multifunctional kinases in cell behaviour. Nature Reviews Molecular Cell Biology (2003) 4(6):446-56.
8. Sunderkotter C., Mosser D., Ridley A., Sorg C., Roth J. Meeting report: molecular mechanisms of inflammation: how leukocytes come, see and seize. European Journal of Cell Biology (2003) 82(7):379-83.

LUDWIG INSTITUTE FOR CANCER RESEARCH

Melbourne Branch of Tumour Biology

Melbourne, Australia

Staff List

Burgess, Antony *Member, Branch Director*
Dunn, Ashley *Member, Associate Branch Director (until April)*
Scott, Andrew *Associate Member, Associate Branch Director, Clinical Program*
Stacker, Steven *Associate Member, Associate Branch Director, Scientific Program*

Angiogenesis Laboratory

Achen, Marc *Assistant Member, Head*
Stacker, Steven *Assistant Member, Head*
Davydova, Natalia *Research Associate (from April)*
Halford, Michael *Post-doctoral Fellow (until April)*
Macheda, Maria *Post-doctoral Fellow (from April)*
Caesar, Carol *Senior Research Officer*
Roufail, Sally *Senior Research Officer*
Chaffer, Christine *Research Officer (until February)*
Inder, Rachel *Research Officer*
Leow, Mei Fen *Research Officer (February to April)*
McDonald, Narelle *Research Officer*
Vranes, Dimitria *Research Officer (from November)*
Kugathasan, Kumudhini *PhD Student*
McColl, Brad *PhD Student*
Loughran, Stephen *UROP Student (from February)*

Colon Molecular & Cell Biology Laboratory

Ernst, Matthias *Assistant Member, Head*
Heath, Joan *Assistant Member, Head*
Abud, Helen *Assistant Investigator*
Jenkins, Brendan *Post-doctoral Fellow*
Ng, Annie Yee-Lee *Post-doctoral Fellow*
Grail, Dianne *Chief Research Officer*
Liu, Yong Shu *Chief Research Officer*
Clay, Fiona Jane *Senior Research Officer (until May)*
Inglese, Melissa *Senior Research Officer*
White, Sara *Senior Research Officer*
Smith, Natasha *Research Officer (from February)*
Richardson, Elsbeth *Research Assistant*
Meyer, Merle *Research Assistant (September to December)*
Christou, Aliki *Visiting Research Assistant (from June)*
Bateman, Trudie *PhD Student*
De Jong-Curtain, Tanya *PhD Student*
Samuel, Michael *PhD Student*
Tomaskovic-Crook, Eva *PhD Student*
Blits, Marjolein *Visiting student (from August)*
Johnsson, Anna-Karin *Visiting Student (from September)*

Cytokine Biology Laboratory

Lieschke, Graham *Assistant Member, Head*
Layton, Judith *Senior Investigator*
McPhee, Dora *Senior Research Officer*
Varma, Sony *Senior Research Officer*
Connell, Fiona *Research Officer (until June)*
Hayman, John *Research Officer*
Crowhurst, Meredith *PhD Student*
Hogan, Benjamin *PhD Student*
Boonchalermvichian, Chaiyaporn *Visiting Student*
Botermans, Claudia *Visiting Student (July to October)*
McCaw, Benjamin *Visiting Student (February to March)*

Epithelial Biochemistry Laboratory

Burgess, Antony *Member, Branch Director, Head*
Nice, Edouard *Associate Member*
Catimel, Bruno *Assistant Investigator*
Walker, Francesca *Associate Investigator*
Lackmann, Martin *Research Associate (until March)*
Clayton, Andrew *Post-doctoral Fellow*
Faux, Maree *Post-doctoral Fellow*
Stenvers, Kaye *Post-doctoral Fellow*
Zhu, Hong-Jian *Post-Doctoral Fellow*
Lin, Feng *Peptide Chemist*
Cody, Stephen *Microscopy Facility Manager*
Cartledge, Kellie *Senior Research Officer (until January)*
Condron, Melanie *Senior Research Officer*
Nerrie, Maureen *Senior Research Officer*
Nheo, Thao Senior *Research Officer*
Orchard, Suzanne *Senior Research Officer*
Rothacker, Julie *Senior Research Officer*
Weinstock, Janet *Senior Research Officer*
Zhang, Hui-Hua *Senior Research Officer*
Catimel, Jenny *Research Officer (from August)*
Gras, Emma *Research Officer*
Kiu, Jessie Hiu *Research Officer*
Ross, Janine *Research Officer*
Tursky, Melinda *Research Officer*
Wu, Fenqiang *Research Officer*
Kleikamp, Sabine *PhD Student*
Vearing, Christopher *PhD student (until March)*
Wang, Bo *PhD Student*
Zamurs, Laura *PhD Student*
Gantelius, Jasper *Visiting Student (until May)*
Patani, Neill *Visiting Student (until January)*
Sandblad, Peter *Visiting Student (from June)*
Siehl, Katjuscha *Visiting Student (June to August)*

Joint ProteomicS Laboratory

(previously Joint Protein Structure Laboratory)

(A Joint Venture of the Ludwig Institute and The Walter and Eliza Hall Instutute of Medical Research)

Simpson, Richard *Member, Head*
Reid, Gavin *Assistant Member*
Ji, Hong *Assistant Investigator*
Tu, Guo-Fen *Assistant Investigator*
Layton, Meredith *Post-doctoral Fellow*
Jordan, Bill *Visiting Post-doctoral Fellow (from November)*
Strahm, Yvan *Visiting Post-doctoral Fellow (until October)*
Moritz, Robert *Proteomics Facility Manager*
Kapp, Eugene *Chief Research Officer*
Clipplingdale, Andrew *Proteomics Research Officer (from June)*
Connolly, Lisa *Proteomics Project Service Manager*
Eddes, James *Senior Research Officer*
Frecklington, David *Senior Research Officer*
Roberts. Kade *Senior Research Officer (from April)*
Church, Nicole *Research Officer*
Hordejuk, Katarzyna *Research Officer*
Gilbert, Sam *Jnr. Mass Spec. Technician (from April)*
Lim, Justin *PhD Student (until May)*
Lioe, Hadi *PhD Student (from February)*
Lim, Kook-Jin *Visiting PhD student*
Goode, Robert *Honors Student*
Tay, Leigh *Honors Student*
Hulett, Joanne *Summer Student (until January)*
Jaw, Juli *Advanced Medical Science Student (from August)*
O'Day, James *UROP Student (until February)*
Sommer, Michael *Visiting Student (until January)*
Bright, Michael *Visiting Student (September until November)*
Streep, Tanya *Visiting Student (May to September)*
Pakin, Simone *Personal Assistant*

Signal Transduction Laboratory

Hibbs, Margaret *Assistant Member, Lab Head*
Harder, Ken *Post-doctoral Fellow*
Kemp, Joanne *Post-doctoral Fellow (from August)*
Casagranda, Franca *Chief Research Officer*
Quilici, Cathy *Chief Research Officer*
Kountouri, Nicole *Senior Research Officer*
Zlatic, Kristina *Senior Research Officer*
Turner, Amanda *Research Officer*
Beavitt, Sarah-Jane *PhD Student*
Naik, Edwina *UROP Student*
Schwarzer, Wibke *Visiting Student (until March)*

Tumour Supressor Laboratory

Maruta, Hiroshi *Associate Member, Head*
He, Hong *Research Associate (until February)*
Nheu, Thao Vuong *Senior Research Officer*
Hirokawa, Yumiko *Research Officer*
Huynh, John *Summer Student (until January)*

Bioinformatics

Hall, Nathan *Bio-Informatics Officer*
McDonald, Joshua *UROP Student*

Information Technology Centre (Parkville Campus)

Tege, Greg *Systems Manager*
Runting, Andrew *Systems Administrator*
Lay, Sami *IT Programmer*
Lai, Victorin *Help-desk Support Officer (from August)*
Witte, Christopher *Help-desk Support Officer (July to November)*

Laboratory Services (Parkville Campus)

Daws, Douglas *Laboratory Manager*
Vremec, Melinda *Laboratory Officer*
Plenter, Robert *Laboratory Officer (until April)*
Batty, John *Laboratory Assistant (from April)*
Cole, Robin *Equipment Officer, Maintenance*
Bradshaw, Derek *Equipment Officer, Maintenance*
Feakes, Valerie *Senior Research Officer, Histology*
Meddings, Penelope *Media Technician*
Helman, Tracy *Animal Facility Manager*
Arnold, Melissa *Technical Assistant, Animal Facility*
Cohen, Jacqueline *Technical Assistant, Animal Facility*
Edmunds, Brett *Technical Assistant, Animal Facility (from November)*
Helman, Adam *Technical Assistant, Animal Facility (until June)*
Kuykhoven, Keilly *Technical Assistant, Animal Facility (from September)*
McKiernan, Sarah *Technical Assistant, Animal Facility (until September)*
Teal, Bianca *Technical Assistant, Animal Facility (from August)*
Tierney, Fay *Technical Assistant, Animal Facility (from November)*
Thorne, Teleah *Technical Assistant, Animal Facility*
Watson, Pieta *Technical Assistant, Animal Facility (until July)*
Webster, Gaye *Technical Assistant, Animal Facility*
Peirce, Susan *Chief Veterinary Officer, Animal Ethics (from April)*
Ginns, Carol *Veterinary Assistant, Animal Ethics (until January)*
Ellet, Felix *Technical Assistant, Aquarium*
Trotter, Andrew *Research Officer, Aquarium (from February)*
Maney, Wayne *Technical Assistant, Wash-up*
Topham, Helen *Technical Assistant, Wash-up*
Vis, Ammy *Technical Assistant, Wash-up*
Zammit, Carmen *Technical Assistant, Wash-up*
Mathews, Peter *Information Officer/Librarian*

Administration (Parkville Campus)

Tsai, Kim *General Manager*
Catanzariti, Nadia *Financial Accountant (from November)*
Fletcher, Chelsea *Assistant Accountant (until June)*
Lancuba, Sofia *Accounts Officer*
Green, Cassey *Payroll/HR Officer*
Clark, Pauline *Human Resources Manager*
Perra, Cristina *Human Resources Officer (from August)*
Thumwood, Cassandra *Scientific Administration Officer*
Jones, Pamela *Personal Assistant*
Stevens, Wendy *Receptionist*
Makris, Polytimi *Administration Assistant/Receptionist*
Luhn, Renate *Senior Administrative Officer*
Near, Christine *Animal Ethics Officer*

MELBOURNE BRANCH CLINICAL PROGRAM

Clinical Program (Parkville and Western Hospital)

(A joint venture of the Ludwig Institute, The Royal Melbourne Hospital and The Western Hospital)

Gibbs, Peter *Senior Clinical Research Fellow*
Johns, Julie *Data Manager/Tissue Bank Coordinator (from March)*

Joint Austin Ludwig Medical Oncology Unit

Cebon, Jonathan *Associate Member, Head*
Davis, Ian *Assistant Member*
Mitchell, Paul *Director Cancer Services, LICR Associate*
Parente, Phillip *Clinical Research Fellow (until February)*
Barrow, Catherine *Clinical Research Fellow (from February)*
Tebbutt, Niall *Clinical Post-doctoral Fellow*
Rudolph, Anne *Personal Assistant*

CANCER VACCINE PROGRAM

Cancer Vaccine Laboratory

Cebon, Jonathan *Associate Member, Head*
Maraskovsky, Eugene *Assistant Member, Head, LICR Associate*
Schnurr, Max *Post-Doctoral Fellow*
Shin, Amanda *Research Officer*
Kong, Alan *Research Officer (from August)*
Junaidi, Hairol *PhD Student (until September)*
Jenderek, Corinna *Visiting student (until September)*

Dendritic Cell Laboratory

Davis, Ian *Assistant Member, Head*
Morris, Leone *Senior Research Officer*
Quirk, Juliet *Senior Research Officer*
Seddon, Lauren *Senior Research Officer (from July)*
Adelaide, Christopher *Research Officer (until April)*

T-Cell Laboratory

Chen, Weisan *Assistant Member, Lab Head*
Qiyuan, Chen *Research Associate*
Jackson, Heather *Manager-Clinical Trials Monitor*
Dimopoulos, Nektaria *Senior Research Officer*
Tai, Tsin Yee *Senior Research Officer*
Kennedy, Gina *Research Officer (from April)*
Luke (nee Beecroft), Tina *Research Officer*
Masterman, Kelly-Anne *Research Officer (until May)*
Toy, Tracey *Research Officer*
Bethune, Barbara *Blood Processor*
Pang, Ken *PhD Student (from February)*

TUMOUR TARGETING PROGRAM

Scott, Andrew *Associate Member, Head*
Lewis, Kath *Personal Assistant*
Smyth, Fiona *Scientific Admin Officer*
Papenfuss, Tony *Physicist (from November)*

Tumour Targeting Laboratory

Lee, F T *Associate Investigator*
Johns, Terrance *Assistant Investigator*
Liu, Zhanqi *Assistant Investigator*
Luwor, Rod *Post-Doctoral Fellow (until August)*
Rayzman, Veronika *Post-doctoral Fellow*
Ng, Christina *Clinical Research Fellow (February to June)*
Potasz, Nicole *Clinical Research Fellow (until January)*
Spirkoska, Violeta *Chief Research Officer*
Butler, Claire *Senior Research Officer (until March)*
Cao, Diana (Dong) *Senior Research Officer (from March)*
Hall, Cathrine *Senior Research Officer*
Mountain, Angela *Senior Research Officer*
Rigopoulos, Angela *Senior Research Officer*
Vitali Angela *Senior Research Officer*
Wang, Dong-mao *Senior Research Officer*
Batrouney, Ahida *Research Officer (October to November)*
Ciccomancini, Melissa *Research Officer*
Henry, Erin *Research Officer (October to November)*
Tavarnesi, Meri *Research Officer*
Vranes, Dimitria *Research Officer (October to November)*
Wang, Dongmao *Research Officer*
Gan, Hui *PhD Student (from February)*
Kelly, Marcus *PhD Student (from February)*
Legge, Sue *PhD Student (until March)*
Marinic, Anna *PhD Student*
Perera, Rushika *PhD Student*
Vernes, Sonja *Honors Student*

Biological Production Facility

Rubira, Michael *BPF Facility Manager*
Murphy, Roger *Associate Investigator, Quality Control Manager*
Cartwright, Glenn *Chief Research Officer, Purification Development Scientist*
Jois, Jennifer *Senior Research Officer, Cell Biologist*
Kypridis, Anna *Senior Research Officer, Purification Process Scientist*
Wirth, Veronika *Senior Research Officer, Quality Control Scientist*
O'Hoy, Kim *Assistant Cell Biologist (from April)*
Cornhels, Jeff *Quality Compliance Officer (from May)*

Tumour Targeting Clinical Trials Group

Potasz, Nicole *Clinical Research Fellow (until January)*
Ng, Christina *Clinical Research Fellow (from February)*
Hopkins, Wendie *Senior Research Nurse*
Schleyer, Paul *Computer Programmer (until August)*
Foo, Serene *MD Student (from February)*
Wheatley, Jennifer *Surgeon, LICR Associate*

LICR Associates - Department of Nuclear Medicine & Centre for PET

Rowe, Chris *Director, LICR Associate*
Hannah, Anthony *Physician, LICR Associate*
Berlangieri, Sam *Physician, LICR Associate*
Tochon-Danguy, Henri *Senior Radiochemist, LICR Associate*
O'Keefe, Graeme *Senior Physicist, LICR Associate*

Cancer Clinical Trials Centre

Hopkins, Wendie *Senior Research Nurse*
Gibbs, Sharen *Research Nurse*
Goldie, Heather *Research Nurse*
Lee, Sunanta *Data Systems Developer*

Joint Pathology/Tissue Bank Group

MacGregor, Duncan *Director, Dept. Anatomical Pathology, LICR Associate*
Murone, Carmel *Scientist, Anatomical Pathology*
Sturrock, Sue *Scientist, Anatomical Pathology*
Browning, Judy *Scientist, Anatomical Pathology*

Laboratory Services (Austin Campus)

Fox, Simon *Assistant Laboratory Manager*
Swift, Karen *Technician, Animal Facility (from February)*
Koul, Usha *Technical Assistant, Wash-up*
Wapshott, Yvonne *Technical Assistant, Wash-up*

Administration (Austin Campus)

Tsai, Kim *General Manager*
Sharples, Catherine *Clinical Operations Manager (from October)*
Catanzariti, Nadia *Financial Accountant (from December)*
Mazzeo, Mary *Financial Accountant (from November)*
Humphrey, Susan *Accounts Officer*

Lazarovska, Vanessa *Receptionist/Secretary*
Tronson, Briony *Receptionist (from June)*
Cox, Shayne *PC/Network Support Officer*

Clinical Trials Australia

(formerly the Centre for Developmental Cancer Therapeutics (CDCT))

(A joint venture of the Ludwig Institute, The Walter and Eliza Hall Institute of Medical Research, The Austin Hospital, the Western Hospital, The Royal Melbourne Hospital and Peter MacCallum Cancer Institute)

Rosenthal, Mark *Director, LICR Associate*
Alt, Carole *Administrator*
Raunow, Heike *Research Nurse*

Branch Director's Report

The Melbourne Branch has continued to combine laboratory and clinical research to transform discoveries into anti-cancer therapeutics. Research at the Branch has continued to develop new concepts in lymphangiogenesis, cancer immunology, cytokine and growth factor signal transduction, proteomics, intestinal biology, colon cancer, lymphoma, melanoma and gastric cancer. The results from human, mouse and zebrafish models of tissue development and tumorigenesis have helped direct the work undertaken in Melbourne on tissue specific markers, cancers and the biochemical events associated with the initiation, progression and maintenance of the tumorigenic state.

The focus for the Melbourne Branch of the Institute is colon biology research, and spans from studies in intestinal biochemistry, genetics and development in vitro, to development and tumorigenesis studies in zebrafish and other models in vivo, through to colon biomarker identification and the testing of innovative cancer therapeutics in humans. These studies encompass the Colon Molecular and Cellular Laboratory, the Epithelial Biochemistry Laboratory, the Joint ProteomicS Laboratory and the Tumor Targetting Laboratory. The use of organ cultures to study intestinal development and the delivery of recombinant genes to intestinal epithelium provide a direct window for viewing the results of perturbing cell regulatory systems in the colon. These studies have been extended to detect factors that influence the embryonic development of the colon.

The Institute's Clinical Program in Melbourne has made substantial achievements over the last 12 months in both clinical and research areas. Research studies exploring the function of the epidermal growth factor receptor, dendritic cell biology and T cell immunology are a focus of the laboratory programs undertaken in Melbourne. A vaccine trial of a protein based vaccine NY-ESO-1 ISCOMATRIX™, conducted in collaboration with CSL Ltd, has shown for the first time conclusive T cell and B cell responses in melanoma patients. These important findings are to be extended to multi-site trials that will test the clinical efficacy of this vaccine. Clinical trials with recombinant antibodies huA33 (colon cancer), cG250 (renal cancer) and hu3S193 (colon, lung and breast cancer) are also showing encouraging results, and the licensing of hu3S193 to Wyeth-Ayerst for an antibody-toxin conjugate was a substantial achievement this year. During 2003, the Institute initiated a partnership with Austin Health for the creation and fundraising for a new Cancer Centre to be named the Olivia Newton-John Cancer Centre, which will incorporate Institute research and clinical programs. The tissue bank program has also been extended to a Human Colon Cancer Initiative which aims to prospectively collect colon cancer specimens for genetic and proteomic analysis, linked to patient bioinformatics, at multiple hospitals throughout Melbourne as a part of a broader LICR program.

Antony W. Burgess

Parkville Campus

Angiogenesis Laboratory

In the Angiogenesis Laboratory, headed jointly by Drs. Steven Stacker and Marc Achen, studies on the regulation of lymphangiogenesis have identified plasmin as an enzyme that converts the pro-forms of the lymphangiogenic growth factors VEGF-C and VEGF-D into the mature, activated dimers. This laboratory forms part of the Institute's Angiogenesis Program, and through collaboration with Dr. Kari Alitalo (Helsinki Affiliate Center), has discovered that VEGF-D is the strongest angiogenesic and lymphangiogenic effector among VEGF family. When VEGF-D is delivered into skeletal muscle using an adenovirusal vector there is a major increase in both lymphangiogenesis and angiogenesis. A link between EGFR inhibitors, the consequential inhibition of VEGF's production and the responses of human tumors to combinations of the EGFR inhibitors and ionizing radiation has been described in a collaborative with scientists from the Peter MacCallum Cancer Centre (Melbourne, Australia). In collaboration with Ark Therapeutics, a European biotechnology company, VEGF-D, in the form of Trinam®, is proceeding to Phase II clinical trial for the prevention of blood vessel blockage after surgery. In collaborative studies on viral (v-) VEGFs, the Angiogenesis Laboratory has shown that despite considerable variations in amino acid sequence, receptor-binding specificities and the ability to induce vascular permeability, the v-VEGFs are uniformly active mitogens for mammalian endothelial cells.

Colon Molecular and Cell Biology Laboratory

The Colon Molecular and Cell Biology Laboratory, headed by Drs. Matthias Ernst and Joan Heath, undertakes studies that are designed to identify and analyse genes involved in colorectal cancer. To generate novel models of colon cancer, a number of transgenic mice based on the A33 antigen promoter have been created, including some designed to generate intestinal epithelial cells containing hypermethylated genomes by over-expression of the DNA methyl transferase genes, *dnmt1* and *dnmt3a*. These mice are helping to confirm whether gene silencing by aberrant methylation plays an important role in colon tumorigenesis. Another A33-transgenic model will enable the analysis of the effect of tumor suppressor gene deletion on intestinal epithelial cell behaviour. A collaboration with Dr. Didier Stanier (University of California) has made possible the initiation of studies with mutant zebrafish that have defects in cell polarization, proliferation and apoptosis. Members of the laboratory have also developed an *in vitro* electroporation system in which embryonic gut explants, that have preserved three-dimensional (3D) tissue architecture, are able to take up expression vectors that encode for fluorescently labelled proteins of interest. This experimental system readily allows the behaviour of individual cells expressing the labelled-proteins to be closely monitored by microscopy, histology, and immunohistochemistry.

Dr Matthias Ernst and his colleagues in the laboratory are continuing to characterize the phenotypes of two mutant mouse strains harboring reciprocal null mutations in each of the two major signalling modules of the cytokine receptor, gp130 ($gp130^{\Delta STAT}$ and $gp130^{757F}$). Analysis of the $gp130^{\Delta STAT}$ mice has revealed a novel role for STAT-mediated interleukin 6-signalling in the promotion of epithelial homeostasis and wound-healing in the colon. In addition, analysis of $gp130^{757F}$ mice has pointed to a critical contribution of gp130-dependent activation of the Ras/Erk-pathway in suppressing gastric hyperplasia.

Cytokine Biology Laboratory

The experimental program in zebrafish genetics at the Branch has two major arms, one of which is the study of haemopoiesis/leukemia and is the central interest of the Cytokine Biology Laboratory, headed by Dr Graham Lieschke. Dr Lieschke's group has completed a large screen for zebrafish mutants with defects in the developmental pathway from mesoderm to myeloid cells. This study has resulted in 32 putative mutants with congenital myeloid-failure syndromes. Transgenic fish are being used to probe the genetic

influences on myeloid development and leukaemia. Positional cloning studies on particular zebrafish have progressed well and laboratory members have recently demonstrated a role for the transcription factor "glial cells missing 2" in branchial arch and gill filament development. In addition, progress has been made with the descriptive characterization and cloning of the mutant *cephalophonos*, and the expression pattern of zebrafish parathyroid hormone genes.

Epithelial Biochemistry Laboratory,

The three major interests of the Epithelial Biochemistry Laboratory, headed by Dr. Tony Burgess, are a molecule called Adenomatous Polyposis Coli (APC), the epidermal growth factor receptor (EGFR) family of tyrosine kinases, and TGFβ signaling. APC is a large, scaffolding molecule known to be associated with the development of colon cancer. Ectopically expressed APC is functional, resulting in the translocation of β catenin from the nucleus and cytoplasm to the cell periphery, and reduces β-catenin/Tcf/LEF transcriptional signaling. E-cadherin is also translocated to the cell membrane, where it forms functional adherens junctions. SW480 cells, a human colon cancer cell line known to be deficient in the APC molecule, have been able to be reverted to a "less cancerous" phenotype by the transfection of full-length APC and show a reduced proliferation rate, a decreased ability to form colonies in soft agar, and an inability to grow tumors in a xenograft mouse tumor model. The effects of APC on cell adhesion processes are being explored in intestinal cultures. Using biosensor measurements, the quantitative dynamics of APC complex formation is currently being studied.

Several members of the Epithelial Biochemistry Laboratory have continued to collaborate with the Division of Health Sciences and Nutrition, CSIRO (Melbourne, Australia) in studies centered on the determination of the 3D structure EGFR family members. In 2003, the laboratory contributed to the elucidation of structure of the extracellular portion of erbB2, which has improved the understanding of ligand binding, conformational change and signaling from hetero-dimeric receptors. In conjunction with the Tumor Targeting Laboratory, the 3D structural information has directed a search for the EGFR epitope recognized by the mab806 (discovered by the late Dr. Elisabeth Stockert at the New York Branch). The mab806 epitope is uniquely positioned at the hinge point of the EGFR; consequently, mab806 can be used to monitor the conformational transitions that occur within the EGFR, when ligand binds. By using fluorescence intensity lifetime (FLIM), differences could be observed in the conformational transitions for two different truncated forms of the extracellular portion of the EGFR. In addition, the interactions and functional consequences of stimulating cells with both EGFR ligands and laminin-10, an activator of integrin receptors, has been observed. Several of the colon cell lines developed at the Branch require the engagement of both receptor systems before proliferation or motogenesis can occur.

Joint ProteomicS Laboratory

The proteomics research at the Branch in the Joint ProteomicS Laboratory (JPSL), headed by Dr. Richard Simpson, continues to improve the technologies for separating and analyzing complex mixtures of proteins and peptides. A new chemistry for controlling the fragmentation of peptides at specific amino acids has been developed. This chemistry assists in both the mass spectrometric identification of peptides and the quantification of protein expression. While two dimensional IEF/SDS gels have been a long standing method for high resolution separation proteins, the capacity and repeatability have limited the use of this technique for high sensitivity proteomics. JPSL are now using a two dimensional solution phase separation system using free flow electrophoresis and reversed-phase high-performance liquid chromatography (HPLC). Members of the JPSL have amassed mass spectrometry (MS/MS) fragmentation spectra for more than 5000 peptides; by analyzing the fragmentation patterns it became clear that some amino acids such as proline and asparagine have unique influences on the fragmentation patterns; consequently, the laboratory have been able to develop identification algorithms capable of faster and more accurate mass spectrometric identification of proteins and peptides.

Signal Transduction Laboratory

A member of the src family of intracellular tyrosine kinases, Lyn, is the focus of research in the Signal Transduction Laboratory, headed by Dr Margaret Hibbs. Lyn has been shown to be important for the regulation of B-lymphocytes through a collaboration with Dr David Tarlinton at the Walter and Eliza Hall Institute of Medical Reasearch (Melbourne, Australia). Lyn-deficient mice have both myeloid and erythroid cell population defects, which are similar to defects in mice lacking the inhibitory phosphatases SHIP-1 and SHP-1. Clearly, Lyn is a critical negative regulator of immune responses. These studies, conducted in collaboration with Dr Gary Anderson, University of Melbourne (Melbourne, Australia), have shown that Lyn-deficient mice develop intense lung eosinophilia, mast cell hyper-degranulation, airway hyper-responsiveness and T_H2 immune deviation, which would expect to pre-dispose animals to asthma. The laboratory has demonstrated that Lyn is expressed in dendritic cells where it plays a major signaling role in biasing immune responses. Furthermore, Lyn-deficient dendritic cells mature inefficiently, exhibit defective inhibitory PIR-B receptor tyrosine phosphorylation, and importantly, can polarize T cells toward a T_H2 phenotype when adoptively transferred into wild type recipients. These results explain how Lyn, which is not expressed in T cells, can influence T cell functional phenotype.

Tumor Suppressor Laboratory

Continued research in the Tumor Suppressor Laboratory, headed by Dr Hiroshi Maruta, has reveled that selective inhibition of the Ser/Thr kinase, PAK-1, is able to block the malignant growth of both ras transformed cells and NF-2 deficient neurofibromatosis cells. This work was based on a previous observation that the tumor suppressor, NF2/merlin, can act as an inhibitor of PAK-1. The central role for PAK-1 appears to be through the oncogene ras, a molecule that is often subject to mutation in cancer resulting in inappropriate activation of this molecule. There has been a great deal of interest and effort in identifying methods of inhibiting activated ras as a novel and specific approach to cancer therapy. Molecules that exhibit PAK-1 inhibitory action that are used in the laboratory are CEP-1347 and the peptide WR-PAK18 and these have no inhibitory activity in cancer cell lines that express NF2.

Austin Hospital Campus

Tumor Targeting Program

Tumor Targeting Laboratory

The development of therapeutics and the biology of the EGFR has been a major focus of the Tumor Targeting Laboratory research program over the last 12 months under the leadership of Dr. Andrew Scott. Through a collaboration with Dr Tim Adams at CSIRO (Melbourne, Australia), Dr. Dane Wittrup at Massachusetts Institute of Technology (Massachusetts, USA), the Epithelial Biochemistry Laboratory of the Melbourne Branch, and the LICR New York Branch, it has been possible to reveal that mAb 806 binds to a novel epitope on the transitional form of EGFR. Binding of the antibody therefore prevents activation of the EGFR and leads to an anti-tumor effect seen in experimental models. Researchers in the laboratory have also confirmed the significance of the PI3-K/Akt pathway to de2-7 EGFR and established for the first time that this truncated receptor can dimerize with, and activate, the wild-type EGFR. Cellular trafficking studies in collaboration with Drs. Ira Mellman and Derek Toomre at Yale University (Connecticut, USA) are continuing. A chimeric form of mAb 806 (ch806) has been constructed and is the subject of preclinical development and characterization. Pre-clinical studies with mAb IIIA4 directed against the

Eph A3 receptor continue in collaboration with Dr Martin Lackmann at Monash University (Melbourne, Australia) and Dr Andrew Boyd at Queensland Institute of Medical Research (Brisbane, Australia), and the development of novel antibodies to CD59, fibroblast activation protein (FAP), and targets on erbB family members are also ongoing.

Clinical Trials

A pilot study of cG250 and low dose subcutaneous IL-2 in patients with advanced renal cell carcinoma have been completed, and a Phase I trial of ^{131}I-huA33 in combination with capecitabine in patients with metastatic colorectal cancer has commenced. A first-in-man Phase I trial of the Le^{y} targeting mAb, hu3S193, is ongoing and has shown promising results with targeting and immune function.

Biological Production Facility

The LICR is unique in having established a Biological Production Facility to manufacture under 'Good Manufacturing Practice' (GMP) conditions study drugs for LICR-sponsored trials worldwide. GMP bioreactor production of the chimeric monoclonal antibody ch806 was completed in 2003. A number of antibodies and peptides, and the tyrosine kinase inhibitor AG1478, were formulated and vialled. A vaccine containing the recombinant protein, NY-ESO-1 and the CHP adjuvant (an aggregate of cholesterol and the carbohydrate pullulan) was also produced.

Centre for Positron Emission Tomography

The research program in molecular imaging continued throughout the year with clinical studies in glioma, melanoma, lymphoma, renal, lung and colon cancer patients conducted. Research studies in tumor hypoxia imaging in glioma, lung and renal cancer patients have demonstrated unique data on this important component of tumor metabolism. The Center's radiochemistry research continued with the application of a novel method of ^{11}C labeling signaling inhibitor compounds.

Royal Melbourne / Western Hospital

Over the last 12 months Dr Peter Gibbs has continued the Branch's collaboration with Cancer Trials Australia to develop an electronic database that captures comprehensive clinical data on all patients with colorectal cancer seen at Royal Melbourne and Western Hospitals. In collaboration with BIO21, a technology platform is being developed that will permit sophisticated analyses of this clinical data and linkage with phenotypic and genomic data from multiple sources. The initial focus of this project will be clinical and research databases in colorectal cancer as part of the human colorectal cancer initiative. There is also a pilot project that links clinical data between multiple hospitals, which will be possible without compromising patient privacy. The tissue bank at Royal Melbourne Hospital has continued to collect tumour samples from patients with colorectal cancer and collection of tumour samples at Western Hospital has just commenced. Another recent development is the collection of blood samples from these patients. These samples will allow prospective studies on putative biomarkers in tumor tissue with matching blood sample, with the added advantage of linked clinical treatment/outcome information.

Joint Austin Ludwig Oncology Unit

The Joint Austin Ludwig Oncology Unit, under the direction of Dr. Jonathan Cebon, is one of Australia's foremost units devoted to clinical care and research, and is focused on the development of new therapies. Early phase trials are performed at the Austin Hospital as a principal site for Cancer Trials Australia (*http://www.cancertrialsaustralia.com*) as well as later phase trials, often undertaken as a participant of one of the Australian cooperative trials groups. The Joint Oncology Unit is a major Australian center for post graduate training in Medical Oncology, and with Dr. Ian Davis as Chair, continues to coordinate the

undergraduate haematology/oncology teaching program for the University of Melbourne.

Highlights for 2003 include conducting a number of innovative clinical trials including: cancer vaccines outlined above and a CpG oligodeoxynucleotide in renal cancer; the tyrosine kinase receptor inhibitors SD8381 in colorectal cancer, Iressa in lung and head & neck cancer, Tarceva in pancreas cancer, and a VEGF receptor kinase inhibitor in hepatocellular carcinoma; and active participation in over 30 clinical trials run by collaborative groups such as the ANZ breast trials group, the Australasian Leukaemia and Lymphoma Group (ALLG), the Trans-Tasman Radiation Oncology Group (TROG) and the Australasian Gastrointestinal Trials Group (AGITG).

Cancer Vaccine Program

The Cancer Vaccine Program takes a comprehensive approach to developing cancer immunotherapeutics. The major strength of the group is this ability to take observations and opportunities from the clinic into the laboratory for further more detailed study, and then design further clinical protocols based on the findings.

In recent years the main focus has been the tumor antigen NY-ESO-1 which has been studied as a protein-based vaccine formulated with the adjuvant ISCOMATRIX™ in collaboration with CSL Ltd (Clinical Trial LUD 99-008). This study found that vaccination induced an integrated and broad based immune response that involved CD4+ and CD8+ T lymphocytes, as well as high titred antibodies. In an unplanned analysis of the patterns of disease relapse it was found that the vaccine appeared to prevent recurrence of melanoma in patients with disease that had been removed surgically but still carried a high risk of recurrence. This has prompted plans to perform a randomized clinical trial to formally the vaccine for its impact disease recurrence. This trial will be performed at major Australian and UK centers. Additionally, protocols have been developed that assess novel methods for enhancing immunity to cutaneous peptide vaccines, with GM-CSF and the Toll-like receptor (TLR)-7 ligand, imiquimod.

Cancer Vaccine Laboratory

The Cancer Vaccine Laboratory, under the joint leadership of Drs. Jonathan Cebon and Eugene Maraskowsky, is evaluating a variety of different dendritic cell subsets both generated *in vitro* or isolated from patients. The impact of exogenous factors on their ontogeny, activation and function has been investigated. The focus on dendritic cell biology has included studies into nucleotide receptors, cytokine production and antigen 'cross-presentation'. These have provided a crucial foundation for understanding the mechanism which underlies the immune response to the NY-ESO-1 vaccine. Furthermore, it has provided Dr. Ian Davis and his team with insights which serve as the basis for novel clinical approaches for dendritic cell immunotherapy. A regional Cancer Vaccine Collaborative has been established, in conjunction with Drs. Rod Dunbar and Mike Findlay in Auckland, New Zealand. Studies of HLA expression in cancer, with a particular focus on the impact of vaccination, have also been conducted in collaboration with the Victorian Transplantation and Immunogenetic Service.

Dendritic Cell Facility

The LICR Dendritic Cell Facility is a GMP-grade facility designed to support the production of dendritic cells for use in the Melbourne Branch Cancer Vaccine Program and is headed by Dr. Ian Davis. The first clinical trial (LUD00-021) was completed in 2003 and included six patients, where dendritic cells were mobilized into peripheral blood by treatment with Flt3L, harvested by leukapheresis, processed in the Facility and administered back into the individual patients. This trial showed that the system used to produce dendritic cells for vaccination was feasible, safe and able to induce strong immune responses in most of the participants. The Facility has also successfully used a new method that allows positive selection of dendritic cells from peripheral blood without Flt3L mobilization. This alternative method will be used for the next trial (LUD2003-003), which will also build on previous experience using the NY-ESO-1 ISCOMATRIX® vaccine, and is expected to begin in mid 2004.

T Cell Laboratory

The research of Dr. Weisan Chen and his T Cell Laboratory has focused on monitoring antigen-specific T cell responses from cancer clinical trial patients in addition to gaining a better understanding of T cell biology in experimental systems. The T Cell Laboratory has continued monitoring HLA-A2 negative patients from trial LUD99-008 for T cell responses, and used combined NY-ESO-1 18-mer and 13-mer synthetic peptide stimulation strategy, which has been proven to be very successful and efficient. Monitoring for trial LUD00-021 has been completed, and commenced for trials LUD00-05 and LUD01-017. During the year many observations have been made including vaccine-induced immune response spreading to other tumor antigen(s); discovery of the first immunodominant HLA-B-restricted T cell epitope and special focus T cell responses to specific region of NY-ESO-1 CT antigen.

Murine T cell research has shown that immunodominance in a secondary response is also affected by multiple factors including immunodomination or antigen presenting cell level competition, the efficiency of cross-presentation for a given T cell epitope and differential antigen presentation. The research conducted in the laboratory has also demonstrated that cross-priming of T cells is a very robust phenomenon and maintains the normal overall immunodominance hierarchy. Using transgenic, knock out animals and influenza model system, the contribution of immunoproteasome to CD8+ T cell development, differentiation and memory induction; the mechanisms of cross-priming; the role of post-translational modification in antigen processing/presentation, and how to enhance memory T cell generation are currently being investigated.

Publications

Primary Research Articles

1. Bhardwaj S., Roy H., Gruchala M., VIIta H., Kholová I., Kokina I., Achen M.G., Stacker S.A., Hedman M., Alitalo K., Ylä-Herttuala S. Angiogenic responses of vascular endothelial growth factors in periadventitial tissue. Human Gene Therapy (2003) 14 (15):1451-62.
2. Cebon J., Jager E., Shackleton M., Gibbs P., Davis I.D., Hopkins W., Gibbs S, Chen Q., Karbach J, Jackson H., MacGregor D.P., Sturrock S., Vaughan H.A., Maraskovsky E., Neumann A, Hoffman E., and Knuth A. Two phase I studies of low dose recombinant human IL-12 with Melan-A and influenza peptides in subjects with advanced malignant melanoma. Cancer Immunity (2003) 3:7
3. Daniels C.B., Lewis B.C., Tsopelas C., Munns S.L., Orgeig S., Baldwin M.E., Stacker S.A., Achen M.G., Chatterton B.E., Cooter R.D. Regenerating lizard tails: a new model for investigating lymphangiogenesis. FASEB Journal (2003) 17 (3):479-81.
4. Dauer M., Obermaier B., Herten J., Pohl K., Rothenfusser S., Schnurr M., Endress S., Eigler A. Mature dendritic cells derived from human monocytes within 48 hours: a novel strategy for dendritic cell differentiation from blood precursors. Journal of Immunology (2003) 170 (8):4069-76.
5. Dauer M., Pohl K., Obermaier B., Meskendahl T., Robe J., Schnurr M., Endres S., and Eigler A. Interferon-alpha disables dendritic cell precursors: dendritic cells derived from interferon-alpha-treated monocytes are defective in maturation and T-cell stimulation. Immunology (2003) 110 (1):38-47.
6. Dyson J.M., Munday A.D., Kong A.M., Huysmans R.D., Matzaris M., Layton M.J., Nandurkar H.H., Berndt M.C., Mitchell C.A. SHIP-2 forms a tetrameric complex with filamin, actin, and GPIb-IX-V: localization of SHIP-2 to the activated platelet actin cytoskeleton. Blood (2003) 102(3): 940-8.
7. Froomes P.R., Sachinidis JI, Ghabrial H., Tochondanguy H., Scott A., Ching M.S., Morgan D.J., Angus P.W. A novel method for determining hepatic sinusoidal oxygen permeability in the isolated perfused rat liver using [15O]O2. Nuclear Medicine and Biology (2003) 30 (2):93-100.
8. Garrett T.P.J., McKern N.M., Lou M.Z., Elleman T.C., Adams T.E., Lovrecz G.O., Kofler M, Jorissen R.N., Nice E.C., Burgess A.W., and Ward C.W. The crystal structure of a truncated ErbB2 ectodomain reveals an active conformation, poised to interact with other ErbB receptors. Molecular Cell (2003) 11 (2):495-505.
9. Gunawardana D.H., Basser R.L., Davis I.D., Cebon J., Mitchell P., Underhill C., Kilpatrick T.J., Reardon K., Green K., Bardy P., Amor P., Ng S., Nation R.L., Begley C.G. A phase I study of recombinant human leukemia inhibitory factor in patients with advanced cancer. Clinical Cancer Research (2003) 9(6):2056-65.
10. Hodder A.N., Drew D.R., Epa V.C., Delorenzi M., Bourgon R., Miller S.K., Moritz R.L.,
11. Frecklington D.F., Simpson R.J., Speed T.P., Pike R.N., Crabb B.S. Enzymic, phylogenetic, and structural characterization of the unusual papain-like protease domain of Plasmodium falciparum SERA5. Journal of Biological Chemistry (2003) 278(48):48169-77.
12. House C.M., Frew I.J., Huang H-L., Wiche G., Traficante N., Nice E.C., Catimel B., and Bowtell D.D.L. A binding motif for Siah ubiquitin ligase. Proceedings of the National Academy of Sciences USA (2003) 100(6):3101-6.
13. Jefford M., Schnurr M., Toy T, Masterman K-A., Shin A., Beecroft T., Tai T.Y., Shortman K.,

Shackleton M., Davis I.D., Parente P., Luft T., Chen W., Cebon J., Maraskovsky E. Functional comparison of DCs generated in vivo with Flt3 ligand or in vitro from blood monocytes: differential regulation of function by specific classes of physiologic stimuli. Blood (2003) 102(5):1753-63.

14. Jefford M., Stockler M.R., Tattersall M.H.N. Outcomes research: what is it and why does it matter?. Internal Medicine Journal (2003) 33(3): 110-18.

15. Johns T.G., Luwor R.B., Murone C., Walker F.E., Weinstock J., Vitali A.A., Perera R.M., Jungbluth A.A., Stockert E., Old L.J., Nice E.C., Burgess A.W., Scott A.M. Antitumor efficacy of cytotoxic drugs and the monoclonal antibody 806 is enhanced by the EGF receptor inhibitor AG1478. Proceedings of the National Academy of Sciences USA (2003) 100(26):15871-6.

16. Jorissen R.N., Walker F., Pouliot N, Garrett T.P.J., Ward C.W., Burgess A.W. Epidermal growth factor receptor: mechanisms of activation and signalling. Experimental Cell Research (2003) 284: 31-53.

17. Jungbluth A.A., Stockert E., Huang H.J.S., Collins V.P., Coplan K.A., Iversen K., Kolb D., Johns T.J., Scott A.M., Cavenee W.K., Old L.J. A monoclonal antibody recognizing human cancers with amplification/overexpression of the human epidermal growth factor receptor. Proceedings of the National Academy of Sciences USA (2003) 100(2):639-44.

18. Kaczmarczyk S.J., Andrikopoulos S., Favaloro J., Domenighetti A.A., Dunn A., Ernst M., Grail D., Fodero-Tavoletti M., Huggins C.E., Delbridge L.M., Zajac J.D., Proietto J. Threshold effects of glucose transporter-4 (GLUT4) deficiency on cardiac glucose uptake and development of hypertrophy. Journal of Molecular Endocrinology (2003) 31(3):449-59.

19. Kapp E.A., Schutz F, Reid G.E, Eddes J.S., Moritz R.L., O'Hair R.A.J., Speed T.J., Simpson R.J. Mining a tandem mass spectrometry database to determine the trends and global factors influencing peptide fragmentation. Analytical Chemistry (2003) 75(22):6251-64.

20. Lan H.Y., Mu W., Tomita N., Huang X.R., Li J.H., Zhu H-J., Morishita R., Johnson R.J. Inhibition of renal fibrosis by gene transfer of inducible Smad7 using ultrasound-microbubble system in rat UUO model. Journal of the American Society of Nephrology (2003) 14(6):1535-48.

21. Li J.H., Huang X.R., Zhu H-J., Johnstone R.W., and Lan H.Y. Role of TGF-beta signaling in extracellular matrix production under high glucose conditions. Kidney International (2003) 63(6):2010-19.

22. Liu Z., Panousis C., Smyth F.E., Murphy R., Wirth V., Cartwright G.A., Johns T.G., Scott A.M. Generation of anti-idiotype antibodies for application in clinical immunotherapy laboratory analyses. Hybridoma and Hybridomics (2003) 22 (4): 219-28.

23. McColl B.K., Baldwin M.E., Roufail S., Freeman C., Moritz R.L., Simpson R.J., Alitalo K., Stacker S.A., Achen M.G. Plasmin activates the lymphangiongenic growth factors VEGF-C and VEGF-D. Journal of Experimental Medicine (2003) 198(6):863-68.

24. Mitchell P., Basser R., Chipman M., Begley G., Grigg A., Cebon J., Davis I., Zalcberg J.R., Ng S., Appia F., Green M. A phase I dose-escalation study of docetaxel with granulocyte colony-stimulating factor support in patients with solid tumours. Annals of Oncology (2003) 14(5):788-94.

25. Mitchell P., Lee F-T., Hall C, Rigopoulos A., Smyth F.E., Hekman A-M., van Schijndel G. M., Powles R., Brechbiel M.W., Scott A.M. Targeting primary human Ph(+) B-cell precursor leukemia-engrafted SCID mice using radiolabeled anti-CD19 monoclonal antibodies. Journal of Nuclear Medicine (2003) 44(7):1105-12.

26. Nandurkar H.H., Layton M.J., Laporte J., Selan C., Corcoran L., Caldwell K.K., Majerus P.W., Mitchell C.A. Identification of myotubularin as the lipid phosphatase catalytic subunit associated with the

3-phosphatase adapter protein, 3-PAP. Proceedings of the National Academy of Sciences USA (2003) 100(15):8660-5.

27. Power B., Doughty L., Shapira D.M., Burns J.E., Bayly A.M., Caine J.M., Liu Z., Scott A.M., Hudson P.J., Kortt A.A. Noncovalent scFv multimers of tumor-targeting anti-Lewis(y) hu3S193 humanized antibody. Protein Science (2003) 12(4):734-47.
28. Rissanen T.T., Markkanen J.E., Gruchala M., Heikura T., Puranen A., Kettunen M.I., Kholová I., Kauppinen R.A., Achen M.G., Stacker S.A., Alitalo K., Ylä-Herttuala S. VEGF-D is the strongest angiogenesic and lymphangiogenesic effector among VEGFs delivered into skeletal muscle via adenoviruses. Circulation Research (2003) 92:1098-1106.
29. Rutanen J., Leppänen P., Tuomisto T.T., Rissanen T.T., Hiltunen M.O., Vajanto I., Niemi M., Häkkinen T., Karkola K., Boone T., Achen M.G., Alitalo K., Ylä-Herttuala S. Vascular endothelial growth factor-D expression in human atherosclerotic lesions. Cardiovascular Research (2003) 59: 971-979.
30. Schnurr M., Toy T., Stoitzner P., Cameron P., Shin A., Beecroft T., Davis I.D.,
31. Cebon J., Maraskovsky E. ATP gradients inhibit the migratory capacity of specific human dendritic cell types: implications for P2Y11 receptor signaling. Blood (2003) 102(2):613-20.
32. Schutz F., Kapp E.A., Simpson R.J., Speed T.J. Deriving statistical models for predicting peptide tandem MS product ion intensities. Biochemical Society Transactions (2003) 31(6):1479-83.
33. Scott A.M., Wiseman G., Welt S., Adjei A., Lee F-T., Hopkins W., Divgi C.R., Hanson L.H., Mitchell P., Gansen D.N., Larson S.M., Ingle J.N., Hoffman E.W., Tanswell P., Ritter G., Cohen L.S., Bette P., Arvey L, Amelsburg A., Vlock D, Rettig W.J., Old L.J. A Phase I Dose-Escalation Study of Sibrotuzumab in Patients with Advanced or Metastatic Fibroblast Activation Protein-positive Cancer. Clinical Cancer Research (2003) 9(5):1639-47.
34. Sitnicka E., Brakebusch C., Martesson I-L., Svensson M., Agace W.W., Sigvardsson M., Buza-Vidas N., Bryder D., Cilio C.M., Ahlenius H., Maraskovsky E., Peschon J.J., Jacobsen S.E.W. Complementary signaling through flt3 and interleukin-7 receptor alpha is indispensable for fetal and adult B cell genesis. Journal of Experimental Medicine (2003) 198(10):1495-1506.
35. Solomon B., Hagekyriakou J., Trivett M.K., Stacker S.A., McColl B.K., Cullinane C. EGFR blockade with ZD1839 ("Iressa") potentiates the antitumor effects of single and multiple fractions of ionizing radiation in human A431 squamous cell carcinoma Epidermal growth factor receptor. International Journal of Radiation Oncology, Biology, Physics (2003) 55(3):713-23.
36. Stenvers K.L., Tursky M.L., Harder K.W., Kountouri N., Amatayakul-Chantler S., Grail D., Small C., Weinberg R.A., Sizeland A.M., Zhu H-J. Heart and liver defects and reduced transforming growth factor beta2 sensitivity in transforming growth factor beta type III receptor-deficient embryos. Molecular and Cellular Biology (2003) 23(12):4371-85.
37. Tahtis K., Lee F.T., Wheatley J.M., Garin-Chesa P., Park J.E., Smyth F.E., Obata Y., Stockert E., Hall C.M., Old L.J., Rettig W.J., Scott A.M. Expression and targeting of human fibroblast activation protein in a human skin/severe combined immunodeficient mouse breast cancer xenograft model. Molecular Cancer Therapy (2003) 2(8):729-37.
38. Ueda N., Wise L.M., Stacker S.A., Fleming S.B., Mercer A.A. Pseudocowpox virus encodes a homolog of vascular endothelial growth factor. Virology (2003) 305(2):298-309.
39. Van Niel G., Mallegol J., Bevilacqua C., Candalh C., Brugière S., Tomaskovic-Crook E., Heath J.K., Cerf-Bensussan N., Heyman M. Intestinal epithelial exosomes carry MHC class II/peptides able to

inform the immune system in mice. Gut (2003) 52:1690-7.

40. Ward A.C., McPhee D.O., Condron M.M., Varma S., Cody S.H., Onnebo S. M. N., Paw B.H., Zon L.I., Lieschke G.J. The zebrafish spi1 promoter drives myeloid-specific expression in stable transgenic fish. Blood (2003) 102(9):3238-40.
41. Wise L.M., Ueda N., Dryden N.H. , Fleming S.B., Caesar C., Roufail S., Achen M.G., Stacker S.A., Mercer A.A. Viral Vascular Endothelial Growth Factors Vary Extensively in Amino Acid Sequence, Receptor-binding Specificities, and the Ability to Induce Vascular Permeability yet Are Uniformly Active Mitogens. Journal of Biological Chemistry (2003) 278(39):38004-14.
42. Zamurs L., Pouliot N., Gibson P.R., Nice E.C. Strategies for the purification of laminin-10 for studies on colon cancer metastasis. Biomedical Chromatography (2003) 17(2-3):201-11.
43. Zhu H-J., Iaria J., Orchard S., Walker F., Burgess A.W. Epidermal growth factor receptor: association of extracellular domain negatively regulates intracellular kinase activation in the absence of ligand. Growth Factors (2003) 21(1):15-30.

Reviews / Book Chapters / Commentaries

1. Achen M.G., Stacker S.A. Vascular endothelial growth factor-D. Encyclopedia of hormones. pp. 559-564, Publisher: Elsevier Editors: Henry, H. and Norman, A.W., 2003.
2. Burgess A.W., Cho H.S., Eigenbrot C., Ferguson K.M., Garrett T.P., Leahy D.J., Lemmon M.A., Sliwkowski M.X., Ward C.W., Yokoyama S. An open-and-shut case? Recent insights into the activation of EGF/ErbB receptors. Molecular Cell (2003) 12(3):541-52.
3. Davis I.D., Jefford M., Parente P., Cebon J. Rational approaches to human cancer immunotherapy. Journal of Leukocyte Biology (2003) 73(1):3-29.
4. Davis I.D., Jefford M., Parente P., Cebon J. Rational approaches to human cancer immunotherapy. Journal of Leukocyte Biology (2003) 73(1):3-29.
5. Lee F.T., Scott A.M. Immuno-PET for tumor targeting. Journal of Nuclear Medicine (2003) 44(8):1282-3.
6. Maruta H., Nheu T.V., He H., Hirokawa Y. Rho family-associated kinases PAK1 and rock. Progress in Cell Cycle Research (2003) 5:203-10.
7. Wong P.K., Campbell I.K., Egan P.J., Ernst M., Wicks I.P. The role of the interleukin-6 family of cytokines in inflammatory arthritis and bone turnover. Arthritis and Rheumatology (2003) 48(5):1177-89.

LUDWIG INSTITUTE FOR CANCER RESEARCH

New York Branch
of Human Cancer Immunology

New York, USA

Staff List

Old, Lloyd J. *Director, Member*
Ritter, Gerd *Associate Director, Associate Member*
Welt, Sydney *Associate Member (until July)*
Gnjatic, Sacha *Assistant Member*
Gure, Ali *Assistant Member*
Jungbluth, Achim *Assistant Member*
Scanlan, Matthew *Assistant Member*
Lee, Frank K.N. *Assistant Investigator, Clinical Trials*
Lee, Sang-Yull *Assistant Investigator, Clinical Trials*
O'donoghue, Joseph *Assistant Investigator Clinical Trials*
Vannelli, Todd *Assistant Investigator, Clinical Trials*
Sharma, Padmanee *Clinical Fellow*
Atanackovic, Djordje *Postdoctoral Fellow (until July)*
Matsuo, Mitsutoshi *Postdoctoral Fellow (until August)*
Sato, Eiichi *Postdoctoral Fellow*
Nishikawa, Hiroyoshi *Research Fellow*
Cohen, Leonard *Research Assistant*
Fortunato, Sheila *Research Assistant*
Williams, Clarence *Research Assistant*
Yin, Beatrice *Research Assistant*
Chua, Ramon *Senior Research Technician (from July)*
Frosina, Denise *Senior Research Technician*
Ritter, Erika *Senior Research Technician*
Santiago, Darren *Senior Research Technician*
Eastlake- Wade, Susannah *Research Technician*
Kelly, David *Research Technician*
Krapivinisky, Evgeniya *Research Technician (from December)*
Murray, Anne *Research Technician*
Venditti, Charis *Research Technician*
Villalobos, Cristina *Research Technician (from December)*

LICR Associates

Chen, Yao-T. *Pathologist (Cornell Medical School. USA)*
Valmori, Danila *Associate Professor of Medical Science and Surgical Science, Columbia-Presbyterian Medical School*
Ayyoub, Maha *Assistant Professor of Medical Science and Surgical Science, Columbia-Presbyterian Medical School*

Secretariat

Rios, Jannette *Research Secretary*

Branch Director's Report

The major objective of the New York Branch is the identification and characterization of suitable antigenic targets for antibody-based and vaccine-based immunotherapies of human cancer. The New York Branch, the smallest of the ten LICR Branches (an average of 26 staff members) utilizes a fully integrated and multidisciplinary approach, with the individual research groups - Antigen Discovery, Biochemistry, Pathology/Morphology, T Cell Immunology and Serological Typing, and Clinical Trials Groups - operating in close collaboration with each other. The highly coordinated activities of the research groups, and their interactions with selected external academic and industrial research partners, are designed such that new discoveries are rapidly transferred into early phase clinical evaluation in cancer patients. The New York Branch is an integral part of the Institute's Clinical Trials Program for immunotherapy of cancer with monoclonal antibodies and vaccines.

As part of LICR's Targeted Antibody Program a series of cell surface antigens have been identified and characterized over the past decade by the New York Branch. Six antigens have been selected for early phase clinical evaluation, and clinical trials with monoclonal antibodies (mAbs) against those antigens are ongoing, or are soon to commence, within the LICR Clinical Trials Program. Currently, a major focus is on the generation and development of non-immunogenic human or chimeric antibody constructs for clinical evaluation. The protocols developed for the clinical study of the newer generation of antibody constructs are designed to provide the following information: pharmacokinetic and quantitative biodistribution properties, tumor localization and imaging characteristics, immunogenicity, and therapeutic activity of antibody alone and in combination with, or as a carrier of, cytotoxic agents.

As part of LICR's Cancer Vaccine Program, the discovery and characterization of new targets is a continuing objective of the Branch with particular emphasis on gene products having characteristics of cancer /testis (CT) antigens. In addition, the Branch's research groups provide critical laboratory support to clinical investigators within the Branch or affiliated with the Institute's Clinical Trials Program in order to monitor, optimize, and characterize the immune responses to cancer vaccines. The Antigen Discovery and Pathology Groups perform the antigen typing of tumors to determine the potential eligibility of a patient to participate in clinical trials, the Biochemistry Group generates monitoring agents, and the T Cell Immunology and Serological Typing Group monitors humoral and cellular immune responses of patients enrolled in the cancer vaccine trials.

The Branch is involved in the immunological monitoring for nearly all clinical trials performed under the auspices of the Cancer Vaccine Collaborative (CVC) which was established in partnership with the Cancer Research Institute (New York, USA) in 2002. Furthermore, the Branch serves as immunological reference laboratory for the LICR's Clinical Trials Program, as well as a training laboratory for immunological monitoring and molecular typing techniques required for the LICR's Cancer Vaccine, and Targeted Antibody Programs.

Lloyd J. Old & Gerd Ritter

Research Report

Antibody Development and Antibody-based Immunotherapy

Over the past decade, a series of cell surface antigens have been identified by the New York Branch. These antigens are being explored as antigenic targets on cancer cells for antibody-based immunotherapy within the LICR Clinical Trials Program. They include: A33, a 43 K glycoprotein with selective expression in normal and malignant epithelium of the gastrointestinal tract (recognized by huAb A33); G250, a glycoprotein expressed by a high percentage of renal cancers (recognized by chAb G250); Le^y, an oligosaccharide epitope expressed on glycolipids and glycoproteins by a wide range of epithelial cancers (recognized by huAb 3S193); GD3, a ganglioside with high expression in melanoma and other neuroectodermal tumors (recognized by chAb KM871); FAP-α, a 95 K glycoprotein strongly expressed in the stromal fibroblasts of epithelial cancers (recognized by mAb F19 and huAbBIBH1); and a novel aberrant form of the epidermal growth factor receptor (EGFR), recognized by mAb 806, which is expressed by a proportion of brain cancers and other tumor types.

Mouse mAb 806 was initially raised, as part of a collaboration with the San Diego Branch, to recognize a truncated (de2-7 deletion) form of EGFR found predominantly in glioblastoma. The de2-7 deletion has also been reported to be found in non-CNS malignancies such as breast and lung carcinomas, and prostate cancers, but the group's findings do not support the presence of the de2-7 deletion mutation in tumors other than glioblastoma. Analysis of mAb 806 gave rise to the surprising finding that mAb 806 showed specificity for overexpressed/amplified EGFR. In a panel of normal tissues mAb 806 showed little or no reactivity with tissues known to express wildtype levels of EGFR, including liver and skin. In contrast, tumors known to overexpress EGFR, e.g. glioblastoma, squamous cell carcinoma of the lung, and head and neck, urinary, and bladder cancers showed strong reactivity by immunohistochemistry. mAb 806 is now undergoing further preclinical characterization at the Melbourne Branch, and a chimeric version of this antibody generated at the Institute's Biological Production Facility in Melbourne is expected to then enter early phase clinical trials, as part of the LICR Clinical Trials Program, in 2004.

Based on evidence that a radiolabeled mouse A33 antibody sensitized patients' tumors to follow-up chemotherapy, a Phase I study of escalating doses of humanized A33 + BOF-Strep chemotherapy was recently completed. Patients with advanced colorectal cancer resistant to at least two chemotherapeutic regimens were entered into four doses levels (5, 10, 25, and 40 mg/m2). The only adverse events, such as neutropenia, and thrombocytopenia, were related to the chemotherapy. No significant toxicities specifically related to the combination of immunochemotherapy were identified. Of the 11 patients completing one treatment cycle, three had radiographic evidence of partial responses, one had a mixed response, and one patient had stable disease for nine months. Still, seven of twelve patients developed anti-human A33 activity (HAHA), according to a novel biosensor-based analysis, specifically developed to allow unambiguous measurement of human anti-human antibodies (HAHA). In collaboration with Dr. Christoph Renner (Homburg Affiliate Center, Germany), several new human or chimeric IgG1 A33 antibodies have been generated to overcome the high frequency of HAHA induction of the first generation antibody, which accelerated antibody serum clearance, blocked tumor targeting and consequently abrogated tumor response. In order to select the best candidate for immunotherapy the genetically engineered antibodies were produced in mammalian expression systems in collaboration with Dr. Florian Wurm (Lausanne Affiliate Center, Switzerland). Antibodies are being subjected at the New York Branch to a detailed analysis, including specificity, cell-surface-reactivity, binding affinity, antigenicity and immunological effector functions.

A new Positron Emission Tomography (PET) Program has been established, as part of the Targeted Antibody Program, at the Branch's host institution, the Memorial Sloan- Kettering Cancer Center (MSKCC) with Dr. Chaitanya Divgi (New York (MSKCC) Affiliate Center), and Drs. Steve Larsen and

Ron Finn from MSKCC. This program will allow quantitative assessment of radiolabeled antibody tumor targeting in real time. The ultimate goal is to replace invasive biopsies to measure tumor antibody uptake. The initial focus is on huAbA33, hu3S193, and chG250 and a first clinical trial has been initiated with 124-I-labeled huAbA33 in patients with colorectal cancer. To study if a smaller size antibody construct may improve the ration of tumor to non-tumor targeting a clinical-grade scFv A33 construct is being produced at the LICR Bioprocess Laboratory at Cornell University, Ithaca and is expected to enter clinical testing in 2004.

Antigen Discovery and Cancer Vaccines

The Antigen Discovery Group has identified a large array of distinct antigens using serological screening of cDNA expression cloning (SEREX) (for details see the LICR Cancer Immunome Database). These antigens fall into different categories including differentiation antigens, splice variants, mutational antigens, overexpressed/amplified antigens, and CT antigens. A new technique based on SEREX, SADA (serum antibody detection array), has been developed for sero-epidemiological analyses, and was used to investigate the serological responses of normal blood donors, and colon or breast cancer patients, to a panel of 278 tumor antigens. Such a method is not only useful for target identification, but may also be of diagnostic and prognostic significance. The antigens recognized by sera from patients with breast or colon cancer included several members of the CT antigen family and other tissue-restricted gene products. One CT antigen in particular, NY-CO-58, was highly overexpressed in 9/9 cases of colon cancer. Another technique to identify potential antigens, RAYS (recombinant antigen expression on yeast surface) is being developed in collaboration with Dr. Renner (Homburg Affiliate Center, Germany).

Identification of novel CT antigens by bioinformatic analysis, a strategy initiated on 2002, is ongoing. Two methods were used, one based on comparison of expressed sequence tags (ESTs) in GenBank, the other based on analysis of data derived from massively parallel signature sequencing (MPSS), a recently developed technique for gene expression profiling. Combining these two approaches and sieving through >1000 genes, ~150 CT candidate genes were identified; their mRNA expression in normal and cancer cells were then evaluated by RT-PCR. Approximately 20 genes were confirmed to have a CT or CT-like expression pattern, including one that belongs to a novel multigene family on chromosome X. Characterization of this and a few other novel CT antigen genes is ongoing.

Another multigene family of CT antigens undergoing characterization is the SSX family. The SSX genes are located on the X chromosome and encode a family of highly homologous nuclear proteins. Thus far, nine (SSX-1 to –9) genes have been described and characterized. Similar to other CT antigens, the expression of most of the SSX genes, including SSX-1 to -5 and SSX-7, is developmentally regulated, being mostly restricted to gametogenic cells but silent in adult normal tissues. Frequent expression of at least one SSX family member is found in several tumor types including head and neck cancer (75%), ovarian cancer (50%), malignant melanoma (43%) and sarcoma (42%). Therefore, antigens of the SSX family are targets of great interest for immunotherapy of cancer. To implement clinical trials SSX-based cancer vaccines, the analysis of natural T cell responses specific for SSX-2, one of the most frequently expressed SSX family members, has been undertaken. An SSX-2 derived epitope located in the 41-49 region of the protein and recognized by tumor-reactive CD8+ T lymphocytes in association with the frequently expressed MHC Class I allele HLA-A2 has been previously identified. High affinity SSX-2 41-49 specific CTL were frequently and exclusively retrieved from HLA-A2+ melanoma patients bearing antigen expressing tumors. During this last year, efforts have been focused on developing methods to analyze SSX-2 specific CD4+ T cells. The group has developed several epitope identification approaches based on the use of synthetic peptides spanning the protein sequence and/or synthetic recombinant proteins in combination with cytokine secretion guided flow cytometry cell sorting of antigen specific CD4+ T cells. These studies have thus far identified two SSX-2 derived CD4+ T cell epitopes. One of these was detected in the majority of SSX-2 expressing melanoma patients analyzed and among tumor infiltrating lymphocytes, indicating

that this sequence may play a dominant role in the induction of cellular CD4+ T cell responses to SSX-2. No specific responses to either epitope were detectable in healthy donors. Interestingly, neither epitope appeared to be presented on the surface of tumor cells at levels sufficient to allow their direct recognition by specific CD4+ T cells. In contrast, they were efficiently processed and presented by autologous dendritic cells upon incubation with SSX-2 recombinant protein or with lysates from SSX-2 expressing tumors. These results support the concept that processing by professional APC is the main pathway through which CD4+ T cell immunoresponse to tumor antigens occurs in vivo. In conclusion, the data confirm the spontaneous immunogenicity of SSX-2 and will be instrumental to the implementation of vaccination trials with SSX antigens in melanoma and other SSX-expressing cancer patients.

For an antigen to serve as a potential target for immunotherapy, it is critical to obtain information on its tissue expression not only on the RNA level, but also on the protein level. Novel mAbs are being prepared for newly discovered antigens to use in comprehensive immunohistochemical expression analyses performed by the Pathology/Morphology Group. The mAbs are prepared by the Branch's Biochemistry Group, or in collaboration with Dr. Boquan Jin (Xi'an, China) and/or Dr. Ivan Gout (Kiev, Ukraine) through the James R. Kerr Program. Extensive immunohistochemical analyses of a range of CT antigens, including MAGE-A1, MAGE-A3, NY-ESO-1, and CT7 has been carried out in a large panel of normal and malignant tissues. Normal tissue expression was restricted to cells undergoing early phases of spermatogenesis. A proportion (20-40%) of a wide range of different tumor types express CT antigens in a heterogeneous/homogeneous fashion. Synovial cell sarcoma showed an unusual high frequency (>80%) of expression with NY-ESO-1 expressed highly homogeneously.

NY-ESO-1, which was discovered in the Branch using SEREX, represents a particularly promising new target antigen for vaccine development, because of its capacity to simultaneously induce both humoral and cellular immune responses in a proportion of cancer patients having NY-ESO-1-expressing tumors. A major proportion of the Branch's research activities on cancer vaccines is focused on this particular antigen, and several clinical vaccine trials targeting the NY-ESO-1 antigen have now been initiated. Besides safety, the main objective of those trials is to assess NY-ESO-1-specific immunity following vaccination with NY-ESO-1. For this purpose, novel types of assays have been developed, allowing sensitive and specific monitoring of humoral and both CD4 and CD8 cellular immune responses to NY-ESO-1. These developments in the monitoring techniques, especially for CD4 T cells, help complete the picture of naturally occurring immune responses to NY-ESO-1 in cancer patients and provide the basis for understanding the nature of responses achieved by immunization. Continued close interactions with Dr. Elke Jäger (Frankfurt Clinical Trials Center) and Dr. Alexander Knuth (Zürich Clinical Trials Center) have expanded the scope of vaccine approaches to target cancer using NY-ESO-1 as a model antigen.

The mechanism of cross-presentation of exogenous antigens by antigen presenting cells is also being studied by the T cell Group. In collaboration with Dr. Ira Mellman (New Haven Affiliate Center, USA), immune complexes of NY-ESO-1 were studied for their capacity to be presented to T cells by various dendritic cell populations. Alternatively, NY-ESO-1 peptides of 30 amino acids were found to be efficiently taken up and processed by a wide variety of non-professional antigen presenting cells, giving rise to the concept of using overlapping long peptides of NY-ESO-1 instead of the whole protein as immunogen in cancer vaccines.

The role of CD1d restricted NKT cells on the quality and size of the adaptive immune response to soluble antigens was studied in collaboration with Dr. Vincenzo Cerundolo (Oxford Affiliate Center, UK) and Dr. Richard Schmidt (Konstanz Affiliate Center, Germany). We found that NKT cells stimulated with the synthetic glycosphingolipid alpha-GalCer significantly enhanced both CD4+ and CD8+ T cell responses to soluble antigens through a direct interaction with dendritic cells in mice. The enhancing effect of NKT cell stimulation did require CD40 signaling but does not require IFN-gamma. This mechanism will be explored in future clinical trials to modulate immune responses induced by vaccination with cancer antigens.

In collaboration with Dr. Nasser Altorki at Weill Medical College at Cornell University, several LICR-sponsored clinical vaccine trials are ongoing in patients with lung cancer. In one of these trials, cancer patients were vaccinated with HLA-A2-restricted peptides 157-165, and 157-167 from NY-ESO-1. Analysis of CD8+ T cell responses elicited by the vaccine showed that an unexpected, cryptic epitope had a dominant reactivity, potentially skewing T cells away from tumor recognition, prompting the removal of peptide 157-167 from clinical studies. A new vaccine study with peptide 157-165 from NY-ESO-1 in incomplete Freund's adjuvant and CpG was initiated in the hope of focusing on stronger tumor specific responses. In addition, a clinical vaccine trial with peptide 157-165 from NY-ESO-1 in incomplete Freund's adjuvant has been initiated at Memorial Sloan-Kettering Cancer Center with Dr. Jakob Dupont in patients with ovarian cancer.

In preparation for vaccine trials in patients with ovarian cancer expression of NY-ESO-1 and LAGE-1 was analyzed by RT-PCR and immunohistochemistry in collaboration with Dr. Kunle Odunsi at Roswell Park Cancer Institute in Buffalo. 43% of 190 ovarian tumor specimens tested expressed NY-ESO-1 and 21% expressed LAGE-1. Co-expression of both antigens was detected in 11% of the samples tested. Thirty percent of patients with NY-ESO-1 or LAGE-1 positive tumors were found to have antibody responses to NY-ESO-1 and LAGE-1. Based on these findings, a study has been initiated at Roswell Park with peptide 157-170 from NY-ESO-1 (also present in LAGE-1), to elicit CD4 and potentially CD8 T cell responses in ovarian cancer patients with tumors expressing NY-ESO-1 or LAGE-1.

In preparation for a vaccine trial in patients with bladder cancer, the frequency of expression of NY-ESO-1, and a closely related CT antigen, LAGE-1, have been studied in over 180 patients with transitional cell carcinomas (TCC). Over 30% of tumors expressed these antigens. Based on these findings, a new vaccine trial has been initiated at Memorial Sloan-Kettering Cancer Center to assess whether immunization with NY-ESO-1 protein plus adjuvant BCG can elicit or augment humoral and cellular immune responses in patients at high risk of relapse after cystectomy.

MAGE-3 is the most commonly expressed cancer testis antigen and thus represents a prime target for cancer vaccines, despite infrequent natural occurrence of MAGE-3-specific immune responses in vivo. In a clinical trial with Dr. Altorki, patients with non-small cell lung cancer patients were vaccinated with full-length MAGE-3 protein, in the presence or absence of saponin-based adjuvant AS02B. Of nine patients receiving Mage-3 protein without adjuvant, three developed marginal Ab titers and another one had a CD8(+) T cell response to HLA-A2-restricted peptide MAGE-3 271-279. In contrast, of eight patients vaccinated with MAGE-3 protein and adjuvant, seven developed high-titered Abs to MAGE-3, and four had a strong concomitant CD4(+) T cell response to HLA-DP4-restricted peptide 243-258. One patient simultaneously developed CD8(+) T cells to HLA-A1-restricted peptide 168-176. The novel monitoring methodology used in this MAGE-3 study establishes that protein vaccination induces clear CD4(+) T cell responses that correlate with Ab production. This development provides the framework for further evaluating integrated immune responses in vaccine settings and for optimizing these responses for clinical benefit

Publications

Primary Research Articles

1. Atanackovic D., Matsuo M., Ritter E., Mazzara G., Ritter G., Jaeger E., Knuth A., Old L.J., Gnjatic S. Monitoring CD4+ T cell against viral and tumor antigens using T cells as novel target APC. Journal of Immunological Methods (2003) 278(1-2):57-66.
2. Ayyoub M., Brehm M., Metthez G., Talbot S., Dutoit V., Taub R.N., Keohan M.L., Gure A.O., Chen Y-T., Williamson B., Jungbluth A.A., Old L.J., Hesdorffer C.S., Valmori, D. SSX antigens as tumor vaccine targets in human sarcoma.. Cancer Immunity (2003) 3:13.
3. Chen Y-T., Alpen B., Ono T., Gure A.O., Scanlan M.J., Biggs III W.H., Arden K., Nakayama E., Old L.J. Identification and characterization of mouse SSX genes: a multigene family on X chromosome with restricted cancer/testis expression. Genomics (2003) 82(6):628-36.
4. Deckert M.P., Renner C., Cohen L.S., Jungbluth A., Ritter G., Bertino J.R., Old L.J., Welt S. A33scFv-Cytosine deaminase: A recombinant protein construct for antibody-directed enzyme-prodrug therapy. British Journal of Cancer (2003) 88(6):937-9.
5. Dhodapkar M.V., Osman K., Teruya-Feldstein J., Fillippa D., Hedvat C., Iversen K., Kolb D., Geller M.D., Comenzo R.L., Coplan K., Chen Y-T., Jungbluth A.A. Expression of cancer/testis (CT) antigens MAGE-A1, MAGE-A3, MAGE-A4, CT-7, and NY-ESO-1 in malignant gammopathies is heterogeneous and correlates with site, stage and risk status of disease. Cancer Immunity (2003) 3:9.
6. Gnjatic S., Atanackovic D., Matsuo M., Jaeger E., Chen Y-T., Lee S.Y., Valmori D., Ritter G., Knuth A., Old L.J. Cross-presentation of HLA class I epitopes from exogenous NY-ESO-1 polypeptides by non-professional antigen presenting cells. Journal Immunology (2003) 170(3): 1191-6.
7. Gnjatic S., Atanackovic D., Jager E., Matsuo M., Selvakumar A., Altorki N.K., Maki R.G., Dupont B., Ritter G., Chen Y-T., Knuth A., Old L.J. Survey of naturally occurring CD4+ T cell responses against NY-ESO-1 in cancer patients: Correlation with antibody responses. Proceedings of the National Academy of Sciences USA (2003) 100(15):8862-7.
8. Hermans I.F., Silk J.D., Gileadi U., Salio M., Mathew B., Ritter G., Schmidt R., Harris A.L., Old L., Cerundolo V. NKT Cells Enhance CD4(+) and CD8(+) T Cell Responses to Soluble Antigen In Vivo through Direct Interaction with Dendritic Cells. Journal Immunology (2003) 171(10):5140-5147.
9. Johns T.G., Luwor R.B., Murone C., Walker F., Weinstock J., Vitali A.A., Perera R.M., Jungbluth A.A., Stockert E., Old L.J., Nice E.C., Burgess A.W., Scott A.M. Antitumor efficacy of cytotoxic drugs and the monoclonal antibody 806 is enhanced by the EGF receptor inhibitor AG1478. Proceedings of the National Academy of Sciences USA (2003) 100(26): 15871-6.
10. Jungbluth A.A., Stockert E., Su Huang HJ., Collins VP., Coplan K., Iversen K., Kolb D., Johns TG., Scott AM., Gullick WJ., Ritter G., Cohen L., Scanlan M.J., Cavenee W.K., Old L.J. A monoclonal antibody recognizing human cancers with amplification/overexpression of the human epidermal growth factor receptor. Proceedings of the National Academy of Sciences USA (2003) 100(2):639-44.
11. Lee S.Y., Obata Y., Yoshida M., Stockert E., Williamson B., Jungbluth A.A., Chen Y-T., Old L.J., Scanlan M.J. Immunomic analysis of human sarcoma. Proceedings of the National Academy of Sciences USA (2003) 100(5): 2651-6.
12. Lohmann C.M., Hwu W.J., Iversen K., Jungbluth A.A., Busam K.J. Primary malignant melanoma of the oesophagus: a clinical and pathological study with emphasis on the immunophenotype of the tumours for melanocyte differentiation markers and cancer/testis antigens. Melanoma Research

(2003) 13(6):595-601.

13. Mischo A., Wadle A., Watzig K., Jager D., Stockert E., Santiago D., Ritter G., Regitz E., Jager E., Knuth A., Old L., Pfreundschuh M., Renner C. Recombinant antigen expression on yeast surface (RAYS) for the detection of serological immune responses in cancer patient. Cancer Immunity (2003) 3: 5.

14. Nakada T., Noguchi Y., Satoh S., Ono T., Saika T., Kurashige T., Gnjatic S., Ritter G., Chen Y-T., Stockert E., Nasu Y., Tsushima T., Kumon H., Old L.J., Nakayama E. NY-ESO-1 mRNA expression and immunogenicity in advanced prostate cancer. Cancer Immunity (2003) 3:10.

15. Nishikawa H., Kato T., Tanida K., Hiasa A., Tawara I., Ikeda H., Ikarashi Y., Wakasugi H., Kronenberg M., Nakayama T., Taniguchi M., Kuribayashi K., Old L.J., Shiku H. CD4+ CD25+ T cells responding to serologically defined autoantigens suppress antitumor immune responses. Proceedings of the National Academy of Sciences USA (2003) 100(19):10902-6.

16. Odunsi K., Jungbluth A., Stockert E., Qian F., Gnajtic S., Tammela J., Intengan M., Beck A., Keitz B., Santiago D., Williamson B., Scanlan M.J., Ritter G., Chen Y-T., Driscoll D., Sood A., Lele S., Old L.J. NY-ESO-1 and LAGE-1 cancer-testis antigens are potential targets for immunotherapy in epithelial ovarian cancer. Cancer Research (2003) 63(18):6076-83.

17. Rodolfo M., Luksch R., Stockert E., Chen Y-T., Collini P., Ranzani T., Lombardo C., Dalerba P., Rivoltini L., Arienti F., Fossati-Bellani F., Old L.J., Parmiani G., Castelli C. Antigen Specific Immunity in Neuroblastoma Patients: Antibody and T Cell Recognition of NY-ESO-1 Tumor Antigen. Cancer Research (2003) 63(20):6948-55.

18. Scott A.M., Wiseman G., Welt S., Lee F-T., Hopkins W., Mitchell P., Adjei A., Gansen D.N., Divgi C.R., Larson S.M., Hoffman EW., Tanswell P., Ritter G., Cohen L., Bette P., Arvay L., Amelsberg A., Vlock D., Rettig W.J., Old LJ. A phase I dose-escalation study of Sibrotuzumab in patients with advanced or metastatic fibroblast activation protein positive cancer. Clinical Cancer Research (2003) 9:1639-1647.

19. Sharma P., Gnjatic S., Jungbluth A., Williamson B., Herr H., Stockert E., Dalbagni G., Donat S.M., Reuter V.E., Santiago D., Ritter G., Chen Y-T., Bajorin D.F., Old L.J. Frequency of NY-ESO-1 and LAGE-1 Expression in Bladder Cancer and Evidence of a New NY-ESO-1 T-cell Epitope in a Patient with Bladder Cancer. Cancer Immunity (2003) 3:19.

20. Tahtis K., Lee F.T., Wheatley J.M., Garin-Chesa P., Park J.E., Smyth F.E., Obata Y., Stockert E., Hall C.M., Old L.J., Rettig W.J., Scott A.M. Expression and targeting of human fibroblast activation protein in a human skin/severe combined immunodeficient mouse breast cancer xenograft model. Molecular Cancer Therapies (2003) 2(8):729-37.

21. Tajima K., Obata Y., Tamaki H., Yoshida M., Chen Y-T., Scanlan M.J., Old L.J., Kuwano H., Takahashi T., Takahashi T., Mitudomi T. Expression of cancer/testis (CT) antigens in lung cancer. Lung Cancer (2003) 42(1):23-33.

22. Welt S., Ritter G., Williams C., Cohen L.S., John M., Jungbluth A., Richards E.C., Old L.J., Kemeny N.E. Phase I study of anti-colon cancer humanized antibody A33. Clinical Cancer Research (2003) 9(4):1338-46.

23. Welt S., Ritter G., Williams C., Cohen L., Jungbluth A., Richards E., Old L.J., Kemeny N.E. Preliminary report of a phase I study of combination chemotherapy and HuA33 immunotherapy. Clinical Cancer Research (2003) 9(4):1347-53.

24. Ayyoub M., Zippelius A., Pittet M.J., Rimoldi D., Valmori D., Cerottini J.C., Romero P., Lejeune F., Lienard D., Speiser D.E. Activation of human melanoma reactive CD8+ T cells by vaccination with an immunogenic peptide analog derived from Melan-A/melanoma antigen recognized by T cells.

Clinical Cancer Research (2003) (2):669-77.

25. Valmori D., Dutoit V., Ayyoub M., Rimoldi D., Guillaume P., Lienard D., Lejeune F., Cerottini J.C., Romero P., Speiser D.E. Simultaneous CD8+ T cell responses to multiple tumor antigen epitopes in a multipeptide melanoma vaccine. Cancer Immunity (2003) 3:15.
26. Rothenfusser S., Hornung V., Ayyoub M., Britsch S., Towarowski A., Krug A., Sarris A., Lubenow N., Speiser D., Endres S., Hartmann G. CpG-A and CpG-B oligonucleotides differentially enhance human peptide-specific primary and memory CD8+ T-cell responses in vitro. Blood (2003) 103:2162-9.
27. Dietrich P.Y., Le Gal F.A., Dutoit V., Pittet M.J., Trautman L., Zippelius A., Cognet I., Widmer V., Walker P.R., Michielin O., Guillaume P., Connerotte T., Jotereau F., Coulie P.G., Romero P., Cerottini J.C., Bonneville M., Valmori D. Prevalent role of TCR alpha-chain in the selection of the preimmune repertoire specific for a human tumor-associated self-antigen. Journal of Immunology (2003) 170(10):5103-9.
28. Dutoit V., Guillaume P., Ayyoub M., Hesdorffer C.S., Luescher I.F., Valmori D. Decreased binding of peptides-MHC class I (pMHC) multimeric complexes to CD8 affects their binding avidity for the TCR but does not significantly impact on pMHC/TCR dissociation rate. Journal of Immunology (2003) 170(10):5110-7.
29. Valmori D., Ayyoub M., Hesdorffer C.S., Keilholz U., Scheibenbogen C. A monoclonal melanoma-specific T-cell population phenotypically indistinguishable from CD3+ LGL-leukemia. Blood (2003) 101:4643-4.
30. Zhao Y., Pinilla C., Valmori D., Martin R., Simon R. Application of support vector machines for T-cell epitopes prediction. Bioinformatics (2003) 19:1978-84.
31. Ayyoub M., Rimoldi D., Guillaume P., Romero P., Cerottini J.C., Valmori D., Speiser D. Tumor-reactive, SSX-2-specific CD8+ T cells are selectively expanded during immune responses to antigen-expressing tumors in melanoma patients. Cancer Research (2003) 63:5601-6.

Reviews / Book Chapters / Commentaries

1. Old L.J. Cancer vaccines 2003: opening address.Cancer Immun. 2003 Dec 12; 3 Suppl 2: 1.PMID: 14670032
2. Oosterwijk E., Brouwers A., Boerman O.C., Larson S.M., Old L.J., Mulders P., Divgi C.R. Monoclonal antibody therapy of kidney cancer. Cancer Treatment Research (2003) 116: 199-212.
3. Oosterwijk E., Divgi C.R., Brouwers A., Boerman O.C., Larson S.M., Mulders P., Old L.J. Monoclonal antibody-based therapy for renal cell carcinoma. Urology Clinics of North America (2003) 30(3): 623-31.

LUDWIG INSTITUTE FOR CANCER RESEARCH

San Diego Branch
of Cancer Genetics

San Diego, USA

Staff List

Cavenee, Webster K. *Director, Member*

Laboratory Of Tumor Biology

Cavenee, Webster K. *Member*

Section of Human Carcinogenesis

Furnari, Frank *Assistant Investigator*
Okumura, Koichi *Visiting Research Scholar I*
Al-Khouri, Anna-Maria *Postdoctoral Fellow (until March)*
Bonavia, Rudy *Postdoctoral Fellow (from April)*
Chen, Yen-Liang *Postdoctoral Fellow*
Lin, Xinyi *Postdoctoral Fellow (from September)*
Mukasa, Akitake *Postdoctoral Fellow*
Steele, Julianne *Associated Research Staff*

Section of Molecular Cytogenetics

Arden, Karen *Associate Investigator*
Hosaka, Taisuke *Postdoctoral Fellow*
Lu, Wen *Postdoctoral Fellow*
Smith, Wendy *PhD Student*
Boyer, Antonia *Associated Research Staff*
Bishop, David *Associated Research Staff*
Sirich, Tammy *MD Student (from April)*

Laboratory of Cancer Genetics

Kolodner, Richard *Member*
Gazzoli, Isabella *Post Graduate Researcher*
Hess, Martin *Postdoctoral Fellow*
Huang, Meng Er *Postdoctoral Fellow*
Mazur, Daniel *Postdoctoral Fellow*
Pennaneach, Vincent *Post Graduate Researcher*
Putnam, Christopher *Postdoctoral Fellow*
Sasaki, Nobuya *Postdoctoral Fellow (until August)*
Schmidt, Kristina *Post Graduate Researcher*
Harrington, Jill *PhD Student*
Kats, Ellen *PhD Student*
Lau, Patrick *PhD Student (until January)*
Mendillo, Marc *PhD Student*
Nardin, Stephanie *PhD Student*
Shell, Scarlet *PhD Student*
Cassel, Dena *Associated Research Staff*
Derry, Katrina *Associated Research Staff (until June)*
Harries, Karin Cecelia *Associated Research Staff (from December)*
Martinez-Barragan, Sandra *Associated Research Staff*
Mueller, James *Associated Research Staff*
Nguyen, Ann Thu *Laboratory Support Staff*

Laboratory of Cell Biology

Cleveland, Don *Member*
Black, Ben *Postdoctoral Fellow*
Boillee, Severine *Postdoctoral Fellow*
Bomont, Pascale *Postdoctoral Fellow (from February)*
Bonday, Zahid *Postgraduate Researcher (until May)*
Clement, Albrecht *Postdoctoral Fellow (until August)*
Foltz, Dan *Postdoctoral Fellow*
Fujimoto, Jiro *Postdoctoral Fellow*
Garcia, Michael *Postdoctoral Fellow*
Kops, Geert *Postdoctoral Fellow*
Lobsiger, Christian *Postdoctoral Fellow*
Mao, Yinghui *Postdoctoral Fellow*
Miller, Timothy *Postdoctoral Fellow (from August)*
Shah, Jagesh *Postdoctoral Fellow*
Van de Velde, Christine *Postdoctoral Fellow*
Yamanaka, Koji *Postdoctoral Fellow*
Kulukian, Anita *PhD Student (from July)*
Silk, Alain *PhD Student*
Weaver, Beth *PhD Student*
Roy, Barnali *Undergraduate Intern*
Wang, Yin *Associated Research Staff*
Ward, Christopher *Associated Research Staff*
Zhao, Ying *Associated Research Staff (until January)*

Laboratory of Chromosome Biology

Desai, Arshad *Assistant Member*
Cheeseman, Iain *Postdoctoral Fellow*
Maddox, Paul *Postdoctoral Fellow (from March)*
Monen, Joost *PhD Student (from July)*
Sandall, Sharsti *PhD Student (from September)*
Hyndman, Francie *Associated Research Staff*

Laboratory of Gene Regulation

Ren, Bing *Assistant Member*
Kim, Tae Hoon *Postdoctoral Fellow*
Li, Zirong *Post Graduate Researcher*
Barrera, Leah *PhD Student (from October)*
Hegler, Joseph *PhD Student (until July)*
Heintzman, Nathaniel *PhD Student*
Maynard, Nathaniel *PhD Student*
Qu, Chunxu *Associated Research Staff*
Van Calcar, Sara *Associated Research Staff*

Laboratory of Mitotic Mechanisms

Oegema, Karen *Assistant Member*
Audhya, Anjon *Postdoctoral Fellow (from September)*
Dammermann, Alexander *Postdoctoral Fellow (from April)*
Maddox, Amy *Postdoctoral Fellow (from March)*

Portier, Nathan *PhD Student (from July)*
Davis, Rochelle *Undergraduate Intern (from December)*
Hsien, Jennifer *Undergraduate Intern*

Laboratory of Proteomic Biology

Zhou, Huilin *Assistant Member*
Gao, Lu *Post Graduate Researcher (from June)*
Smolka, Marcus *Post Graduate Researcher*
Zhou, Weidong *Post Graduate Researcher (from October)*
Chang, Justin *PhD Student (from July)*
Ponte-de-Albuquerque, Claudio *PhD Student (from July)*
Kwo, Henry *Undergraduate Intern (from October)*
Liu, Shi *Undergraduate Intern (from October)*
Ryan, Jennifer *Associated Research Staff*

General Services

Reigle, Brian *Technical Support LAN Services*
Weger, John *Research Associate (Core Sequencing)*
Ness, Stefanie *Associated Research Staff (Core Sequencing)*
Wu, Joann *Associated Research Staff (Core Sequencing)*

Administration and Secretariat

Peacock, Dianne *Branch Administrator*
McPhail, Natalie *Branch Controller (from March)*
Goulding, Ruth *Branch Grants Accounting Manager (from July)*
Munoz-Mack, Alma *Branch Grants Accounting Manager*
Cramer, Michelle *Branch Human Resources Manager (from April)*
Gray, Barbara *Accounts Payable Specialist*
Inglese, Jane *Payroll Specialist*
Cardiel, Blanca A. *Grants Accountant*
Garrett, Delvin *Grants Accountant (from March)*
Callahan, Kimberly *GL Accountant*
Piandes, Gary *Facilities Coordinator (until September)*
Cano, Maya *Undergraduate Intern (from January to September)*
Vergara, Christy *Administrative Assistant (from March)*
Birk, Heather *Administrative Assistant*
Kern, Eric *Administrative Assistant*
Montgomery, Ann *Administrative Assistant*
Van Roekel, Alexis *Administrative Assistant*

Branch Director's Report

During 2003, we have continued to make discoveries of importance; some of these are summarized in the text of this Report. Branch staff received international awards and honors and we continue in roles of significance in scientific societies and journal editorial and program review responsibilities. Many postdoctoral fellows and graduate students have moved on to independent positions in academia or industry after having finished their training with us. Another measure of our success is the large and steadily increasing level of competitive grant support won by Branch staff. Thus, the Branch continues as a vibrant and challenging environment for training and internationally recognized research.

Our junior faculty members continue to excel as they build their research groups and efforts. For example, Dr. Karen Oegema was selected for a Pew Foundation Scholar Award, Dr. Arshad Desai was chosen to receive a Damon Runyon Cancer Research Foundation Scholar Award, Dr. Frank Furnari has received Scholar Awards from both the V Foundation and the Sidney Kimmel Foundation for Cancer Research and Dr. Bing Ren was selected as one of the lead participants in the ENCODE national project of the National Institutes of Health. These extremely competitive awards speak volumes to the quality of people and science in the Branch. There have been new synergies between our research groups driven by complementary science and sharing of frontline technologies. The quality, accomplishment and breadth of expertise of Branch staff has been praised and welcomed by The Dean of our Medical School, Dr. Edward Holmes, as well as the Chairs of our academic Departments, and our partnership with the University has never been stronger. We continue to appreciate our wonderful physical and intellectual environment and look forward to a continuing trajectory in our scientific discovery.

Webster K. Cavenee

Research Report

Laboratory of Tumor Biology

The Laboratory is headed by Dr. Webster Cavenee and comprises two Sections with complementary interests. The Section of Molecular Cytogenetics is led by Dr. Karen Arden and is focused on three general areas. The first is the etiologic role of the alveolar rhabdomyosarcoma-associated PAX3-FKHR fusion gene. Discoveries this year suggest the intimate involvement of this gene in establishing precursor cells for malignant conversion. The second is the normal function of the FKHR sub-family of genes, each of which has been associated with cancer. Two of these genes, FKHR1 and FKHR2, have been shown this year to play intimate roles in the development of the vascular system and in ovarian maturation, respectively. The third area is the role of DNA methylation in prostate cancer development and progression and several potential DNA targets have been isolated. The Section of Human Carcinogenesis, led by Dr. Frank Furnari, is investigating the genetic lesions involved in advanced stage glioma. In these areas, the group has shown this year: 1) the commonly amplified and truncated epidermal growth factor receptor gene (ΔEGFR) elicits paracrine signals to other tumor cells thereby driving tumor heterogeneity; and, 2) the phosphatase and tensin (PTEN) homology gene product interacts with other proteins which posttranslationally modify it and regulate its activity.

Laboratory of Cancer Genetics

The Laboratory, headed by Dr. Richard D. Kolodner is focused on three general areas: 1. the use of the yeast Saccharomyces cerevisiae as a model organism for identification of genes that function to suppress the accumulation of mutations and other types of genome rearrangements in DNA; 2. the development of mice containing mutations in genes that suppress the accumulation of mutations and other types of genome rearrangements in DNA and the analysis of the role of these genes in suppressing cancer susceptibility; and 3. gaining an understanding of the role of defects in these genes in inherited and sporadic cancers in humans.

Genetic studies have led to the demonstration that germline mutations in human genes encoding the major DNA mismatch repair system cause an inherited cancer susceptibility syndrome (HNPCC). In addition, some sporadic cancers develop defects in the mismatch repair gene MLH1 at an early step in tumorigenesis. Using the S. cerevisiae model system, two different advances have been made: for the first time, a biochemical role for proliferating cell nuclear antigen (PCNA) in mismatch repair has been elucidated by demonstrating that PCNA binds to the MSH2-MSH6 mismatch recognition complex and then transfers it to the mispaired base; and a model system for investigating polygenic interactions between mismatch repair defects has been developed and used to show that some human HNPCC causing mutations are actually weak mutations that interact genetically with other weak mutations to cause polygenic loss-of-function defects. Through the generation of mutant mice combined with studies in which inherited mutations in the human MSH6 gene have been modeled in S. cerevisiae, it has been possibly to identify separation-of-function mutations that inactivate mismatch repair but do not cause defects in the mismatch repair protein dependent DNA damage response. These mutations cause significantly increased cancer susceptibility demonstrating for the first time that increased mutation rates due to mismatch repair defects are sufficient to cause the development of cancer. In addition, the laboratory has developed a novel mutator assay in the yeast S. cerevisiae that allows detection of genome rearrangements like those seen in cancer cells. Using this assay, a number of genes were identified that function to prevent genome rearrangements.

Laboratory of Cell Biology

The Laboratory, headed by Dr. Don Cleveland, is focused on two general areas: how chromosomes are faithfully moved into each daughter cell at cell division; and, the molecular genetics of axonal growth and mechanisms of human motor neuron disease, especially the disease, familiarly known as Lou Gehrig's disease, or ALS. The mitotic checkpoint is the cell cycle control mechanism that is the primary protection against development of aneuploidy. This is achieved by the generation at unattached centromeres of a diffusible "stop anaphase" signal. In the past year, the laboratory has identified the mechanism of activation and silencing of this essential mammalian checkpoint. Selectively weakening of the checkpoint in adult mice has allowed a test of a central question in tumor development: does aneuploidy drive carcinogenesis? A second interest is in the mechanism leading to selective killing of motor neurons in ALS. In almost every example of inherited human neurodegenerative disease, the question of selective vulnerability of individual neurons has been perplexing, since the mutant gene products are widely expressed. Using modeling in mice, an explanation has been identified: toxicity arises from the convergence of mutant damage within the target neurons and within the neighboring non-neuronal cells.

Laboratory of Chromosome Biology

The Laboratory, headed by Dr. Arshad Desai, is focused on understanding the mechanisms that segregate chromosomes during cell division. Specifically, kinetochores, macromolecular assemblies that form on chromosomes to connect them to spindle microtubules during cell division, are being analyzed. The kinetochore-microtubule connection is central to accurate segregation of chromosomes and prevention of aneuploidy. This interface is also the target of anti-mitotic chemotherapeutic drugs, such as taxol. In the past year, the laboratory has combined functional genomics with proteomics in the early C. elegans embryo to identify nine new kinetochore components. In vivo functional assays are now being employed to define the precise contributions of each component to kinetochore function in C. elegans and these are being extended to analyze selected components in mammals. The group is also studying the mechanisms that specify kinetochore formation at a localized site on chromosomes. The specification event involves formation of specialized chromatin containing a histone H3 variant. Using an unbiased functional genomic strategy, a novel protein involved in chromosomal targeting of this histone variant has been identified and its mechanism of action is being defined.

Laboratory of Gene Regulation

The Laboratory, headed by Dr. Bing Ren, investigates how the complex gene regulatory networks in mammalian cells control cellular proliferation and differentiation. The research is divided into two general areas: (1) Development of genomics and bioinformatics tools that allow genome wide identification of regulatory targets for transcription factors. Specifically, the group has developed the genome wide location analysis (GWLA) technology to detect in vivo protein-DNA interactions in mammalian cells; (2) Application of these tools to study of transcription factors that play critical roles in tumorigenesis. GWLA was employed to examine the target genes for two oncogenic pathways: c-Myc in Burkitt's lymphoma cells, and β-catenin in colon cancers. This led to the novel finding that over-expressed c-myc binds to a large number of gene promoters, suggesting novel molecular mechanisms for c-myc function in malignant transformation. Targets of β-catenin have also been identified; one encodes a small peptide known to be overexpressed in colon tumors and many other solid tumors and that the group has now demonstrated plays a key role in the oncogenic transforming activity of β-catenin.

Laboratory of Mitotic Mechanisms

The Laboratory, headed by Dr. Karen Oegema, is focused on understanding the morphogenetic transformations required for cell division. The group uses the early embryo of the soil nematode Caenorhabditis elegans as a model system because of the advantages it offers for the molecular analysis of mitosis. RNA-mediated interference (RNAi) in C. elegans makes it feasible to analyze the first mitotic division of embryos depleted of any targeted gene product. The group is combining RNAi-based functional genomics with single-cell high-resolution microscopy assays and biochemical characterization of native protein complexes to study three aspects of cell division: centrosome duplication and maturation; cleavage furrow assembly and membrane dynamics during cytokinesis; and, kinetochore specification and assembly (a collaboration with the laboratory of Arshad Desai). In 2003, a fluorescence-based assay for centriole assembly was developed and, by combining it with RNAi, a role for four centriolar components and two pericentriolar material proteins in centrosome duplication was discovered. In a fluorescence screen of embryonic lethal genes of unknown function, two novel proteins that are required for spindle assembly were identified and are being characterized. A combination of live and fixed assays have also been employed to characterize the functions of two structural components of cleavage furrows, anillin and the septins.

Laboratory of Proteomic Biology

The Laboratory, headed by Dr. Huilin Zhou, is focused on the analysis of protein phosphorylation events in DNA damage response pathways and the role of protein sumoylation in gene silencing and the DNA damage response. First, using yeast as a model system, an approach to quantitatively analyze phosphorylation changes to several protein kinases involved in the DNA damage response pathway has been established. The laboratory is now systematically mapping phosphorylation patterns of most proteins implicated in DNA damage response pathways in an effort to understand the role of phosphorylation in regulating DNA damage response. In addition, human homologs whose dysfunction leads to various human diseases are being examined. Secondly, a number of protein substrates for sumoylation modification have been identified and the link between protein sumoylation, gene silencing and DNA repair is being tested.

Publications

Primary Research Articles

1. Chen Y-T., Alpen B., Ono T., Gure A.O., Scanlan M.A., Biggs III W.H., Arden K.C., Nakayama E., Old L.J. Identification and Characterization of Mouse SSX Genes: A Multigene Family on X Chromosome with Restricted Cancer/Testis Expression. Genomics (2003) 82:628-36.
2. Clement A., Nguyen M., Roberts E.A., Garcia M.L., Boillee S., Rule M., McMahon A.P., Doucette W., Siwek D., Ferrante R.J., Brown R.H., Julien J-P., Goldstein L.S.B., Cleveland D.W. Wild Type Nonneuronal Cells Extend Survival of SOD1 Mutant Motor Neurons in ALS Mice. Science (2003) 302:113-117.
3. Desai A., Rybina S., Müller-Reichert T., Shevchenko A., Shevchenko A., Hyman A., Oegema K. KNL-1 Directs Assembly of the Microtubule-Binding Interface of the Kinetochore in C. elegans. Genes and Development (2003) 17:2421-2435.
4. Garcia M.L., Lobsiger C.S., Shah S.B., Deerinck T.J., Crum J., Yound D., Ward C.M., Crawford T.O., Gotow T., Ellisman M.H., Calcutt N.A., Cleveland D.W. NF-M is an Essential Target For the Mylein-Directed "Outside-In" Signaling Cascade that Mediates Radial Axonal Growth. Journal of Cell Biology (2003) 163:1011-1020.
5. Gazzoli I., Kolodner R.D. Regulation of the Human MSH6 promoter by the Sp1 Transcription Factor and Alteration of Promoter Activity and Expression by Polymorphisms. Molecular and Cellular Biology (2003) 23:7992-8007.
6. Guo P., Hu B., Gu W., Xu L., Wang D., Huang H-J.S., Cavenee W.K., Cheng S-Y. Platelet-Derived Growth Factor-B Enhances Glioma Angiogenesis by Stimulating Vascular Endothelial Growth Factor Expression in Tumor Endothelia and by Promoting Pericyte Recruitment. The American Journal of Pathology (2003) 162:1083-1093.
7. Hoffman H.M., Gregory S., Mueller J.L., Tresierras M.A., Broide D.H., Wanderer A.A., Kolodner R.D. Fine Structure Mapping of CIAS1: Identification of an Ancestral Haplotype and a Common FCAS Mutation L353P. Human Genetics (2003) 112:209-216.
8. Hu B., Guo P., Fang Q., Tao H-Q., Wang D., Nagane M., Huang H-J.S., Gunji Y., Nishikawa R., Alitalo K., Cavenee W.K., Cheng S-Y. Angiopoietin-2 Induces Human Glioma Invasion through the Activation of Matrix Metalloprotease-2. Proceedings of the National Academy of Sciences USA (2003) 100:8904-8909.
9. Huang M-E., Rio A.G., Nicolas A., Kolodner R.D. A Genome-Wide Screen in Saccharomyces cerevisiae for Genes that Suppress the Accumulation of Mutations. Proceedings of the National Academy of Sciences USA (2003) 100:11529-11534.
10. Jagmohan-Changur S., Poikonen T., Vilkki S., Launonen V., Wikman F., Orntoft T.F., Møller P., Vasen H., Tops C., Kolodner R.D., Mecklin J-P., Järvinen H., Bevan S., Houlston R.S., Aaltonen L.A., Fodde R., Wijnen J., Karhu A. EXO1 Variants Occur Commonly in Normal Populations: Evidence Against a Role in Hereditary Nonpolyposis Colorectal Cancer. Cancer Research (2003) 63:154-158.
11. Jungbluth A.A., Stockert E., Huang H-J.S., Collins V.P., Coplan K., Iversen K., Kolb D., Johns T.J., Scott A.M., Gullick W.J., Ritter G., Cohen L., Scanlan M.J., Cavenee W.K., Old L.J. A Monoclonal Antibody Recognizing Human Cancers with Amplification/Overexpression of the Human Epidermal Growth Factor Receptor. Proceedings of the National Academy of Sciences USA (2003) 100:639-644.
12. Kantake N., Sugiyama T., Kolodner R.D., Kowalczykowski S.C. The Recombination-Deficient

Mutant RPA (rfa1-t11) is Displaced Slowly from Single-Stranded DNA by Rad51 Protein. Journal of Biological Chemistry (2003) 278:23410-23417.

13. Kirkham M., Müller-Reichert T., Oegema K., Grill S., Hyman A.A. SAS-4 is a C. elegans Centriolar Protein that Controls Centrosome Size. Cell (2003) 112:575-587.
14. Lau P.J., Kolodner R.D. Transfer of the MSH2-MSH6 Complex from Proliferating Cell Nuclear Antigen to Mispaired Bases in DNA. Journal of Biological Chemistry (2003) 278:14-17.
15. Li Z., Van Calcar S., Qu C., Cavenee W.K., Zhang M., Ren B. A Global Transcriptional Regulatory Role for C-myc in Burkitt's Lymphoma Cells. Proceedings of the National Academy of Sciences USA (2003) 100:8164-8169.
16. Mao Y., Abrieu A., Cleveland D.W. Activation and Silencing of Mitotic Checkpoint Signaling by Modulating BubR1 Kinase Activity with CENP-E. Cell (2003) 114: 87-98.
17. Myung K., Pennaneach V., Kats E., Kolodner R.D. Chromatin Assembly During DNA Replication Functions in the Maintenance of Genome Stability. Proceedings of the National Academy of Sciences USA (2003) 100:6640-6645.
18. Myung K., Kolodner R.D. Induction of Genome Instability by DNA Damage in Saccharomyces cerevisiae. DNA Repair (2003) 2:243-258.
19. Nakae J., Kitamura T., Kitamura Y., Biggs W.H., Arden K.C., Accili D. The Forkhead Transcription Factor Foxo1 Regulates Adipocyte Differentiation. Developmental Cell (2003) 4:119-129.
20. Schmidt M.H.H., Furnari F.B., Cavenee W.K., Bögler O. Epidermal Growth Factor Receptor Signaling Intensity Determines Intracellular Protein Interactions, Ubiquitination, and Internalization. Proceedings of the National Academy of Sciences USA (2003) 100:6505-6510.
21. Shimodaira H., Yoshioka-Yamashita A., Kolodner R.D., Wang J.Y.J. Interaction Between Mismatch Repair Protein PMS2 and the P53-Related Transcription Factor P73 in the Apotosis Response to Cisplatin. Proceedings of the National Academy of Sciences USA (2003) 100:2420-2425.
22. The HGCP/CGAP Consortium. The Generation and Utilization of a Cancer Oriented Representation of the Human Transcriptome, Using Expressed Sequence Tags. Proceedings of the National Academy of Sciences USA (2003) 100:13418-13423.
23. Weaver B.A.A, Putkey F.R., Cleveland D.W. CENP-E is Essential for the Mammalian Spindle Assembly Checkpoint to Prevent Single Chromosome Loss. Journal of Cell Biology (2003) 162:551-563.
24. Wei K., Clark A., Wong E., Kane M., Mazur D.J., Parris T., Kolas N.K., Russell R., Hou Jr H., Kneitz B., Yang G., Kunkel T.A., Kolodner R.D., Cohen P.E., Edelmann W. Inactivation of Murine Exonuclease 1 Results in Defective DNA Mismatch Repair, Increased Cancer Susceptibility and Male and Female Sterility. Genes and Development (2003) 17:603-614.
25. Xia C-H., Roberts E.A., Her L-S., Liu X., Williams D.S., Cleveland D.W., Goldstein L.S.B. Abnormal Neurofilament Transport Caused by Targeted Disruption of Neuronal Kinesin Heavy Chain KIF5A. Journal of Cell Biology (2003) 161:55-66.
26. Yamanaka K., VandeVelde C., Bertini E., Boespflug-Tanguy O., Cleveland D.W. Unstable Mutants in the Peripheral Endosomal Membrane Component ALS2 Cause Early Onset Motor Neuron Disease. Proceedings of the National Academy of Sciences USA (2003) 100: 16041-16046.

Reviews / Book Chapters / Commentaries

1. Cavenee W.K. Preparing for Serendipity. Cancer Biology and Therapy (2003) 2:213-215.
2. Cavenee W.K. The Recessive Nature of Dominance. Genes, Chromosomes & Cancer (2003) 38:322-325.
3. Cleveland D.W., Mao Y., Sullivan K.F. Centromeres and Kinetochores: from Epigenetics to Mitotic Checkpoint Signaling. Cell (2003) 112:407-421.
4. Dammermann A., Desai A., Oegema K. The Minus End in Sight. Current Biology (2003) 13: R614-624.
5. Li Z., Ren B. Analysis of Gene Expression Regulatory Circuitry. In: Microarray Technology and Its Applications, UR Müller, and D Nicolau, eds. (2003) Springer Verlag, New York.
6. Louis D.N., Cavenee W.K. Molecular Biology of Central Nervous System Tumors. In: Cancer: Principles and Practice of Oncology, VT DeVita, S Hellman and SA Rosenberg, eds. (2003). Lippincott-Raven Publishers, Philadelphia, 7th Edition.
7. Maddox A.S., Oegema K. Deconstructing Cytokinesis. Nature Cell Biology (2003) 5:773-776.
8. Maddox A.S., Oegema K. Closing the GAP: a Role for a RhoA GAP in Cytokinesis. Molecular Cell (2003) 11:846-848.
9. Miller T.M., Cleveland D.W. Has Gene Therapy Arrived for ALS? Nature Medicine (2003) 9:1256-1257.
10. Zhou H., Boyle R., Aebersold R. Quantitative Protein Analysis by Solid Phase Isotope Tagging and Mass Spectrometry. In: Protein-protein Interactions. H Fu, ed. (2003) Humana Press. Totowa, New Jersey.
11. Zhou H., Boyle R., Aebersold A. Site Specific, Stable Isotope Labeling of Cysteinyl Peptides in Complex Peptide Mixtures. In: Cell Biology: A Laboratory Handbook. 3rd Edition. J Celis, ed. (2003) Elsevier Science. London, UK.

LUDWIG INSTITUTE FOR CANCER RESEARCH

São Paulo Branch
of Cancer Biology and Epidemiology

São Paulo, Brazil

Staff List

Brentani, Ricardo R. *Member, Director*

Cell and Molecular Biology Group

Brentani, Ricardo R. *Member, Director*
Torloni, Humberto *Associate Member*
Martins, Vilma R. *Assistant Member*
Castro, Rosa Maria R. P. S. *PhD Student*
Costa, Fabricio Falconi *PhD Student*
Couto, Cinthia G. C. *PhD Student (from August)*
Freitas, Adriana R.O. *PhD Student*
Hajj, Glaucia Noeli Maroso *PhD Student*
Lee, Kil Sun *PhD Student (to July)*
Lima, Flávia Regina Souza *PhD Student*
Lopes, Marilene Hohmuth *PhD Student*
Muras, Angelita Gonzalez *PhD Student*
Silva, Ricardo Luis Alves *PhD Student (until January)*
Kindlmann, Kathleen *M.Sc.Student*
Landemberger, Michele Christine *M.Sc. Student*
Rodrigues, Carolina C. *M.Sc.Student (from February)*
Takiishi, Tatiana *Under Graduate Student (from September)*
Fonseca, Rogério da Silva *Under Graduate Student (until January)*
Arantes, Camila P. *Under Graduate Student (from July)*
Lembke, Carolina G., *Under Graduate Student (from September)*
Nascimento, Carlos F. *Technician*
Nomizo, Regina *Technician*
Nonogaki, Suely *Technician*
da Silva, Miyuki Fukuda *Technician*
Ferreira, Severino Da Silva *Technician*

Cancer Genetics Group

Caballero, Otávia L. S. *Assistant Member*
de Oliveira, André Luiz Vettore *Senior Investigator*
Bulgarelli, Adriana *PhD Student*
Góes, Fernanda Costa G. De S. *PhD Student (from June)*
Picolli, Fabio de Simoni *PhD Student (until January)*
Santos, Milena J. S Flória Lima *PhD Student (to November)*
Sredni, Simone Treiger *PhD Student*
de Oliveira, Mariana Brait Rodrigues *M.Sc.Student*
Dellamano, Márcia *M.Sc.Student*
Kagohara, Luciane T. *M.Sc.Student (from February)*
Vidal, Daniel O. *M.Sc.Student (from February)*
Mello, Barbara P. *Under Graduate Student (from September)*
de Carvalho, Fabrício *Technician*
de Moura, Ricardo Pereira *Technician*
da Cunha, Danielle Renzoni *Technician*
Paixão, Valéria Aparecida *Technician*
Seixas, Andrea Quintella de Andrade *Technician*

Laboratory of Molecular Biology and Genomics

Camargo, Anamaria Aranha *Assistant Member*
Carraro, Dirce Maria *Senior Investigator*
Verbisck, Newton Valério *PhD Student*
Bettoni, Fabiana *M.Sc.Student*
Parmigiani, Raphael Bessa *M.Sc.Student*
Silva, Ana Paula Medeiros *M.Sc.Student*
Ierardi, Daniela Filippini *M.Sc.Student*
Ferreira, Elisa N. E *Under Graduate Student (from February)*
Geraldo, Murilo V. *Under Graduate Student (from July)*
Granato, Mariana Facchini *Under Graduate Student*
Bastos, Elen P. *Technician (from March)*
Camargo Filho, Fernando *Technician (from February)*
Kaiano, Jane Haruko L. *Technician*
Monteiro, Elisangela *Technician*
Padovani, Reimar *Technician (from October)*
Salim, Ana Christina de M. *Technician*
Passetti, Fabio *Technician (to March)*
Pires, Lilian Campos *Technician*

Computational Biology Group

Souza, Sandro José *Associate Member*
Barbosa, Elza H. A. *PhD Student (from March)*
de Souza, Jorge Estefano Santana *PhD Student*
Kuva, Silvia Maria *PhD Student (until May)*
Leerkes, Maarten *PhD Student*
Vibranovski, Maria Dulcetti *PhD Student*
Sakabe, Noboru Jo *M.Sc.Student*
Slager, Natanja Sara Kirschbaum *M.Sc.Student*
Galante, Pedro A. F. *Under Graduate Student*
Lopes, Graziela Mie P. *Under Graduate Student (until August)*
Scatolini Jr., Odair *Under Graduate Student (until July)*
Fabri, Artur Penha *Technician (until September)*
Oliveira, Rodrigo S. *Technician (from March)*

Molecular Immunology Group

Reis, Luiz Fernando Lima *Assistant Member*
de Carvalho, Alex Fiorini *Senior Investigator (Microarray)*
Abrantes, Eduardo Fernandes *PhD Student*
Diniz, Suzana Nogueira *PhD Student*
Martins, Waleska Kerllen *PhD Student (from May)*
Meireles, Sibele Inacio *PhD Student (until June)*
Muto, Nair H. *PhD Student (from September)*
Gomes, Luciana Inácia *PhD Student*
Stolf, Beatriz Simonsen *PhD Student*
Temini, Lara *PhD Student*
Barreto, Bianca D. *M.Sc.student*
Coló, Anna Estela Luiza *M.Sc.student*
Esteves, Gustavo Henrique *M.Sc.student (until April)*

Pagotto, Ana Helena *M.Sc.student*
Marques, Mariana M. *Under Graduate Student (from September)*
Neves Filho, Emerson *Under Graduate Student*
Dias, Adriana Abalen M. *Technician (from August)*
Marques, Sarah M. *Technician (from April)*
Mendonça Neto, Chamberlein E. *Technician (Microarray)*
Mota, Louise D. De C. *Technician (from June)*
Santos, Mariana M.s. *Technician (from May)*
Silva, André A., *Technician (from September)*
Spilborghs, Graziela M. G. T. *Technician (from May)*

Virology Group

Villa, Luisa Lina *Member*
de Souza, Patricia Savio *PhD Student*
Gonzalez, Laura C. S. *PhD Student*
Junes, Katiana de S. *PhD Student*
Thomman, Patrícia *PhD Student*
Trevisan, Andrea *PhD Student*
Bolpetti, Aline Do N. *M.Sc.Student (from September)*
Rabachini, Tatiana *M.Sc.Student*
Silvestre, Rodrigo Vellasco D. *M.Sc.Student*
De Paula, Lenice Galan *Nurse*
Pinto, Maria Luiza B. G. *Nurse*
Andreoli, Maria A. A. *Technician (from August)*
Costa, Maria Cecília *Technician*
Ferreira, Silvaneide A. *Technician*
Miyamura, Romulo A. *Technician*
Pereira Sobrinho, João S. *Technician*
Pierulivo, Enrique Mario Boccardo *Technician (from May)*
Prado, Jose Carlos Mann *Technician*
Rossi, Adriana Cristina Machado *Technician (until June)*
Santos, Monica I. R. *Technician*
Mendonça, Roseli de Souza *Laboratory Worker*

Laboratory Support - General

Carneiro, Izabel Costa *Laboratory Worker*
Sampaio, Sirleia Miranda *Laboratory Worker*
Sousa, Leonel C. B. *Photographic Artist*
da Cruz, João Aquilau *Animal Keeper (until October)*
Gonçalves, Wanderley Lourenço *Assistant Animal Keeper*
Lima, Ricardo R. F. *Assistant Animal Keeper (from November)*
Silva, Domingos S. M. *Assistant Animal Keeper*
Silva, Eudes *Maintenance Supervisor*
Monteiro, Luiz Antonio *Maintenance Assistant*
Silva, Francisco Sampaio *Maintenance Assistant*

Administration and Secretariat

Campos, Paulo Roberto *Administrator (from May)*
Walter, Antje Luise *Assistant Administrator*
Coelho, Viviani Ap. Martins *Accountant*
Souza, Maria Conceição *General Services*
Rocha, Roseli Z. *Buyer - Importations*
da Rocha, Rubens Antonio *Storekeeper*
Monteiro, Jaqueline *Executive Secretary*
Costa, Gisely da *Research Secretary (Microarray) (until January)*
Rodrigues, Aline P. *Research Secretary (Immunology) (from February)*
Hering, Maria Stella Leme *Research Secretary (Virology)*
Lopes, Fernanda M.C. *Research Secretary (Human Cancer Genome) (from August)*
Pereira, Ana Cláudia *Research Secretary (Computational Biology)*
Ribeiro, Eliane Lopes *Research Secretary (Cellular And Molecular Biology)*
Pereira, Carla Regina F. *Secretary*
da Silva, Raquel Hessel *Project Clerk (Virology)*
Capelucci, Renata Juliana Souza *Office Clerk*
de Sousa, Andréa Ramos *Office Clerk*
de Souza, Raquel Rodrigues *Office Clerk*
Paraguassú, Erica *Office Clerk*
Rocha, Juliana Freitas *Office Clerk*
Cristino Júnior, Elias Lopes *Office Boy*
Fietzek, Birgit *Receptionist*

Branch Director's Report

The São Paulo Branch's expertise in genomics projects is internationally recognized through ORESTES, and now the Transcript Finishing Initiative (TFI), both of which were collaborative projects partly-funded by FAPESP (The State of São Paulo) and LICR. The Branch has also been involved in other genome sequencing and expression projects, including the successful implementation of a virtual genome institute named ONSA (Organization for Nucleotide Sequencing and Analysis). Additionally, the Branch has played a major role in the Brazilian Genome Network, set up by the Brazilian Ministry of Science and Technology, which involves laboratories from almost all states in Brazil. This Network has already sequenced and annotated the genome of the free living bacteria Chromobacterium violaceum (The Brazilian National Genome Project Consortium, 2003) and is currently sequencing the genomes of Mycoplasma synoviae and Mycoplasma hypopneumoniae.

Branch investigators are also involved in research that aims to elucidate gene expression, and molecular markers for cancer diagnosis, prognosis and cancer management, involving predicting toxicity and response to therapy. Cutting-edge technologies, such as Massively Parallel Signature Sequencing (MPSS) and microarrays also carried out as a joint collaboration with FAPESP, are generating high throughput expression data from various types of tumors collected at the HOSPITAL DO CANCER our long-standing partner, and complex bioinformatics have been developed in close collaboration with scientists from the Institute of mathematics at USP and are being used to analyze the results.

Of particular note in the research towards the diagnosis and treatment of cancer, is the crucial research being conducted on cervical cancer caused by the human papilloma virus. The interplay between HPV transforming genes E6 and E7 and TNF expression is being currently evaluated.

Finally, the characterization of the cellular prion protein (PrPc) remains a priority at the Branch. Apart from its etiology in spongiform encephalopathies, such as variant Creutzfeld-Jakob Disease (vCJD) or 'mad cow's' disease, it is becoming increasingly clear that PrPc's may have a role in epilepsy and cognitive function. We have also contributed to the elucidation of it's role in neuronal differentiation mediated by its interactions with extracellular glycoproteins laminin and vitronectin,as well as its neuroprotective effect through its interaction with extendin or STI1. The analysis of PrPc is therefore crucial for furthering the understanding of several neurological disorders.

Ricardo R. Brentani

Research Report

Laboratory of Molecular Biology and Genomics

The Laboratory, led by Dr. Anamaria Camargo, has been analyzing expressed and genomic sequences to identify novel human genes. The application of this approach has led to the identification of novel human genes on chromosomes 22 and 21 and at the Hereditary Prostate Cancer Locus 1 (HPC1) at 1q25. The same approach was also applied in a large collaborative project known as the Transcript Finishing Initiative (TFI), which was jointly funded by the Ludwig Institute and FAPESP, and involved 35 research groups from the state of São Paulo. The Initiative was concluded on July 2003 and final results are being considered for publication. A total of 59,975bp transcribed sequences were generated, and the structures of 211 transcripts were defined. Members of the Laboratory have also been directly involved in the implementation of ONSA, which was responsible for sequencing the Xylella fastidiosa genome. The Laboratory is responsible for the finishing phase of both the ONSA and Brazilian National Genome Network projects.

The group continues to concentrate on the characterization of new human genes related to cancer. This is being achieved using both in silico and experimental approaches. For the in silico approach, expressed and genomic sequences are being analyzed in order to identify putative Cancer-Testis antigens, exclusively expressed in normal testis and tumors. Differential methylation will be used as the major experimental approach to select candidate genes related to cancer. In addition, expression data collected by MPSS will be analyzed to identify novel genes regulated by c-erbB2. Techniques for the analysis of differential methylation such as Differential Methylation Hybridization (DMH) and techniques for the characterization of novel genes such as GLGI are being implemented in the laboratory.

Virology Group

The Virology Group, headed by Dr. Luisa Villa, continues to pursue the key elements involved in the natural infection by human papillomavirus (HPV) and its association with cervical neoplasia. Studies on the intratypic variability of HPV-16 to another high-risk type, namely HPV-18 were extended, and the differential oncogenic potential of certain molecular variants of HPV-18 were linked to differences in the regulatory region of these variants, which affected the P105 promoter activity. The search for HPV types linked to a higher incidence of cervical tumors in some geographic areas is now focusing on HPV-58, another high-risk type that is the third most frequent HPV type found in cervical samples from the Ludwig-McGill study of the natural history of HPV and cervical neoplasia. The HPV 58 isolates in our cohort study exhibit substantial intratype variability. Further studies should clarify if these variants are differentially associated with cervical neoplasia. One area of analysis is whether the antigenic variations naturally found in viral proteins could interfere in the proliferative responses against HPV-16 peptides, and therefore, to the immune response against these viruses. Furthermore, investigation is underway to determine the presence of neutralizing antibodies in the sera of women naturally exposed to HPV. This information may contribute to the understanding of the role of such antibodies in protecting from HPV infection after virus-like particle (VLP) vaccination, as reported in recent clinical trials. Preliminary data indicate that women with neutralizing antibodies are more likely to harbor a persistent HPV-16 infection during the first year of follow-up.

The Group has previously shown that high viral load of high-risk type HPV is associated with persistent infection and an increased risk of development of cervical neoplasia. This important correlate of disease is being further analyzed, by studying the integration status of the viral genomes in the cervical specimens of participants of our cohort study. By means of Real-Time PCR, the Group has shown that integration was more strongly associated with high viral load specimens than in low viral load ones, implying that evaluation of the integration status of HPV16 may play an adjunctive role in cervical cancer screening and

risk prediction.

Finally, the Group is investigating the role of micronutrients and antioxidants in HPV clearance. Analyses of serum carotenoids, tocopherols and derivatives of retinoic acid (RA) were conducted on samples from the Ludwig/McGill cohort, and the results support the Group's previous findings of decreased risk of persistent HPV infection with increased dietary intake of lutein/zeaxanthin. Consumption of certain carotenoids, as well as higher endogenous RA concentrations leading to increases in serum levels, may decrease the duration of an oncogenic HPV infection thereby decreasing risk of cervical disease in high-risk women.

Laboratory of Cancer Genetics

The activities of the Laboratory of Cancer Genetics, led by Dr. Andrew Simpson, are focused on the identification of potential targets of cancer therapy; namely genes that are expressed specifically in certain tumors and/or tissues, and are not expressed in essential normal tissues. Massively parallel signature sequencing (MPSS), a technique that can generate millions of signature tags sufficient to provide a 10-fold clone coverage of the transcripts present in a human cell, was used to obtain data from 33 different normal tissues for the identification of genes predicted to be prostate-specific and not present in essential normal tissues. A candidate group of genes from the dataset is now being extensively characterized. MPSS data was also used to the search for cancer-testis (CT) antigens; genes with mRNA expression profiles restricted to cancer and testis. Eight genes (from 141 with this pattern of expression) were selected for validation, and the expression profile restricted to normal testis and cancer could be confirmed in four cases. The Group is currently producing recombinant proteins to generate specific monoclonal antibodies against all the validated prostate-specific and CT candidate genes identified by MPSS. These antibodies will be used to confirm by immunohistochemistry the expression specificity and further evaluate whether these genes can be used as targets for cancer therapy.

Other studies in the laboratory focus on the identification of molecular markers for tumor progression or diagnosis in breast cancer. The Laboratory has undertaken analyses using the methodology and results of serial analysis of gene expression (SAGE). Publicly-available data from a breast tumor database resulted in the identification of 172 genes at least four-fold overexpressed in breast cancer libraries compared to the normal breast libraries. SAGE is also being used to identify genes correlated with aggressiveness or good response to chemotherapy in Wilms' Tumor (WT), which is the most frequent childhood renal cancer. The DNA methylation states of the promoters of genes involved in cancer are also being analyzed in samples of bladder carcinomas, and pediatric acute lymphocytic leukemia (ALL) and myelodisplastic syndrome (MDS)

Cellular and Molecular Biology Group

The Cellular and Molecular Biology Group, led by Dr. Ricardo Brentani, evaluate the importance of the Cellular Prion protein (PrPc) in human diseases other than spongiform encephalopathies. Some years ago the Group described that PrPc gene ablated animals have a higher sensitivity to seizure in vivo, suggesting that total or partial modification of PrPc function might be related to epilepsy. Recently, a PrPc polymorphism at codon 171 (Asn->Ser), absent in controls, was found to be heterozygous in 23% of patients with Mesial Temporal Lobe Epilepsy due to hyppocampal sclerosis (MTLE-HS) and in 13% of patients with malformations of cortical development. Furthermore, patients carrying the Asn171Ser variant had a five times higher chance to continue to have seizures after temporal lobectomy than those carrying the normal allele. These results suggest that the PrPc variant allele at codon 171 (Asn171Ser) is associated with epileptogenesis in these two types of human epilepsy. This polymorphism is localized within the laminin γ1 chain binding site at the PrPc molecule, previously described by the Group. Work is underway to evaluate the cellular and molecular mechanisms involved in the alteration of PrPc physiological functions due to the amino acid change at codon 171 and the epileptogenesis process.

The Group has also described that old (nine month) PrPc gene ablated animals have impairment in short and long-term memory retention that is not observed in younger mice (3 months) with the same genotype. These data are consistent with recent data from the literature indicating that some polymorphisms in the PrPc gene are associated with worse cognition in elderly patients. The mechanisms related to the role of PrPc in cognition are also under evaluation.

Computational Biology Group

The Computational Biology Group, led by Dr. Sandro Souza, continues to use bioinformatics resources to explore the complexity of the human transcriptome, particularly the alternative splicing of transcripts. The Group has shown that the ORESTES collection of human cDNAs is enriched with splicing variants that affect the structure of the corresponding protein. This is probably due to the biased distribution of the ORESTES sequences towards the central part of the transcripts as well as its normalization capacity. This makes the ORESTES technology an excellent platform for the exhaustive screening of the variability found within the human transcriptome. The Group has evaluated the distribution of intron retention events in the human transcriptome. Although many isolated cases of splicing variants with biological significance have been reported, it remains doubtful whether a significant fraction of the events have biological significance, or are spurious products from the splicing machinery. This is especially true for intron retention that is believed to be largely derived from unspliced or partially spliced pre-mRNAs. Through a large-scale analysis of intron retention in more than 20,000 human genes, the Group concluded that a significant fraction of the detected events is not spurious and probably reflect biological significance. This conclusion is supported by a non-random distribution of the events, with a significant fraction of splicing events conserved in mouse, and coding for domains in the corresponding proteins.

One important component of the Group's activities is the maintenance of multi-group collaborations for large-scale projects. This was exemplified by an active participation in the 'Jamborestes', a jamboree for the annotation of all sequences produced by the Human Cancer Genome Project and the Cancer Genome Anatomy Project. Additionally, the Group is also participating in the H-Invitational, an initiative from the Japanese government to annotate all human full-length sequence, and The Transcript Finishing Initiative with other groups in the Branch.

Laboratory of Inflammation

During the last two years the research projects of the Laboratory, headed by Dr. Luiz Reis, were transferred to the newly established laboratory of animal experimentation at Hospital do Cancer (the Branch's host institution) and are now under the coordination of Dr. Adriana Dias, an independent investigator at the Hospital. In collaboration with Dr. Dias' group, the Laboratory functionally characterizing PTX-3 (formerly identified as TSG-14) using transgenic and knock-out mice.

The Laboratory's major focus is now the application of gene expression profiling in cancer-related projects. During the last three years, the Laboratory has dedicated a major effort to the establishment of the cDNA microarray technology and related protocols, as well as the exploitation of the clone collection (approximately 1,200,000 clones) generated during the HCGP. In 2003, the Hospital do Cancer decided to invest a major effort in creating an array-dedicated bioinformatics, which is coordinated by Dr Helena Brentani. There is now a complete platform covering clone collection, tumor bank, RNA bank, microarray production, data submission, on-line quality control for dye-swap hybridizations, and data analysis. The availability of a dedicated bioinformatics effort has allowed us to prepare new, project oriented array collections, with very stringent criteria for selecting clones to be immobilized. The Laboratory's goals are now concentrated on the development of molecular tools for diagnosis and prognosis, based on signature expression, particularly for head and neck, gastroesophageal, and breast cancers.

Publications

Primary Research Articles

1. Abrantes E.F., Pires E.G., Carvalho A.F., Savino W., Reis L.F.L. Identification, structural characterization and tissue distribution of TSG-5 a new TNF-stimulated gene. Genes and Imunity (2003) 4:298-311.
2. Anelli A, Brentani R.R., Gadelha A.P., Albuquerque A.A., Soares F. Correlation of p53 status with outcome of neoadjuvant chemotherapy using paclitaxel and doxorubicin in stage III-B breast cancer. Annals of Oncology (2003) 14(3): 428-432.
3. Badal, V., Chuang, L.S.H., Tan, E.H.H., Badal, S., Villa, L.L., Wheeler, C.M., Li, B.F.L., Bernard, H.U. CpG methylation of Human Papillomavirus type 16 DNA in cervical cancer cell lines and in clinical specimens: Genomic hypomethylation correlates with carcinogenic progression. Journal of Virology (2003) 77: 6227-6234.
4. Brentani H., Caballero O.L., Camargo A.A., Silva A.M., Silva Junior W.A., Dias Neto E., Grivet M., Gruber A., Guimarães P.E.M., Hide W., Iseli C., Jongeneel V., Kelso J., Nagai M.A., Ojopi E.P.B. , Osório E.C., Reis E.M.R., Riggins G.J., Simpson A.J.G., Souza S.J., Brian, Stevenson B.J., Strausberg R.L., Tajara E.H., Verjovski S.A. The Generation and Utilization of a Cancer Oriented Representation of the Human Transcriptome, Using Expressed Sequence Tags Proceedings of the National Academy of Sciences USA (2003) 100(23):13418-13423.
5. Camargo A.A, Simpson A.J.G. Collaborative research networks work Journal of Clinical Investigation (2003) 112:468-471.
6. Caruso P., S. I. Meireles, Luiz F. L. Reis, T. Mauad T., Martins M.A., Deheinzelin D. Low tidal volume ventilation induces proinflammatory and profibrogenic responsein lungs of rats. Intensive Care Medicine (2003) (10):1808-11.
7. Carraro D.M., Camargo A.A., Salim A.C.M., Grivet M., Vasconcelos A.T., Simpson A.J.G. PCR assisted contig extension – PACE: a stepwise strategy for bacterial genome closure Biotechniques (2003) 34(3):626-8.
8. Carvalho, A.F., Neves, E.J., Hirata Jr. R., Reis, L.F.L. A glass slide-based agarose gel electrophoresis for determining the efficiency of RNA amplification and Cy-dye incorporation during RNA labeling. Biotechniques (2003) 35(4):688-690.
9. Coelho, F.R.G., Mann-Prado, J.C., Pereira-Sobrinho, J.S., Hamada, G., Landman, G., Pinto, Aa, Nonogaki, S., Villa, L.L. Estrogen and progesterone receptos in normal tissue, cervical intraepithelial neoplasia and invasive carcinoma of the uterine cervix. Brazilian Journal of Medical and Biological Research (2003) 37(1):83-88.
10. Coitinho A.S., Roesler R., Martins V.R., Brentani R.R., Izquierdo I. Cellular prion protein ablation impairs behavior as a function of age. Clinical Neuroscience (2003) 14 (10): 1375-1379.
11. Gomes I.L., Silva, R.L.A., Stolf, B.S., Cristo, E.B., Hirata Jr. R., Soares, F.A., Reis, L.F.L., Neves, E.J., Carvalho, A.F. Comparative analysis of Amplified and non-amplified RNA for Hidridization in cDna microarray. Analytical Biochemistry (2003) 321:248-255.
12. Jacchieri S.G., Torquato, R., Brentani, R.R. A Structural Study of the Binding of Flagellin by Toll-Like Receptor 5. Journal of Bacteriology (2003) 185: 4243-4247.
13. Logullo A.F., Nonogaki S., Miguel R.E., Kowalski L.P., Nishimoto I.N., Pasini F.S., Frederico M.H.H.,

Brentani R.R., Brentani M.M. Transforming growth factor B1 (TGFB1) expression in head and neck squamous cell carcinoma patients as related to prognosis. Journal of Oral Pathology and Medicine (2003) 32: 139-145.

14. Mackay A., Jones C., Dexter T., Silva R.L., Bulmer K., Jones A., Simpson P., Harris R.A., Jat P.S., Neville A.M., Reis L.F., Lakhani S.R., O'Hare M.J. cDNA microarray analysis of genes associated with ERBB2 (HER2/neu) over expression in human mammary luminal epithelial cells. Oncogene (2003) 22(17):2680-8.
15. Magalhães P.P., Queiroz D.M., Camargo A.A., Simpson A.J.G. Cag A status of helicobacter pylori infection and p53 gene mutations in gastric adenocarcinoma. Carcinogenesis (2003) 24:145.
16. Meireles S.I., Carvalho A.F., Hirata Jr. R., Montagnini A.L., Martins W.K., Runza F.B., Termini L., Chamberlein N., Silva R.L.A., Soares F. A., Neves E.J., Reis L.F.L. Differentially expressed genes in gastric tumors identified by cDNA array. Cancer Letters (2003) 190:199-211.
17. Nonnenmacher B., Pintos J., Bozzetti MC, Mielzinskalohnas I, Lorincz AT, Ikuta N, Schwartsmann, Villa LL, Schiller JT, Franco E. Epidemiologic correlates of antibody response to Human Papillomavirus among women at low risk of cervical cancer. International Journal of STD & AIDS (2003) 14: 258-265.
18. Osório E.C., de Souza J.E., Oliveira P.S.L., de Souza S.J. pp-Blast: a pseudo-parallel Blast. Brazilian Journal of Medical and Biological Research (2003) 36:1-2.
19. Palazzi M.A., Yunes J.A., Cardinalli I.A., Stangenhaus G.P., Ferreira A.S., Sobrinho J.S.P., Villa L.L. Detection of oncogenic Human Papillomavirus in sporadic retinoblastoma. Acta Opthalmologica Scandinia (2003) 81(4):396-398.
20. Picconi M.A., Alonio L.V., Sichero L., Mbayed V., Villa L.L., Gronda J., Campos R., Teyssie A.R. Human Papillomavirus type-16 variants in quechua aboriginals from Argentina. Journal of Medical Virology (2003) 69: 546-552.
21. Rabelo-Santos S.H., Zeferino L.C., Villa L.L., Sobrinho J.P., Amaral R.G., Magalhães A.V. Human papillomavirus prevalence among women with cervical intraepithelial neoplasia III and invasive cervical cancer form Goiânia-Brazil. Memórias do Instituto Oswaldo Cruz (2003) 98:181-184.
22. Rocha J.C., Silva R.L.A., Mendonça B.B., Marui S., Simpson A., Camargo A.A. High frequency of novel germline mutations in the VHL gene in the heterogeneous population of Brazil. Medical Genetics (2003) 40(3):e31, 2003
23. Rousseau M.C., Villa L.L., Costa MC, Abrahamovicz M., Rohan TE, Franco E. Occurrence of cervical infection with Multiple Human Papillomavirus types is associated with age and cytologic abnormalities. Sexually Transmitted Diseases (2003) 581-587.
24. Rousseau M.C., Villa L.L., Costa M.C., Abrahamovicz M., Rohan T.E., Franco E.L. Predictors of cervical coinfection with multiple human papillomavirus types. Cancer Epidemiology, Biomarkers & Prevention (2003) 12: 1029-1037.
25. Sakabe N.J., de Souza J.E.S., Galante P.E.A., de Oliveira P.S.L, Passett F., Brentani H., Osório E.C., Zaiats A.C., Leerkes M.R., Kitajima J.P., Brentani R.R., Strausberg R.L., Simpson A.J.G., de Souza S.J. ORESTES are enriched in rare exon variants affecting the encoded proteins Comptes Rendus Biologies (2003) 326:979-985.
26. Schlecht N.F., Platt, R.W., Negassa, A., Duarte-Franco, E., Rohan, T.E., Ferenzy A., Villa L.L. Modeling the time dependence of the assocoation between Human Papillomavirus infection and cervical cancer precursor lesions. American Journal of Epidemiology (2003) 158 (9):878-886.

27. Schlecht, N.F., Platt Rw, Duarte-Franco E, Costa M.C., Sobrinho J.P, Prado, J.C.M, Ferenczy,A., Rohan, T.E., Villa, L.L., Franco, E.L. Human Papillomavirus Infection as a Predictor of Progression and Regression of Cervical Cancer Precursor Lesions. Journal of the National Cancer Institute (2003) 95(17):1336-1343.

28. Schlecht NF., Trevisan A., Duarte-Franco E., Rohan T., Ferenczy A., Villa L.L., Franco E.L. Viral Load as a Predictor of Lesion Risk in the natural history of Cervical Intraepithelial Neoplasia. International Journal of Cancer (2003) 103(4):519-524.

29. Silva A.P.M., Salim A.C.M., Bulgarelli A., de Souza J.E.S., Caballero O., Iseli C., Stevenson B.J., Jongeneel C.V., de Souza S.J., Simpson A.J.G., Camargo A.A. Identification of 9 transcripts and two RGSL genes within the hereditary prostate cancer region (HPC1) at 1q25. Gene (2003) 310:49-57.

30. Souza, S.J. The emergence of a synthetic theory on intron evolution. Genetica (2003) 118:117-121.

31. Souza P.S.A., Villa, L.L. Genetic susceptibility to infection with Human Papillomavirus and development of cervical cancer in women in Brazil. Mutation Research (2003) 544: 375-383.

32. Sredni S.T., Soares F.A., de Caballero O.L., de Camargo B., Carpentieri D.F., Nichols K., Chou P.M., Mathews M., Pawel B., Huff D. The expression of WT1 in the differentiation of rhabdomyosarcoma from other pediatric small round blue cell tumors. Modern Pathology (2003) 16(11): 1178-9.

33. Sredni S.T., Zerbini M.C.N., Latorre M.R., Alves V.A.F. p53 as a prognostic factor in adrenocortical tumors of adults and children. Brazilian Journal of Medical and Biological Research (2003) 36:23-27

34. Sredni S.T., Alves V.A.F., Latorre M.R., Zerbini M.C.N. Adrenocortical tumours in children and adults: a study of pathological and proliferation features Pathology (2003) 35:130-135.

35. Vasconcelos A.T.R., Almeida D.F., Hungria M., Guimarães C.T., Antônio R.V., Almeida F.C., Almeida L.G.P., Almeida R.,Gomes A.A.A., Andrade E.M., Araripe J., Araújo M.F.F., Astolfi-Filho S., Azevedo V., Baptista A.J., Bataus L.A.M., Batista J.S., Belo A., Van den Berg C., Bogor M., Bonattor S., Bordignons J., Brigido M.M., Britot C.A., Brocchi M, Burity H.A., Camargo A.A, Cardoso D.D.P, Carneiro N.P., Carraro D.M., Carvalho C.M.B., Cascardo J.C.M., Cavada B.S., Chueire L.M.O., Creczynski-Pasa T.B., Cunha-Junior N.C., Fagundes N., Falcão C.L., Fantinatti F., Farias I.P., Felipe M.S.S., Ferrari L.P., Ferro J.A., Ferro M.I.T., Franco G.R., Freitas N.S.A., Furlan L.R., Gazzinelli R.T., Gomes E.A., Gonçalves P.R., Granjeiro T.B., Gratapaglia D., Grizard E.C., Hanna E.S., Jardim S.N., Laurino J., Leoi L.C.T., Lima L.F.A., Loureiro N.F., Lyra M.C.C.P., Madeira H.M.F., Manfio G.P., Maranhão A.Q., Martins W.S., Mauro S.M.Z., Medeiros S.R.B., Meissner R.V., Moreira M.A.N., Nascimento F.F., Nicolas M.F., Oliveira J.G., Oliveira S.C., Paixão R.F.C., Parente J.A., Pedrosa F.O., Pena S.D.J., Pereira J.O., Pereira M.., Pinto L.S.R.C., Pinto L.S., Porto J.I.R., Potrich D.P., Ramalho Neto C.E., Reis A.L.M.M., Vettore A., Simpson A.J.G. The complete genome sequence of chromabacterium violaceum reveals remarkable and exploitable bacterial adaptability. Proceedings of the National Academy of Sciences (2003) 100(20) 11660-11665.

36. Vêncio R., Brentani H., Pereira C.A. Using credibility intervals instead of hypothesis tests in SAGE analysis. Bioinformatics (2003) 19:1-4.

37. Vettore A.L., da Silva F.R., Kemper E.L., Souza G.M., da Silva A.M., Ferro M.I., Henrique-Silva F., Giglioti E.A., Lemos M.V., Coutinho L.L., Nobrega M.P., Carrer H., Franca S.C., Bacci Junior M., Goldman M.H., Gomes S.L., Nunes L.R., Camargo L.E., Siqueira W.J., Van Sluys M.A., Thiemann O.H., Kuramae E.E., Santelli R.V., Marino C.L., Targon M.L., Ferro J.A., Silveira H.C., Marini D.C., Lemos E.G., Monteiro-Vitorello C.B., Tambor J.H., Carraro D.M., Roberto P.G., Martins V.G., Goldman G.H., de Oliveira R.C., Truffi D., Colombo C.A., Rossi M., de Araujo P.G., Sculaccio S.A., Angella A., Lima M.M., de Rosa Junior V.E., Siviero F., Coscrato V.E., Machado M.A., Grivet L., Di

Mauro S.M., Nobrega F.G., Menck C.F., Braga M.D., Telles G.P., Cara F.A., Pedrosa G., Meidanis J., Arruda P. Analysis and funstional annotation of an expressed sequence tag collection for tropical crop sugarcane. Genome Research (2003) 13(12):2725-2735.

38. Villa L.L. Vaccines against papillomavirus infections and disease – Salud Pública de México (2003) 45(2):318-323.

39. Walz R., Castro R.M.R.P.S., Velasco T.R., Alexandre Jr.V., Lopes M.H., Leite J.P., Santos A.C., Assirati Jr. J.Á., Wichert-Ana L., Bustamante V.C.T., Bianchin M.M., Maciag P.C., Ribeiro K.B., Guarnieri R., Araujo D., Caballero O., Moura R., Salim ACM, Kindlmann K., Landenberger NC, Marques Jr. W., Fernandes R.M.F., Serafini L.N., Machado H.R., Carlotti Jr C.G., Brentani R.R., Sakamoto A.C., Martins V.R. Surgical outcome in mesial temporal sclerosis correlates with prion protein gene variant. Neurology (2003) 61:1204-1209, 2003.

40. Zanker K.S., Brentani R.R., Mihich E. International symposium on new leads in experimental and clinical gene targeting in cancer. Journal of Cancer Research Clinical Oncology (2003) 129:258-261.

Reviews / Book Chapters / Commentaries

1. Boccardo E., Villa L.L. Vírus e Câncer. In: Bases da Oncologia. Eds.: Maria Mitzi Brentani, Francisco Ricardo Gualda Coelho e Luiz Paulo Kowalski. Editora Marina e Tecmed Editora., cap 6. - pp. 87 111, 2003.

2. Camargo, A.A. & Costa, F.F. Onocogenes e Genes Supressores de Tumor. In: Bases da Oncologia. (ed. Brentani M.M., Coelho F.R.G., Kowalski L.P.). Editora Lemar, São Paulo, SP. Pags71-86, 2003

3. Iftner T., Villa L.L. Human Papillomavirus Technologies, Chapter 12, Journal of the National Cancer Institute Monographs, 31:80-88, 2003

4. Lee K.S., Linden R., Prado M.A.M., Brentani R.R., Martins V.R. Towards cellular receptor for prions. Reviews in Medical Virology (2003) 13:399-408.

LUDWIG INSTITUTE FOR CANCER RESEARCH

Stockholm Branch
of Molecular and Cellular Biology

Stockholm, Sweden

Staff List

Pettersson Ralf. F. *Director, Member*

Protein Transport Group

Pettersson, Ralf. F. *Head*
Fuxe, Jonas *Postdoctoral Fellow, part-time*
Neve, Etienne *Postdoctoral Fellow*
Sollerbrant, Kerstin *Postdoctoral Fellow*
Bergström, Anita *Senior Technician*
Svensson, K. *Senior Technician (until May)*
Mirza, Momina *Ph.D. Student*
Raschperger, Elisabeth *Ph.D. Student*
Vincent, Theresa *Ph.D. Student (from August)*
Överby, A. *Ph.D. Student (from June)*

Developmental Biology Group

Eriksson, Ulf *Head, Member*
Li, Xuri *Assistant Investigator*
Fieber, Christina *Postdoctoral Fellow*
Rezaian, Soheilla *Technician (from May)*
Åkerblom, Barbara *Senior Technician*
Ehnman, Monika *Ph.D. Student (from August)*
Folestad, Erika *Ph.D. Student*
Fredriksson, Linda *Ph.D. Student*
Li, Hong *Ph.D. Student*
Lidén, Martin *Ph.D. Student*
Pontén, Annica *Ph.D. Student*
Tryggvason, K. *Ph.D. Student*

Yeast Cell and Molecular Biology Group

Ljungdahl, Per O. *Head, Associate Member*
Andersson, Jessica *Postdoctoral Fellow*
Heessen, Stijn *Postdoctoral Fellow (from November)*
Martinez Rodriguez, Paula *Postdoctoral Fellow*
Nyman, Tomas *Postdoctoral Fellow*
Zargari, A. *Postdoctoral Fellow (until August)*
Andréasson, Claes *Ph.D. Student*
Boban, Mirta *Ph.D. Student*
Kota, Jhansi *Ph.D. Student*

Gene Expression Group

Perlmann, Thomas *Head, Member*
Benoit, Gerard *Postdoctoral Fellow*
Borgius, Lotta *Postdoctoral Fellow*
Malewicz, Michal *Postdoctoral Fellow (from February)*
Mata de Urquiza, A. *Postdoctoral Fellow (until March)*
Muhr, Jonas *Postdoctoral Fellow*
Petersson, Susanna *Postdoctoral Fellow*

Werme, Martin *Postdoctoral Fellow*
Wu, X. *Postdoctoral Fellow*
Joodmardi, Eliza *Technician*
Bylund, Magdalena *Ph.D. Student*
Friling, Stina *Ph.D. Student*
Hermanson, Elisabeth *Ph.D. Student*
Lengquist, Johan *Ph.D. Student*
Nordzell, Mariette *Ph.D. Student*
Sandberg, Magnus *Ph.D. Student (from October)*
Volakakis, Nikolaos *Ph.D. Student (from October)*

Technical Support

Tollman, Inger *Laboratory Manager*
Anderling, Mats *Systems Administrator*
Enrione Barrera, Erika *Laboratory Assistant*

Administration & Secretariat

Linderholm, Charlotte *Administrator*
Freidenfelt, Birgitta *Secretary and Assistant to the Administrator*

Branch Director's Report

The research program of the four groups at the Stockholm Branch spans quite broad areas in the general field of cell and molecular biology. Research interests during the past year have centered on the following topics: Regulation of export of membrane proteins from the endoplasmic reticulum (ER); function of the coxsackie- and adenovirus receptor; mechanisms of cellular nutrient assessments and metabolic compartmentalization in yeast; cell biology of retinoids (vitamin A compounds); mechanisms of action of nuclear receptors, including the retinoid receptors and a subclass of orphan receptors, particularly in the context of the central nervous system; characterization of novel growth factors and their receptors regulating the formation of new blood vessels (angiogenesis).

Ralf F. Pettersson

Research Report

Protein Transport Group

During the past year, research in the Protein Transport Group, led by Dr. Ralf Pettersson, has focused on three separate projects: (i) the role of transmembrane lectin receptors in the export of soluble glycoproteins from the endoplasmic reticulum (ER) in mammalian cells, (ii) the analyses of the normal function of the Coxsackie- and Adenovirus Receptor (CAR), and (iii) the development of a reverse genetics system for members of the *Bunyaviridae* family of RNA viruses.

(i) The three-dimensional crystal structure of the non-calcium-bound form of p58/ERGIC-53, a receptor facilitating the export of a subset of glycoproteins from the ER to the Golgi complex, was solved to 1.46 A resolution in 2002. During the past year, the active, calcium-bound form of p58 was also successfully solved. The structure revealed striking similarities with plant leguminous lectins, but also some interesting differences in regard to metal-binding ions. A new member of the mammalian membrane-bound ER lectins was identified and characterized. This lectin was related to a previously characterized protein, VIP-36, and hence named VIP-36-like protein (VIPL). It localizes to the ER and may be involved in the regulated export of a subclass of secretory proteins.

(ii) About two years ago, the Group initiated a new program to study the normal cellular function of CAR. This receptor is required for the uptake of adenoviruses and coxsackie B viruses onto cells. CAR is a member of a growing family of cell surface proteins (called the CTX-family) belonging to the Ig superfamily. Members include CTX, JAM A-D, A33, ESAM, and BT-IgSF. In a yeast two-hybrid screen, the PDZ-domain protein called LNX (Ligand of Numb protein X) was found to bind to the cytoplasmic tail of CAR. Two splice variants of CAR, both terminating in a PDZ-binding motif (TVV and SIV, respectively), were shown to be important for binding. CAR bound to the second (out of four) PDZ-domain of LNX. CAR localizes to tight junctions of epithelial cells. Since PDZ-proteins are known to organize cell surface proteins, LNX may serve such a function. CAR expression was found to inversely correlate with the malignancy grade of primary human astrocytomas (grades II-IV), suggesting that CAR might function as a tumor suppressor gene. A new member of the CTX-family was identified and characterized. Since it is most closely related to CAR it was named CLMP (CAR-like Membrane Protein). It also localizes to tight junctions of polarized epithelial cells and mediates homophilic cell-cell interaction.

(iii) A long-standing interest in the molecular and cell biology of a large family of RNA viruses, the *Bunyaviridae*, has recently resulted in the development of a polymerase I-dependent "reverse genetics system". The system has allowed us to analyze in detail the role of individual nucleotides of the promoter region of the three viral genomic RNA segments. The focus is currently on the identification of the packaging signals that enable the virus to package its genome into virions. The long-term aim is to be able to create infectious virus entirely from cloned RNA segments.

Yeast Cell and Molecular Biology Group

The Yeast Group, led by Dr. Per Ljungdahl, has continued to explore the molecular basis of how eukaryotic cells assess the availability of nutrients present in their growth environments. The focus is on the regulation of amino acid uptake in baker's yeast *Saccharomyces cerevisiae*, which offers an attractive system to pursue basic mechanisms of signal transduction. The Group has previously shown that Stp1p and Stp2p are homologous and redundant transcription factors that are synthesized as latent cytoplasmic proteins with N-terminal regulatory domains. In response to extracellular amino acids, the plasma membrane localized SPS-sensor, composed of three proteins, induces an endoproteolytic processing event that cleaves away the regulatory domains. The shorter forms of Stp1p/Stp2p are targeted to the nucleus were they bind and activate transcription of amino acid permease genes. As many questions remain as to the precise mechanisms governing the activity of latent factors in metazoan cells, the work in yeast is intended to

serve as paradigms for understanding similar regulatory networks that function to control proper growth and development of mammalian cells.

During the last year, the team made three noteworthy advances in our understanding of amino acid based signaling in yeast. First, they found that two conserved motifs within the N-terminal domains of Stp1p/Stp2p are required for proper nuclear exclusion and proteolytic processing, respectively. These motifs function in parallel; mutations that abolish processing abrogate signaling, whereas mutations that interfere with cytoplasmic retention result in constitutive derepression of SPS-sensor regulated genes independently of processing. The N-terminal regulatory domain of Stp1p is transferable and confers full SPS-sensor pathway control when fused to an unrelated synthetic transcription factor. Our analysis suggests that the regulation of Stp1p/Stp2p occurs with greater similarity to the activation of the well-studied latent metazoan factor NFκB/Rel than previously thought. Second, the team found that a novel membrane protein (Asi1p) functions to block unprocessed Stp1p/Stp2p from gaining access to the nucleus. This latter finding provides the first clue to what appears to be a completely new mode of gene regulation. Finally, the Group discovered that the opportunistic human fungal pathogen *Candida albicans* possesses all of the components of amino acid signaling found in baker's yeast. A *Candida* strain lacking the SPS-sensing pathway has a reduced capacity to take up amino acids, and is unable to undergo proper developmental switching in response to inducing amino acids. Strikingly, this mutant strain is unable to efficiently mount virulent infections in a mouse model. The analysis strongly suggests that *C. albicans* cells use amino acids, presumably as nitrogen sources, during growth in mammalian hosts.

Developmental Biology Group

The research program of the Group, led by Dr. Ulf Eriksson, focuses on the biology of members of the vascular endothelial growth factor/platelet-derived growth factor (VEGF/PDGF) family, and their role in angiogenesis, tissue repair, and tumorigenesis.

The VEGF-B project. Vascular endothelial growth factor B (VEGF-B), identified by the group in 1995, is expressed in many tissues, and is abundant in heart, muscle and brain, but its exact physiological role is unclear. Using several tumor models in bone marrow-reconstituted mice carrying genetically tagged bone marrow, the team has shown that the matrix-bound isoform VEGF-B_{167} is able to attract bone marrow-derived cells more efficiently than the freely soluble isoform VEGF-B_{186}. Thus, VEGF-B contributes to the stromal reaction in tumors. In collaboration with others, it has been that VEGF-B_{167}, delivered as a recombinant protein via osmotic mini-pumps, or generated by an adenoviral infection, induces a potent angiogenic response in a heart infarction model, while it is inactive in a hind limb ischemia model. The differential effects seen suggest that VEGF-B is a relatively heart-specific angiogenic growth factor with very few observed side effects. Future aims are to mechanistically address the roles of VEGF-B in therapeutic and pathological angiogenesis.

The PDGF project. The Group previously identified two genes as novel members of the platelet-derived growth factor (PDGF) family. These factors, denoted PDGF-C and PDGF-D, display a unique structural organization, and unique receptor binding properties. The presence of an N-terminal CUB domain in both factors is novel among the PDGFs, and proteolytic removal of the CUB domain is necessary for biological activity. The research team identified tissue plasminogen activator (tPA) as a specific activator of PDGF-C, but not PDGF-D. tPA interacts directly with both domains of PDGF-C, and the interaction with the CUB domain is necessary for proteolytic activation. Studies using tPA-deficient primary fibroblasts suggest that activated fibroblasts express both PDGF-C and tPA, and that the growth of the cells in culture is dependent on an autocrine growth stimulatory loop involving tPA-mediated activation of PDGF-C. This stimulatory loop may have a role in angiogenesis, tumor stroma reactions, and in tissue regeneration, including diabetic wound healing. Efforts are also underway to identify the specific protease(s) that is involved in activation of PDGF-D. PDGF-C treatment in the heart infarction and hind limb models results in the stimulation of angiogenesis in both tissues, and in muscle regeneration in the limb. At least in part

the biological effects of PDGF-C are the result of stimulation of vascular progenitor cells. Similar studies involving PDGF-D are under way.

Gene Expression Group

The Gene Expression Group, led by Dr. Thomas Perlmann, is analyzing nuclear receptors; ligand-regulated transcription factors that bind steroid hormones, thyroid hormone, retinoids and other small and lipophilic signaling molecules. These receptors are fascinating proteins for several different reasons: First, they are excellent tools for understanding how genes are regulated since small molecule ligands can be used to switch these transcription factors between active and inactive states. Second, classical nuclear receptor signaling pathways, e.g. steroid hormone and retinoid receptors, influence many biological pathways important in development and adult physiology. Accordingly, their significance in disease, including cancer, is critical. Third, nuclear receptors include a large number of related but less well characterized orphan receptors lacking identified ligands. The existence of these proteins is intriguing and suggests that additional unexplored nuclear receptor-mediated signaling pathways remain to be characterized.

Nurr1 is an orphan nuclear receptor, previously shown by the Gene Expression Group to be critically involved in dopamine cell development. Dopamine cells are clinically important since they degenerate in Parkinson's disease and are important in several other disorders, including schizophrenia. The Gene Expression Group has continued the characterization of how Nurr1 regulates the process of dopamine cell differentiation and found that the cyclin dependent kinase (CDK) inhibitor p57Kip2 is a regulated target gene of Nurr1. Moreover, p57Kip2 functions by an unusual mechanism that does not involve inhibition of CDKs, but instead requires direct protein-protein interaction with Nurr1. Thus, in the context of dopamine cell development p57Kip2 functions as a transcriptional co-factor. The group also presented surprising data showing that Nurr1's ligand binding domain lacks a cavity for ligand-binding. Thus, Nurr1 defines the first ligand-independent member of the nuclear receptor family. However, despite the inability to interact with cognate ligands, Nurr1 functions in ligand-induced signaling as a heterodimerization partner with the retinoid X receptor. In an additional study that was completed during 2003, the group showed that these heterodimers can promote the survival of neuronal cells; such a function that might be of significance in neurodegenerative disease. Ongoing studies aim to test this hypothesis.

Finally, the Group is also studying the mechanisms by which neurons are formed from stem cells (neurogenesis). This process involves the activity of proneural basic helix-loop-helix transcription factors. It was found that the HMG-box transcription factors Sox1-3 are critical determinants of neurogenesis. Using chick *in ovo* electroporation technology, it was found that Sox1-3 keep neural cells undifferentiated by counteracting the activity of proneural proteins. The data suggest that the generation of neurons from stem cells depends on the inhibition of Sox1-3 expression by proneural proteins.

Publications

Primary Research Articles

1. Bertl A., Ramos J., Ludwig J., Lichtenberg-Fraté H., Reid J., Bihler H., Calero F., Martínez P., Ljungdahl P.O. Characterization of potassium transport in wild-type and isogenic yeast strains carrying all combinations of *trk1*, *trk2*, and *tok1* null mutations. Molecular Microbiology (2003) 47:767-780.
2. Bylund M., Andersson E., Novitch B., Muhr J. Vertebrate neurogenesis is counteracted by Sox1-3 activity. Nature Neuroscience (2003) 6 (11):1162-1168.
3. Bäckman C., You Z-B., Perlmann T., Hoffer B.J. Elevated locomotor activity without altered striatal contents in Nurr1 heterozygous mice after acute exposure to methamphetamine. Behavioral Brain Research (2003) 143:95-100.
4. Eitner F., Ostendorf T., Kretzler M., Cohen C.D. (for the ERCB-Consortium), Eriksson U., Gröne H-J., Floege J. PDGF-C expression in the developing and normal adult human kidney and in glomerular diseases. Journal of the American Society of Nephrology (2003) 14:1145-1153.
5. Evstafieva A.G., Belov G.A., Rubtsov G.Y.P., Kalkum M., Joseph B., Chichkova N.V., Sukhacheva E.A., Bogdanov A.A., Pettersson R.F., Agol V.I., Vartapetian A.B. Apoptosis-related fragmentation, translocation, and properties of human prothymosin alpha. Experimental Cell Research (2003) 284:211-223.
6. Flick K., Hooper J., Schmaljohn C., Pettersson R.F., Feldmann H., Flick R. Rescue of Hantaan virus minigenomes. Virology (2003) 306:219-224.
7. Fuxe J., Liu L., Malin S., Philipson L., Collins V.P., Pettersson R.F. Expression of the coxsackie- and adenovirus receptor in human astrocytic tumors and xenografts. International Journal of Cancer (2003) 103:723-729.
8. Hermanson E., Joseph B., Castro D., Lindqvist E., Aarnisalo P., Wallen A., Benoit G., Hengerer B., Olson L., Perlmann T. Nurr1 regulates dopamine synthesis and storage in MN9D dopamine cells. Experimental Cell Research (2003) 288:324-334.
9. Joseph B., Marchetti P., Lefebvre O., Schraen-Maschke S., Méreau-Richard C., Formstecher P. The novel retinoid AHPN/CD437 induces a rapid but incomplete apoptotic response in human myeloma cells. Biochimica et Biophysica Acta (2003) 1593:277-283.
10. Joseph B., Wallén-Mackenzie Å., Benoit G., Murata T., Joodmardi E., Okret S., Perlmann T. p57kip2 cooperates with Nurr1 in developing dopamine cells. Proceedings of the National Academy of Sciences USA (2003) 100:15619-15624.
11. Li H., Fredriksson L., Li X., Eriksson U. PDGF-D is a potent transforming and angiogenic growth factor. Oncogene (2003) 22:1501-1510.
12. Molinas C.R., Campo R., Dewerchin M., Eriksson U., Carmeliet P., Koninckx P.R. The role of vascular endothelial growth factor and placental growth factor in basal adhesion formation and in CO_2 pneumoperitoneum-enhanced adhesion formation following laparoscopic surgery in transgenic mice. Fertility and Sterility (2003) 80: Suppl 2, 803-811.
13. Neve E.P.A., Svensson K., Fuxe J., Pettersson R.F. VIPL, a VIP36-like membrane protein with a putative function in the export of glycoproteins from the endoplasmic reticulum. Experimental Cell Research (2003) 288:70-83.

14. Pontén A., Li X., Thorén P., Aase K., Sjöblom T., Östman A., Eriksson U. Overexpression of PDGF-C in heart induces cardiac fibrosis, hypertrophy and dilated cardiomyopathy. American Journal of Pathology (2003) 163:673-682.

15. Sockanathan S., Perlmann T., Jessell T.M. Retinoid receptor signaling in postmitotic motor neurons regulates rostrocaudal positional identity and axonal projection pattern. Neuron (2003) 40:97-111.

16. Sollerbrant K., Raschperger E., Mirza M., Engström U., Philipson L., Ljungdahl P., Petterson R.F. The Coxsackievirus and Adenovirus Receptor (CAR) forms a complex with the PDZ domain-containing protein Ligand-of-Numb protein-X (LNX). Journal of Biological Chemistry (2003) 278:7439-7444.

17. Tille J.C., Wang X., Lipson K.E., McMahon J., Ferrara N., Zhu Z., Hicklin D.J., Sleeman J.P., Eriksson U., Alitalo K., Pepper M.S. Vascular endothelial growth factor (VEGF) receptor-2 signaling mediates VEGF-$C_{\Delta N \Delta C}$- and VEGF-A -induced angiogenesis in vitro. Experimental Cell Research (2003) 285:286-298.

18. Veitonmäki N, Fuxe J, Hultdin M., Roos G., Pettersson R.F., Cao Y. Immortalization of bovine capillary endothelial cells by hTERT alone involves inactivation of endogenous $p16^{INK4}$/pRB. FASEB Journal (2003) 17:764-766.

19. Velloso L.M., Svensson K., Pettersson R.F., Lindqvist Y. The crystal structure of the carbohydrate recognition domain of the glycoprotein sorting receptor p58/ERGIC-53 reveals a novel metal binding site and conformational changes associated with calcium ion binding. Journal of Molecular Biology (2003) 334:845-851.

20. Wang Z., Benoit G., Liu J., Prasad S., Aarnisalo P., Liu X., Xu H., Walker P.C., Perlmann T. Structure and function of Nurr1 reveals a novel class of ligand-independent nuclear receptors. Nature (2003) 423:555-560.

21. Wallén-Macenzie Å., Mata de Urquiza A., Petterson S., Rodriguez F.J., Friling S., Wagner J., Ordentlich P., Lengqvist J., Heyman R.A., Arenas E., Perlmann T. Nurr1-RXR heterodimers mediate RXR ligand-induced signaling in neuronal cells. Genes & Development (2003) 17:3036-3047.

22. Werme M., Hermanson E., Carmine A., Buervenich S., Zetterström R.H., Thorén P., Ögren S-O., Olson L., Perlmann T., Brené S. Brain reward circuitry is regulated by Nurr1. European Journal of Neuroscience (2003) 17: 2418-2424.

Reviews / Book Chapters / Commentaries

1. Eriksson U., Li X. VEGF-B. In "Encyclopedia of Hormones", Henry H & Norman A (Eds.), Academic Press, 2003.

2. Li X., Eriksson U. Novel PDGF family members: PDGF-C and PDGF-D. Cytokine & Growth Factor Reviews (2003) 14:91-98.

3. Lidén M., Tryggvason K., Eriksson U. Structure and function of retinol dehydrogenases of the short chain dehydrogenase/reductase (SDR) superfamily. Molecular Aspects of Medicine (2003) 24:403-406.

4. Mata de Urquiza A., Perlmann T. In vivo reporter systems for receptor and ligand activities. Methods in Enzymology (2003) 364:463-75.

5. Mata de Urquiza A., Perlmann, T. Structure and function of retinoid receptors RAR and RXR. In "Cellular proteins and their fatty acids in health and disease", Dutta-Roy AK and Spener F, (Eds). Wiley-CSH, Weinheim, Germany, 191-207, 2003.

6. Tryggvason K. Retinoid processing in vivo – characterization and structure-function analysis of

retinol dehydrogenases. PhD Thesis, Karolinska Institute, Stockholm 2003.

7. Wallén Å., Perlmann T. Transcriptional control of dopamine neuron development. Annals of the New York Academy of Sciences (2003) 991:48-60.

LUDWIG INSTITUTE FOR CANCER RESEARCH

Uppsala Branch
of Growth Regulation

Uppsala, Sweden

Staff List

Heldin, Carl-Henrik *Director*

Growth Regulation Group

Östman, Arne *Associate Member, Group Head*
Hellberg, Carina *Assistant Investigator*
Furuhashi, Masao *Postdoctoral Fellow*
Kappert, Kai *Postdoctoral Fellow (from August)*
Micke, Patrick *Postdoctoral Fellow*
Pietras, Kristian *Postdoctoral Fellow (until February)*
Sjöblom, Tobias *Postdoctoral Fellow (until August)*
Bäckström, Gudrun *Senior Technical Assistant*
Sandström Leppänen, Jill *Technical Assistant*
Hägerstrand, Daniel *Ph.D. Student*
Persson, Camilla *Ph.D. Student (until June)*
Sandin, Åsa *Ph.D. Student (from May)*
Sundquist, Christina *Ph.D. Student (from September)*

Signal Transduction Group

Demoulin, Jean-Baptiste *Postdoctoral Fellow, Section Head (to October)*
Chiara, Federica *Postdoctoral Fellow*
Johannesen, Lene *Postdoctoral Fellow (until July)*
Rorsman, Charlotte *Senior Technical Assistant*
Kallin, Anders *Ph.D. Student*

Cytoskeletal Regulation Group

Aspenström, Pontus *Assistant Member, Group Head*
Hájková, Lucie *Postdoctoral Fellow (joint with Molecular Signaling Group)*
Saras, Jan *Postdoctoral Fellow (to March)*
Ruusala, Aino *Senior Technical Assistant*
Edlund, Sofia *Ph.D. Student (until June)*
Fransson, Åsa *Ph.D. Student*
Johansson, Ann-Sofi *Ph.D. Student*
Richnau, Ninna *Ph.D. Student*

Gene Targeting Group

Heuchel, Rainer, *Assistant Member, Group Head*
Li, Ronggui, *Postdoctoral Fellow*
Åhgren, Aive, *Senior Technical Assistant*

Molecular Signaling Group

Dikic, Ivan, Assistant Member *Group Head (until January)*
Hájková, Lucie *Postdoctoral Fellow (joint with Cytoskeletal Regulation Group) (until June)*
Soubeyran, Philippe *Postdoctoral Fellow (until September)*
Ivankovic-Dikic, Inga *Postdoctoral Fellow (until June)*
Haglund, Kaisa *Ph.D. Student (until June)*
Husnjak, Koraljka *Visiting Ph.D. Student (to February)*
Kowanetz, Katarzyna *Ph.D. Student*

Szymkiewicz, Iwona *Ph.D. Student (to June)*
Zapart, Grzegorz *Ph.D. Student (January to March)*

TGF-β Signaling Group

Moustakas, Aristidis *Assistant Member, Group Head*
Niimi, Hideki *Postdoctoral Fellow*
Valcourt, Ulrich *Postdoctoral Fellow*
Morén, Anita *Senior Technical Assistant*
Kowanetz, Marcin *Ph.D. Student*
Pardali, Katerina *Ph.D. Student*
Ritvos, Olli *Visiting Investigator (March to August)*

Integrated Signaling Group

Souchelnytskyi, Serhiy *Assistant Member, Group Head*
Dubrovska, Anna *Postdoctoral Fellow (from April)*
Iwahana, Hiroyuki *Postdoctoral Fellow*
Lomnytska, Marta *Postdoctoral Fellow (from September)*
Stasyk, Taras *Postdoctoral Fellow (until July)*
Yakymovych, Ihor *Postdoctoral Fellow*

Apoptotic Signaling Group

Landström, Maréne *Assistant Member, Group Head*
Edlund, Sofia *Postdoctoral Fellow (from July)*
Kozakai, Takaharu *Postdoctoral Fellow (until March)*
Lee, So Young *Postdoctoral Fellow (from April)*
Zhang, Shouting *Postdoctoral Fellow (from August)*
Grimsby, Susanne *Senior Technical Assistant*

Gene Expression Group

Ericsson, Johan *Assistant Member, Group Head*
Grönroos, Eva *Assistant Investigator*
Bengoechea Alonso, Maria Teresa *Postdoctoral Fellow*
Sundqvist, Anders *Postdoctoral Fellow*
Lukiyanehuk, Vasyl *Ph.D. Student (from November)*
Simonsson, Maria *Ph.D. Student*

Matrix Biology Group

Heldin, Paraskevi *Associate Investigator, Group Head*
Asteriou, Trias *Postdoctoral Fellow*
Li, Yuejuan *Postdoctoral Fellow (from October)*
Takahashi, Yoshinori *Postdoctoral Fellow*
Kamiryo, Masaru *Ph.D. student (from July)*
Li, Lingli *Ph.D. Student*

Protein Structure Group

Hellman, Ulf *Member, Group Head*
Engström, Ulla *Senior Technical Assistant*
Wernstedt, Christer *Senior Technical Assistant*

Technical Support

Ejdesjö, Bengt *Purchasing Officer*
Hedberg, Ulf *IT support*
Hermansson, Lars-Erik *Service Engineer*
Pettersson, Gullbritt *Laboratory Assistant*
Schönquist, Inger *Laboratory Assistant*

Administration & Secretariat

Hallin, Eva *Finance and Administration Manager*
Schiller, Ingegärd *Secretary*

Branch Director's Report

Malignant cells are characterized by perturbations in the signaling pathways that regulate cell growth, chemotaxis and migration. The aim of the work at the Uppsala Branch is to elucidate the molecular mechanisms involved in the regulation of these events, and to use such knowledge for the development of useful treatment regimens for cancer patients. During 2003, the Branch was comprised of 11 groups working on different aspects of signal transduction mechanisms. Important themes of the work are, as before, studies on platelet-derived growth factor (PDGF), a major mitogen for connective tissue cells, and transforming growth factor β (TGF β), which inhibits the growth of most cell types.

During the year, the Head of the Molecular Signaling Group, Dr. Ivan Dikic, left to take up a position as professor at Frankfurt University, the Head of the Signal Transduction Group, Dr. Jean-Baptiste Demoulin, left for a Group Leader position at the Catholic University of Louvain in Brussels, and the Head of the Growth Regulation Group, Dr. Arne Östman, left for a professorship at the Karolin's Institute in Stockholm. We wish Ivan, Jean-Baptiste and Arne good luck in their future work and we look forward to future interactions with them. Aspects of the PDGF-related programs of these groups will be continued at our Branch in a novel group with Carl-Henrik Heldin as Group Leader, and with Johan Lennartsson as Head for a section on Signal Transduction, and Carina Hellberg as Head for a section of Translational Research. The aim of this group will be to elucidate the molecular mechanism for PDGF induced cell growth and chemotaxis, and to explore the utility of PDGF antagonists in cancer treatment using preclinical models and patient studies.

Carl-Henrik Heldin

Research Report

Growth Regulation Group

In the Growth Regulation Group, led by Dr. Arne Östman, work continued along two lines: platelet-derived growth factor (PDGF) receptors as cancer drug targets, and; the function and regulation of protein tyrosine phosphatases (PTPs), which are PDGF antagonists. In addition, a project aimed at tumor stroma characterization was initiated, with the ultimate goal of identifying novel drug targets for this tumor compartment. The role of PDGF in the growth of human glioblastoma multiforme (GBM) was investigated by treating primary GBM cultures with the PDGFR receptor kinase inhibitor Glivec. The tumor's sensitivity to the inhibitor correlated with the levels of PDGF receptor expression, and gene-expression analyses showed that the expression of less than five genes could predict this response. The findings identify a PDGF-dependent GBM subset, characterized by high PDGF receptor expression and a specific gene expression pattern.

Most solid tumors show perivascular PDGFβ receptor expression, and therefore the role of PDGF in tumor pericyte recruitment was also analyzed. In tumors overexpressing PDGFβ receptor, increased pericyte coverage was found, in the absence of increased vessel density and increased tumor growth. Thus it appeared that PDGF contributes to tumor growth by enhancing tumor vessel function. This suggested targeting of PDGF-dependent pericyte recruitment as a novel way to enhance the anti-angiogenic effects of, for example, vascular endothelial growth factor (VEGF) antagonists. In experimental tumor models, inhibition of PDGF receptors in tumor stroma enhanced the tumor-specific uptake of chemotherapy drugs. A study investigating if these interesting findings can be reproduced in a clinical setting has been initiated, and is expected to be completed during the autumn of 2004.

Signal Transduction Group

A first line of research of the Signal Transduction Group, led by Dr. Jean-Baptiste Demoulin, dealt with the mechanism of activation of the PDGF receptor tyrosine kinase using crystallography and mutagenesis. A panel of PDGF β receptor mutants with progressive C-terminal truncations was used, and it was found that allosteric inhibition of the PDGF β-receptor by its C-terminal tail is one of the mechanisms involved in keeping the receptor inactive in the absence of ligand. The group also analyzed the role of signaling proteins associated with the PDGF receptor, mainly Gab1 and NHERF. It was found that Gab1 played a selective role in regulation of the mitogen-activated protein kinases Erk and p38 downstream of the PDGF β-receptor, and contributed to cytoskeletal reorganization and chemotaxis in response to PDGF.

Finally, the regulation of gene expression by PDGF in fibroblasts was analyzed using cDNA microarrays produced by the Sanger Center/LICR/Cancer Reserch UK Microarray Consortium. Among the significantly regulated transcripts up-regulated by PDGF after 24 h of treatment was a cluster of genes involved in fatty acid and cholesterol biosynthesis, all of which are controlled by sterol regulatory element-binding proteins (SREBP). SREBP activation was dependent on the phosphatidylinositol 3 kinase (PI3K) pathway, and the expression of these genes correlated with increased membrane lipid production. In conclusion, our results suggest that growth factors induce membrane lipid synthesis via the activation SREBP and PI3K.

Cytoskeletal Regulation Group

The work in the Cytoskeletal Regulation Group, led by Dr. Pontus Aspenström, focused on studies of the molecular mechanisms that control cell migration and cell growth under normal physiological conditions as well as during disease. Signaling pathways involving the Rho family of small GTPases, and particularly the selective activation of Cdc42 and RhoA, have been found to be of particular importance for the organization of the actin cytoskeleton and thereby the control of cell morphogenesis and cell migration.

The group showed that the inhibitory Smad7 is required for the TGF-β-induced activation of Cdc42 and that the PI3K signaling pathway is required for the TGF-β-induced mobilization of the actin cytoskeleton. The group is characterizing several novel Rho GTPase family members, including the Cdc42-related protein Wrch1. The group is also analyzing the recently identified mammalian protein WIRE (for WIP-related), which was shown to participate in the mobilization of the actin cytoskeleton and internalization of the PDGF β-receptor. WIRE has been shown to be associated with the Wiskott-Aldrich syndrome protein (WASP) family, now recognized as important regulators of actin polymerization. WIRE, therefore, constitutes a possible link between the actin cytoskeleton and receptor endocytosis machinery.

Gene Targeting Group

This group, led by Dr. Rainer Heuchel, uses gene targeting in the mouse in an effort to explore the in vivo importance of specific signaling pathways initiated by different growth factors.

In collaboration with another laboratory, the Gene Targeting Group generated mice with point mutated PDGF β-receptors that were unable to bind, and therefore activate, PI3K upon ligand stimulation, a prerequisite to signal actin cytoskeletal rearrangements, proliferation and inhibition of apoptosis in cell culture experiments. Surprisingly, these mice had no obvious phenotype, but showed a defect in the regulation of the interstitial fluid homeostasis. In order to further restrict signaling from the PDGF-β-receptor, an additional point mutation was introduced, such that neither PI3K nor phospholipase-Cγ (PLCγ) were able to bind to the activated PDGFR-β. Although double mutant mouse embryonic fibroblasts exhibited reduced proliferation and migration in response to PDGF, mutant mice showed no overt phenotype. However, in a model of experimental glomerulonephritis, mesangial cell defects were observed, and it was also shown that the double mutant cells were defective in colonizing the vascular smooth muscle cell compartment. In order to investigate the possible involvement of the PDGF β-receptor in disease, two mouse models with point mutations in the activation loop of the kinase domain were generated. Analogous mutations have been found in patients with hereditary papillary renal carcinoma and mastocytosis, and gastric carcinomas. The characterization of these mice is ongoing.

The group also investigated the promoter of the mouse Smad7 gene, which is induced by TGF-β, and found an essential DNA binding site for several TGF-β activated Smads in the promoter sequence, which is also required for the cooperation of these Smads with Sp1 and Ap1 transcription factors in order to guarantee an efficient TGF-β response from the Smad7 promoter. The group has generated mice with a null mutation in the Smad7 gene in collaboration with another laboratory, and the phenotype of the Smad7 knockout animals is currently under investigation.

Molecular Signaling Group

The Molecular Signaling Group, led by Dr. Ivan Dikic, is dedicated to studying the molecular mechanisms by which signal transduction mediated by growth factor activated receptor tyrosine kinases (RTKs) control cell growth and differentiation and the reasons why they are deregulated in diseases such as cancer.

Several lines of evidence have converged to show that RTK ubiquitination is important both for receptor internalization and for degradation in lysosomes, which leads to the down-regulation of the RTK's signaling. The group has recently shown that the protein Cbl directs monoubiquitination, rather than polyubiquitination, of activated EGF and PDGF receptors in mammalian cells. Furthermore, a single ubiquitin attached to EGF receptors was sufficient to mediate internalization as well as degradation of receptors. The group are currently addressing questions that deal with the role of monoubiquitin signals in endocytic sorting of cargo (i.e. EGF receptor and associated receptor complexes), the functions of ubiquitin binding proteins that serve as ubiquitin receptors along the endosomes, and the mechanisms that define the specificity in determining mono-, multi- or poly-ubiquitination of distinct Cbl substrates. The group has previously shown that binding of the adaptor protein CIN85 to Cbl and recruitment of endophilins in complexes with activated EGF receptors is critical for EGF receptor internalization. More

recently, they showed that CIN85 binds to the distal carboxyl-terminus of Cbl, recognizing a novel motif present in several CIN85 effectors. The group is now utilizing mouse models, and cells derived therefrom, to understand how components of these modules regulate cell functions in vivo.

TGF-β Signaling Group

The TGF-β Signaling Group, led by Dr. Aristidis Moustakas, investigates signaling pathways that regulate cell growth and differentiation in response to TGF-β, and pays special attention to processes that contribute to tumor cell invasiveness and metastasis.

The highly conserved TGF-β signaling engine consists of plasma membrane serine/threonine kinase receptors and Smad proteins, their cytoplasmic effectors, that upon activation by the receptors, translocate to the nucleus and regulate gene expression. The group has analyzed the mechanism of Smad3 nuclear export and identified exportin-4 and the Ran GTPase as the major transporting factors for Smad3, a receptor-activated (R-) Smad. The group has also studied the regulation of Smad4, which serves as the common effector for all TGF-β superfamily pathways. In carcinomas, specific amino acid substitutions in Smad4 lead to its enhanced poly-ubiquitination and proteolysis, and the group identified Smurf1 as an E3 ubiquitin ligase that induces poly-ubiquitination of wild-type Smad4 with the assistance of the adaptor protein Smad7. The characterization of Smurf1 is continuing, and Smurf1 expression levels in carcinomas that express mutant Smad4 will be examined to validate the relevance of the biochemical model in human cancer. Finally, Smad proteins cooperate with several nuclear factors in order to regulate transcription, and several of these and other related proteins, such as YY1 and p21, are being characterized to understand their regulation and function in TGF-β signaling.

TGF-β plays a tumor suppressor role in early stages of carcinogenesis, yet it promotes carcinoma cell invasiveness and metastasis. Using mammary epithelial models that exhibit this dual response to TGF-β, the group focussed on regulation of proliferation and epithelial-mesenchymal transition (EMT), a change in cell differentiation that precedes tumor cell migration and metastasis. It found that chronic exposure of mammary epithelial cells to TGF-β leads to sensitization and relative resistance to growth inhibition, while cells remain in the fibroblastic phenotype, thus exhibiting sustained and long-term EMT. Additionally it was shown that the EMT response is specific to TGF-β and is not induced by BMP members of the superfamily, and that Smads play critical roles during EMT. Using cDNA microarrays, the group identified several novel gene targets of the pathway with interesting functional characteristics that makes them candidate effectors of EMT and promoters of cell invasiveness. Using human mammary carcinoma cells that have no Smad4, and comparative cDNA microarray analysis of the TGF-β and BMP pathways, the team also found that gene regulation downstream of these pathways primarily depends on the presence of Smad4. Two prominent targets of this screen were the transcriptional regulators Id2 and Id3 that block both cell cycle arrest and EMT responses to TGF-β. Both genes were shown to be repressed by TGF-b and induced by BMP. These findings explain why TGF-β is a potent inducer of growth inhibition and EMT in epithelial cells, while BMP is not. The functional analysis of the genes identified by these microarray analyses is ongoing.

Integrated Signaling Group

Activities in the Integrated Signaling Group, led by Dr. Serhiy Souchelnytskyi, are concentrated on proteome profiling of breast epithelial cell transformation, and on studies of TGF-β signaling in cancer. The group has developed a proteomics platform based on use of two-dimensional gel electrophoresis and proteome image analysis to analyze protein expression, turnover, phosphorylation, and glycosylation. Protein identification by mass spectrometry is performed in collaboration with Dr. Hellman at the Uppsala Branch. Study of post-translational protein modifications is the main challenge of modern proteomics, and the group has developed and modified several approaches to monitor the phospho- and glyco-proteome of human epithelial breast cancer cells.

In various proteomics-based projects, the group has identified over 200 proteins affected by TGF-β. Phosphoproteome profiling of TGFβ/Smad signaling in human breast cancer cells has led to the identification of 35 proteins, while glycoproteome profiling has led to the identification of 27 proteins affected by TGFβ1. Functional studies of one of the novel targets showed a TGF-β1-dependent regulation of apoptosis-induced fragmentation of genomic DNA. The group found that Smad3 forms complexes with BRCA1 and BRCA2, and that direct interaction of BRCA1 and Smad3 resulted in synergy in transcriptional regulation, but Smad3 inhibited BRCA1 dependent repair of DNA double-strand breaks. The opposing effects of TGF-β/Smad3 and BRCA1 on DNA repair may modulate sensitivity of tumors to chemotherapeutic drugs. The observation that DNA damage repair can be regulated by TGF-β/Smad proteins, through interactions with BRCA1, Rad51, Rag1, and ORC5T, unveiled a novel pathway in TGF-β signaling which targets directly regulators of genomic DNA integrity. In other proteomics studies related to TGF-β/Smad crosstalk pathways, numerous other proteins have been identified, including: 54 proteins were altered by TGFβ1 in human endothelial cells; proteins affected by TGFβ family receptors; and 33 proteins associated with type II BMP receptor.

The group's proteomics platform has also been used to study protein-protein complexes, and for discovery of prognostic markers of human breast and ovarian cancers. The group has identified 23 proteins as potential markers of human breast and ovarian cancer in plasma obtained from 43 patients. Collection of plasma samples for a large cohort-validation, and follow-up of the primary cohort of patients whose plasma was used for the proteomics-based screen, is on-going. Additionally, proteome profiling of the effect of expression of the non-coding RNA DD3, a marker of prostate cancer, has revealed changes in expression of 47 proteins.

Apoptotic Signaling Group

The aim of the Apoptotic Signaling Group, led by Dr. Maréne Landström, is to elucidate the role of Smad7 in the TGF-β–induced apoptotic signalling pathway. Smad7 is a target gene of TGF-β and the group has demonstrated that Smad7 is required for TGF-β–induced apoptosis in prostate cancer cells as well as in human keratinocytes. The group has previously shown that 2-methoxyestradiol (2-ME) has both anti-angiogenic and direct cytotoxic effects on several tumor cell types, and is now investigating the role of Smad7 in the apoptotic pathway induced by 2-ME in human prostate cancer cells. Increased expression of Smad2, Smad3 and Smad4, as well as the inhibitory Smad6 and Smad7, was observed in normal and malignant prostate epithelial cells in vivo, preceding apoptosis induced by androgen withdrawal.

The group has also recently reported that Smad7 facilitates the TGF-β activating kinase 1 (TAK1), mitogen activated protein kinase kinase 3 (MKK3) and p38 mitogen activated protein (MAP) kinase pathway, presumably by acting as an adaptor protein bringing the kinases close to each other, and the group is attempting to elucidate the molecular mechanism(s) by which TGF-β and Smad7 activate this signaling pathway. The group is investigating possible novel target proteins downstream of the p38-Smad7 complex, using microarray analyses on cells overexpressing Smad7.

Smad7 is predominantly localized in the nucleus of resting cells, but stimulation of cells with TGF-β causes a rapid export of Smad7 to the cytoplasm, where it interacts with the TGF-β–activated receptor-complex. At longer timepoints after TGF-β stimulation of cells, Smad7 accumulates in the nucleus again. The group is investigating whether the apoptotic effect of Smad7 is dependent on its possibility to interact with other proapoptotic proteins which can shuttle between the nucleus and the cytoplasm. In a collaboration with Dr. Aspenström, the team found that Smad7 expression is required for TGF-β-mediated cytoskeletal regulation which occurs mainly via the small GTPase Cdc42, and the investigation of the role of Smad7 in TGF-β–dependent regulation of cytoskeletal processes is continuing.

Gene Expression Group

The Gene Expression Group, led by Dr. Johan Ericsson, has demonstrated that Smad7 interacts with the transcriptional coactivator p300, resulting in the acetylation of Smad7 on two lysine residues in its N-terminus. Acetylation or mutation of these lysine residues stabilizes Smad7 and protects it from TGF-β-induced degradation, as the acetylation prevents ubiquitination by the ubiquitin ligase Smurf1. Thus, the group's data indicate that competition between ubiquitination and acetylation of overlapping lysine residues constitutes a novel mechanism to regulate protein stability. Additionally, the group showed that SREBP transcription factors are acetylated by their p300/CBP coactivators, which colocalize with SREBPs in nuclear speckles in vivo. Coexpression with p300 dramatically increased the expression of both SREBP1a and SREBP2 and this effect depended on the acetyltransferase activity of p300. The team demonstrated that the acetylated residue in SREBP1a is also targeted by ubiquitination, indicating that acetylation of SREBPs regulate their stability. Thus, the group's studies define acetylation-dependent stabilization of transcription factors as a novel mechanism for coactivators to regulate gene expression. A potential application from these findings is that compounds that enhance the stability of SREBPs should increase the levels of transcriptionally active SREBP, which would lead to an enhanced expression of the low density lipoprotein (LDL) receptor gene and, thereby, increased clearance of LDL from the circulation. The most common treatment for elevated cholesterol levels in humans is a group of drugs called statins, which block cholesterol synthesis and, therefore, activate SREBPs. Other compounds that enhance SREBP stability could potentially be used to enhance the cholesterol-lowering activities of statins.

Matrix Biology Group

The Matrix Biology Group, led by Dr. Paraskevi Heldin, studies the molecular mechanisms whereby the polysaccharide hyaluronan promotes tumor growth and invasion. First, the regulation of hyaluronan synthases (Has1, Has2 and Has3) and hyaluronidases (PH-20, Hyal1 and Hyal2) in response to growth factors released by tumor cells were studied. Platelet-derived growth factor-BB (PDGF-BB) was shown to have a potent stimulatory effect, which was partly mediated by activation of protein kinase C. Among the three Has isoforms studied, the Has2 isoform is more markedly upregulated or suppressed in response to external stimuli. Notably, TGF-ß1 was found to stimulate Has activities but to strongly inhibit hyaluronidase activities. Additional studies revealed that newly synthesized hyaluronan is retained on the cell surface either by sustained attachment to the Has or through its interactions with hyaluronan receptors, such as CD44. The data show that hyaluronan is therefore crucial for the assembly of cell microenvironment or pericellular matrices, which influence critical cell functions such as growth and migration.

The group also compared the biological properties of a non-hyaluronan producing mesothelioma cell line with those of the same cells made to produce hyaluronan after transfection of Has2 cDNA. The data indicate that increased synthesis of hyaluronan leads to an increased malignant phenotype of mesotheliomas and facilitated their aggressive spread in a CD44-dependent manner. These results were further confirmed by additional studies in a colon carcinoma model both in vitro and in vivo. The interesting and novel finding in these studies was that Has2 overproduction promoted tumorigenicity, whereas Hyal1 overexpression suppressed tumor development. Given the opposite effects of hyaluronan synthesizing and hyaluronan degrading enzymes, studies on the molecular mechanism that regulate their activities are warranted as the information obtained may offer novel approaches for hyaluronan-based therapies.

Studies have also been initiated to investigate how hyaluronan fragments affects neovascularization at the molecular level. The team studied the gene expression profile, using microarrays, of endothelial cells in response to hyaluronan fragments in the absence or presence of inhibitory CD44 antibodies. The effect of the fragments on the induction of tubuli structures was compared to the effect of the angiogenic factor, basic fibroblast growth factor (bFGF). Several distinct but common classes of genes were up- or down-

regulated after stimulation with hyaluronan fragments or bFGF during capillary endothelial cell assembly into tube-like structures. The roles in endothelial cell differentiation of the genes regulated by hyaluronan fragments are currently under investigation.

Protein Structure Group

The Protein Structure Group, led by Dr. Ulf Hellman, has close interactions with the other Groups within the Uppsala Branch and at the Stockholm Branch, as well as with external research groups. The primary work of the Group involves peptide synthesis, radio-labeled amino acid sequencing, and mass spectrometry.

High quality peptides are produced using Fmoc chemistry on an Applied Biosystems 433A instrument. The Group also prepares peptides carrying various modifications, i.e. phosphorylation, acetylation, oxidation etc., which have been most useful for the different Groups, being used for the generation of anti-peptide antibodies, as substrates or inhibitors, or as ligands in affinity chromatography experiments. All products are quality control analyzed by the matrix-assisted-laser-desorption-ionization-time-of-flight-mass-spectrometer (MALDI-ToF-MS).

Mass spectrometry is used to identify proteins of interest from biochemical experiments. Over 95% of the samples for analysis by MALDI-ToF-MS come as bands or spots from one- or two-dimensional gels followed by in-gel tryptic digestion. With Coomassie-visible material only a few percent is used for analysis; with silver stained material, often all the samples must be applied, and with weak silverstain, the sample must be concentrated and desalted on a hand-made reversed phase column in sub microlitre scale. Determining protein identity by peptide mass fingerprinting (PMF) is a routine procedure, provided the target protein's sequence is available in a sequence data bank. After generation of a proteolytic digest and analysis by MALDI-ToF-MS, the Group utilizes a search engine (ProFound or MASCOT are preferred) to scan sequence data banks. If a clear mass spectrum is obtained, a protein identity with high significance is usually obtained. When this is not the case, a protein's identity may be established by determining the amino acid sequence of one or more tryptic peptides. A sequence homology search is, in contrast to PMF, tolerant to amino acid substitutions.

Fragment analysis of peptides in MALDI-ToF-MS by post source decay (PSD) sequencing is an established procedure for MALDI instruments equipped with a reflector. The process in standard MALDIs is time-consuming, rather imprecise and very difficult to interpret, and the technique is therefore not often used. However the Group's new Ultraflex is a dedicated top-of-the-line MALDI instrument with a special feature for PSD. The TOF/TOF technology allows PSD spectra to be generated in seconds, so it is possible to scan through several peptides from one digest in a short time. The easy and quick PSD combines extremely well with the Chemically Assisted Fragmentation (CAF) derivatization protocol, leading to unsurpassed easily interpreted spectra. CAF-PSD identification is used for the characterization of unknown peptide species, as well as for analysis of modified peptides.

Publications

Primary Research Articles

1. Abramczyk O., Zien P., Zielinski R., Pilecki M., Hellman U., Szyszka R. The protein kinase 60S is a free catalytic CK2α' subunit and forms an inactive complex with superoxide dismutase SOD1. Biochemical and Biophysical Research Communications (2003) 307: 31-40.
2. Arsura M., Panta G.R., Bilyeu J.D., Cavin L.G., Sovak M.A., Oliver A.A., Factor V., Heuchel R., Mercurio F., Thorgeirsson S.S., Sonenshein G.E. Transient activation of NF-κB through a TAK1/IKK kinase pathway by TGF-β1 inhibits AP-1/SMAD signaling and apoptosis: implications in liver tumor formation. (2003) 22: 412-425.
3. Autero M., Heiska L., Rönnstrand L., Vaheri A., Gahmberg C.G., Carpén O. Ezrin is a substrate for Lck in T cells. FEBS Letters (2003) 535: 82-86.
4. Blaukat A., Micke P., Kalatskaya I., Faussner A., Müller-Esterl W. Downregulation of bradykinin B2 receptor in human fibroblasts during prolonged agonist exposure. American Journal of Physiology Heart and Circulatory Physiology (2003) 284: H1909 1916.
5. Chou W-C., Prokova V., Shiraishi K., Valcourt U., Moustakas A., Hadzopoulou-Cladaras M., Zannis V.I., Kardassis D. Mechanism of a transcriptional cross talk between transforming growth factor-β-regulated Smad3 and Smad4 proteins and orphan nuclear receptor hepatocyte nuclear factor-4. Molecular Biology of the Cell (2003) 14: 1279-1294.
6. Demoulin J-B., Seo J.K., Ekman S., Grapengiesser E., Hellman U., Rönnstrand L. and Heldin C-H. Ligand-induced recruitment of Na+/H+ exchanger regulatory factor to the PDGF (platelet-derived growth factor) receptor regulates actin cytoskeleton reorganization by PDGF. Biochemical Journal (2003) 376: 505-510.
7. Díaz-Enrich M.J., Ibarguren I., Hellman U., Villamarín J.A. Characterization of a type I regulatory subunit of cAMP-dependent protein kinase from the bivalve mollusk Mytilus galloprovincialis. Archives of Biochemistry and Biophysics (2003) 416: 119-127.
8. Dib K., Melander F., Axelsson L., Dagher M-C., Aspenström P., Andersson T. Down-regulation of Rac activity during β2 integrin-mediated adhesion of human neutrophils. Journal of Biological Chemistry (2003) 278: 24181 24188.
9. Dixelius J., Mäkinen T., Wirzenius M., Karkkainen M.J., Wernstedt C., Alitalo K., Claesson-Welsh L. Ligand-induced vascular endothelial growth factor receptor-3 (VEGFR-3) heterodimerization with VEGFR-2 in primary lymphatic endothelial cells regulates tyrosine phosphorylation sites. Journal of Biological Chemistry (2003) 278: 40973-40979.
10. Edlund S., Bu S., Schuster N., Aspenström P., Heuchel R., Heldin N-E., ten Dijke P., Heldin C-H., Landström M. Transforming growth factor-β1 (TGF-β)-induced apoptosis of prostate cancer cells involves Smad7-dependent activation of p38 by TGF-β-activated kinase 1 and mitogen-activated protein kinase kinase 3. Molecular Biology of the Cell (2003) 14: 529-544.
11. Eriksson E., Dons L., Gigliotti Rothfuchs A., Heldin P., Wigzell H., Rottenberg M.E. CD44-regulated intracellular proliferation of Listeria monocytogenes. Infection and Immunity (2003) 71: 4102-4111.
12. Fransson Å., Ruusala A., Aspenström P. Atypical Rho GTPases have roles in mitochondrial homeostasis and apoptosis. Journal of Biological Chemistry (2003) 278: 6495-6502.
13. Gazit A., Yee K., Uecker A., Böhmer F-D., Sjöblom T., Östman A., Waltenberger J., Golomb G., Banai

S., Heinrich M.C., Levitzki A. Tricyclic quinoxalines as potent kinase inhibitors of PDGFR kinase, Flt3 and Kit. Bioorganic & Medicinal Chemistry (2003) 11: 2007-2018.

14. Giandomenico V., Simonsson M., Grönroos E., Ericsson J. Coactivator-dependent acetylation stabilizes members of the SREBP family of transcription factors. Molecular and Cellular Biology (2003) 23: 2587-2599.
15. Gizatullina Z.Z., Grapengiesser E., Shabalina I.G., Nedergaard J., Heldin C-H., Aspenström P. Effect of transforming growth factor-β on calcium homeostasis in prostate carcinoma cells. Biochemical and Biophysical Research Communications (2003) 304: 643-649.
16. Groth A., Lukas J., Nigg E.A., Silljé H.H.W., Wernstedt C., Bartek J., Hansen K. Human Tousled like kinases are targeted by an ATM- and Chk1 dependent DNA damage checkpoint. The EMBO Journal (2003) 22: 1676-1687.
17. Haglund K., Sigismund S., Polo S., Szymkiewicz I., Di Fiore P.P., Dikic I. Multiple monoubiquitination of RTKs is sufficient for their endocytosis and degradation. Nature Cell Biology (2003) 5: 461-466, 2003.
18. Itoh S., Thorikay M., Kowanetz M., Moustakas A., Itoh F., Heldin C-H., ten Dijke P. Elucidation of Smad requirement in transforming growth factor-β type I receptor-induced responses. Journal of Biological Chemistry (2003) 278: 3751-3761.
19. Ivanov K.I., Puustinen P., Gabrenaite R., Vihinen H., Rönnstrand L., Valmu L., Kalkkinen N., Mäkinen K. Phosphorylation of the potyvirus capsid protein by protein kinase CK2 and its relevance for virus infection. The Plant Cell (2003) 15: 2124-2139, 2003.
20. Jacobson A., Salnikov A., Lammerts E., Roswall P., Sundberg C., Heldin P., Rubin K., Heldin N-E. Hyaluronan content in experimental carcinoma is not correlated to interstitial fluid pressure. Biochemical and Biophysical Research Communications (2003) 305: 1017-1023.
21. Jandt E., Denner K., Kovalenko M., Östman A., Böhmer F-D. The protein-tyrosine phosphatase DEP-1 modulates growth factor-stimulated cell migration and cell-matrix adhesion. Oncogene (2003) 22: 4175-4185.
22. Kaivo-Oja N., Bondestam J., Kämäräinen M., Koskimies J., Vitt U., Cranfield M., Vuojolainen K., Kallio J.P., Olkkonen V.M., Hayashi M., Moustakas A., Groome N.P., ten Dijke P., Hsueh A.J., Ritvos O. Growth differentiation factor-9 induces Smad2 activation and inhibin B production in cultured human granulosa-luteal cells. Journal of Clinical Endocrinology and Metabolism (2003) 88: 755-762.
23. Karger E.M., Frolova O.Y., Fedorova N.V., Baratova L.A., Ovchinnikova T.V., Susi P., Makinen K., Rönnstrand L., Dorokhov Y.L., Atabekov J.G. Dysfunctionality of a tobacco mosaic virus movement protein mutant mimicking threonine 104 phosphorylation. Journal of General Virology (2003) 84: 727–732.
24. Kowanetz K., Terzic J., Dikic I. Dab2 links CIN85 with clathrin-mediated receptor internalization. FEBS Letters (2003) 554: 81-87.
25. Kowanetz K., Szymkiewicz I., Haglund K., Kowanetz M., Husnjak K., Taylor J.D., Soubeyran P., Engström U., Ladbury J.E., Dikic I. Identification of a novel proline-arginine motif involved in CIN85-dependent clustering of Cbl and down-regulation of epidermal growth factor receptors. Journal of Biological Chemistry (2003) 278: 39735-39746.
26. Kruljac-Letunic A., Moelleken J., Kallin A., Wieland F., Blaukat A. The tyrosine kinase Pyk2 regulates Arf1 activity by phosphorylation and inhibition of the Arf-GTPase activating protein ASAP1. Journal of Biological Chemistry (2003) 278: 29560-29570.

27. Kurisaki K., Kurisaki A., Valcourt U., Terentiev A.A., Pardali K., ten Dijke P., Heldin C-H., Ericsson J., Moustakas A. Nuclear factor YY1 inhibits transforming growth factor β- and bone morphogenetic protein-induced cell differentiation. Molecular and Cellular Biology (2003) 23: 4494-4510.

28. Lennartsson J., Wernstedt C., Engström U., Hellman U., Rönnstrand L. Identification of Tyr900 in the kinase domain of c-Kit as a Src-dependent phosphorylation site mediating interaction with c-Crk. Experimental Cell Research (2003) 288: 110-118.

29. Lindblom P., Gerhardt H., Liebner S., Abramsson A., Enge M., Hellström M., Bäckström G., Fredriksson S., Landegren U., Nyström H.C., Bergström G., Dejana E., Östman A., Lindahl P., Betsholtz C. Endothelial PDGF-B retention is required for proper investment of pericytes in the microvessel wall. Genes and Development (2003) 17: 1835-1840.

30. Lorenzo C., Salinas G., Brugnini A., Wernstedt C., Hellman U., González-Sapienza G. Echinococcus granulosus antigen 5 is closely related to proteases of the trypsin family. Biochemical Journal (2003) 369: 191-198.

31. Lundin L., Rönnstrand L., Cross M., Hellberg C., Lindahl U., Claesson-Welsh L. Differential tyrosine phosphorylation of fibroblast growth factor (FGF) receptor-1 and receptor proximal signal transduction in response to FGF-2 and heparin. Experimental Cell Research (2003) 287: 190-198.

32. Markova B., Herrlich P., Rönnstrand L., Böhmer F.D. Identification of protein tyrosine phosphatases associating with the PDGF receptor. Biochemistry (2003) 42: 2691-2699.

33. Micke P., Basrai M., Faldum A., Bittinger F., Rönnstrand L., Blaukat A., Beeh K.M., Oesch F., Fischer B., Buhl R., Hengstler J.G. Characterization of c-kit expression in small cell lung cancer: prognostic and therapeutic implications. Clinical Cancer Research (2003) 9: 188-194.

34. Morén A., Hellman U., Inada Y., Imamura T., Heldin C-H., Moustakas A. Differential ubiquitination defines the functional status of the tumor suppressor Smad4. Journal of Biological Chemistry (2003) 278: 33571-33582.

35. Nore B.F., Mattsson P.T., Antonsson P., Bäckesjö C-M., Westlund A., Lennartsson J., Hansson H., Löw P., Rönnstrand L., Smith C.I.E. Identification of phosphorylation sites within the SH3 domain of Tec family tyrosine kinases. Biochimica et Biophysica Acta (2003) 1645: 123-132.

36. Pant V., Mariano P., Kanduri C., Mattsson A., Lobanenkov V., Heuchel R., Ohlsson R. The nucleotides responsible for the direct physical contact between the chromatin insulator protein CTCF and the H19 imprinting control region manifest parent of origin-specific long-distance insulation and methylation-free domains. Genes & Development (2003) 17: 586-590.

37. Parussini F., García M., Mucci J., Agüero F., Sánchez D., Hellman U., Åslund L., Cazzulo J.J. Characterization of a lysosomal serine carboxypeptidase from Trypanosoma cruzi. Molecular and Biochemical Parasitology (2003) 131: 11-23.

38. Pietras K., Stumm M., Hubert M., Buchdunger E., Rubin K., Heldin C-H., McSheehy P., Wartmann M., Östman A. STI571 enhances the therapeutic index of epothilone B by a tumor-selective increase of drug uptake. Clinical Cancer Research (2003) 9: 3779-3787.

39. Pontén A., Li X., Thorén P., Aase K., Sjöblom T., Östman A., Eriksson U. Transgenic overexpression of platelet-derived growth factor-C in the mouse heart induces cardiac fibrosis, hypertrophy, and dilated cardiomyopathy. American Journal of Pathology (2003) 163: 673-682.

40. Ribom D., Larsson A., Pietras K., Smits A. Growth factor analysis of low-grade glioma CSF: PDGF and VEGF are not detectable. Neurological Sciences (2003) 24: 70-73.

41. Rollman O., Jensen U.B., Östman A., Bolund L., Gustafsdottir S.M., Jensen T.G. Platelet-derived

growth factor (PDGF) responsive epidermis formed from human keratinocytes transduced with the PDGFβ–receptor gene. Journal of Investigative Dermatology (2003) 120: 742-749.

42. Sabounchi-Schütt F., Åström J., Hellman U., Eklund A., Grunewald J. Changes in bronchoalveolar lavage fluid proteins in sarcoidosis: a proteomics approach. European Respiratory Journal (2003) 21: 414-420, 2003.
43. Sollerbrant K., Raschperger E., Mirza M., Engström U., Philipson L., Ljungdahl P.O., Pettersson R.F. The coxsackievirus and adenovirus receptor (CAR) forms a complex with the PDZ domain-containing protein ligand-of-numb protein-X (LNX). Journal of Biological Chemistry (2003) 278: 7439-7444.
44. Soubeyran P., Barac A., Szymkiewicz I., Dikic I. Cbl/ArgBP2 complex mediates ubiquitination and degradation of c-Abl. Biochemical Journal (2003) 370: 29 34.
45. Stoika R., Yakymovych M., Souchelnytskyi S., Yakymovych I. Potential role of transforming growth factor β1 in drug resistance of tumor cells. Acta Biochimica Polonica (2003) 50: 497-508, 2003.
46. Storgaard Sørensen C., Syljuåsen R.G., Falck J., Schroeder T., Rönnstrand L., Khanna K.K., Zhou B-B., Bartek J., Lukas J. Chk1 regulates the S phase checkpoint by coupling the physiological turnover and ionizing radiation-induced accelerated proteolysis of Cdc25A. Cancer Cell (2003) 3: 247-258.
47. Sundqvist A., Ericsson J. Transcription-dependent degradation controls the stability of the SREBP family of transcription factors. Proceedings of the National Academy of Sciences USA (2003) 100: 13833-13838.
48. Suzuki K., Yamamoto T., Usui T., Suzuki K., Heldin P., Yamashita H. Expression of hyaluronan synthase in intraocular proliferative diseases: regulation of expression in human vascular endothelial cells by transforming growth factor-β. Japanese Journal of Ophthalmology (2003) 47: 557-564.
49. Tentes I., Asimakopoulos B., Hellman U., Nikolettos N., Kortsaris A., Kontoleon-Vakalopoulou E. Subcellular fractionation and electrophoretic analysis of proteins from the regenerating nerve in rabbits following treatment with triiodothyronine (T3). In Vivo (2003) 17: 601-608.
50. Tienthai P., Kimura N., Heldin P., Sato E., Rodriguez-Martinez H. Expression of hyaluronan synthase-3 in porcine oviducal epithelium during oestrus. Reproduction, Fertility and Development (2003) 15: 99-105.
51. Tienthai P., Yokoo M., Kimura N., Heldin P., Sato E., Rodriguez-Martinez H. Immunohistochemical localization and expression of the hyaluronan receptor CD44 in the epithelium of the pig oviduct during oestrus. Reproduction (2003) 125: 119-132.
52. Troncoso M.F., Zolezzi P.C., Hellman U., Wolfenstein-Todel C. A novel trypsin inhibitor from Peltophorum dubium seeds, with lectin-like properties, triggers rat lymphoma cell apoptosis. Archives of Biochemistry and Biophysics (2003) 411: 93-104.
53. Usui T., Nakajima F., Ideta R., Kaji Y., Suzuki Y., Araie M., Miyauchi S., Heldin P., Yamashita H. Hyaluronan synthase in trabecular meshwork cells. British Journal of Ophthalmology (2003) 87: 357-360.
54. Voytyuk O., Lennartsson J., Mogi A., Caruana G., Courtneidge S.A., Ashman L.K., Rönnstrand L. Src family kinases are involved in the differential signaling from two splice forms of c-Kit. Journal of Biological Chemistry (2003) 278: 9159-9166.
55. Wester K., Asplund A., Bäckvall H., Micke P., Derveniece A., Hartmane I., Malmström P-U., Pontén F. Zinc-based fixative improves preservation of genomic DNA and proteins in histoprocessing of human tissues. Laboratory Investigation (2003) 83: 889-899.

56. Wollberg P., Lennartsson J., Gottfridsson E., Yoshimura A., Rönnstrand L. The adapter protein APS associates with the multifunctional docking sites Tyr-568 and Tyr-936 in c-Kit. Biochemical Journal (2003) 370: 1033-1038.

57. Zuo S., Hellman U., Lundahl P. On the oligomeric state of the red blood cell glucose transporter GLUT1. Biochimica et Biophysica Acta (2003) 1618: 8-16.

Reviews / Book Chapters / Commentaries

1. Dikic I. Mechanisms controlling EGF receptor endocytosis and degradation. Biochemal Society Transactions (2003) 31: 1178-1181.

2. Dikic I., ad Giordano S. Negative receptor signaling. Current Opinion in Cell Biology (2003) 15: 128-135.

3. Dikic I., Szymkiewicz I., Soubeyran P. Cbl signaling networks in the regulation of cell function. Cellular and Molecular Life Sciences (2003) 60: 1805-1827.

4. Edlund S. Mechanisms for TGF-β-mediated regulation of the actin filament system and apoptosis. Acta Universitatis Upsaliensis. Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine. Eklundshofs Grafiska, Uppsala (2003) 1245: pps. 1-73.

5. Haglund K., Di Fiore P.P., Dikic I. Distinct monoubiquitin signals in receptor endocytosis. Trends in Biological Sciences (2003) 28: 598-603.

6. Heldin C-H. Protein tyrosine kinase receptor signaling overview. In: Handbook of Cell Signaling, Bradshaw R and Dennis E, eds. Academic Press, San Diego, CA, 1: pps. 391-396, 2003.

7. Heldin C-H. Platelet-derived growth factor (PDGF). In: Encyclopedia of hormones and related cell regulators, Henry HL and Norman AW, eds. Elsevier Science (USA), 3: pps. 231-237, 2003.

8. Heldin P. Importance of hyaluronan biosynthesis and degradation in cell differentiation and tumor formation. Brazilian Journal of Medical and Biological Research (2003) 36: 967-973.

9. Itoh F. Regulation of TGF-β/Smad signaling through Smad interacting proteins. Acta Universitatis Upsaliensis. Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine. Eklundshofs Grafiska, Uppsala (2003) 1296: pps. 1-55.

10. Kallin A. Modulation of PDGF receptor signaling via the phosphatase SHP-2 and the docking protein Gab1. Acta Universitatis Upsaliensis. Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine. Eklundshofs Grafiska, Uppsala (2003) 1300: pps. 1-69.

11. Moustakas A., Heldin C-H. The nuts and bolts of IRF structure. Nature Structural Biology (2003) 10: 874-876.

12. Moustakas A., Heldin C-H. Ecsit-ement on the crossroads of Toll and BMP signal transduction. Genes & Development (2003) 17: 2855-2859.

13. Persson C. Protein tyrosine phosphatases as regulators of receptor tyrosine kinases. Acta Universitatis Upsaliensis. Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine. Eklundshofs Grafiska, Uppsala (2003) 1232: pps. 1-52.

14. Pietras K., Sjöblom T., Rubin K., Heldin C-H., Östman A. PDGF receptors as cancer drug targets. Cancer Cell (2003) 3: 439-443.

15. Richnau N. RICH-1, a multi-functional RhoGAP domain-containing protein, involved in regulation o fthe actin filament system and membrane-trafficking. Acta Universitatis Upsaliensis. Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine. Eklundshofs Grafiska, Uppsala (2003) 1282: pps. 1-75.

16. Souchelnytskyi S. Proteomics in studies of signal transduction in epithelial cells. J. Mammary Gland Biol. Neoplasia (2003) 7: 359-371.

17. Szymkiewicz I. Cbl in regulation of growth factor receptor endocytosis and actin dynamics. Acta Universitatis Upsaliensis. Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine. Eklundshofs Grafiska, Uppsala (2003) 1243: pps. 1-58.

18. ten Dijke P., Heldin C-H. TGF-β receptrs. In: Cytokine Reference, Oppenheim JJ and Feldmann M, eds. Academic Press, pps. 1871-1884, 2003.

LUDWIG INSTITUTE FOR CANCER RESEARCH

Clinical Trials Program

Office of Clinical Trials Management

Directors Report

Immunotherapy with monoclonal antibodies and cancer vaccines continued to be the focus of the Ludwig Institute Clinical Trials Program in 2003. The Cancer Vaccine Collaborative (CVC), initiated in 2001 between the Cancer Research Institute and LICR to leverage the strength of each organization, now plays a major role in funding and conducting cancer vaccine clinical trials at centers in the U.S.A., Europe, Japan, and Australia. This collection of clinical centers, have all demonstrated success for immunotyping and immune monitoring. The monoclonal antibody clinical program pursued radioimmunotherapy studies with metals and chelates, embarked upon imaging studies utilizing positron emitting tomography (PET) to better understand the biology of antibodies, and concentrated on process development programs needed for high yield production cell lines.

The number of clinical trial concepts reviewed by the clinical investigation committee during 2003 remained stable at around 20. Although the number of active clinical studies increased to nearly 40 at one point in 2003, there is now an effort to begin to reduce this number by closing trials that are not accruing patients at a reasonable rate or that have objectives that have become outdated.

Three Investigational New Drug Applications were submitted and activated by the Institute in 2003, bringing the total number of INDs to 13. Additionally the first submission to the Recombinant Advisory Committee was made for a protocol using vaccinia and fowlpox vectors expressing NY-ESO-1. The regulatory environment continues to be challenging particularly in light of the EU Directive that implements an entirely new process for investigational agents in Europe. Both regulators and Sponsors are finding their way through the maize. This impacts not only the conduct of clinical trials but also the importation of study reagents into the EU.

The heart of the Institute's production of clinical study reagents remains with the LICR Biological Production Facility in Melbourne. The facility and the expert staff are responsible for production of monoclonal antibodies, and the testing, storage and distribution of clinical study reagents, as well as for preparing the production documents for the regulators. The Bioprocess Production Facility on the campus of Cornell University in Ithaca, dedicated in November 2002, was successfully validated during 2003 and will initiate its first protein GMP production in early 2004. With the movement by the Institute to using full length protein as a study agent, rather than peptides, the Ithaca facility is now an even more important component of the clinical vaccine program.

The implementation of the Ludwig Electronic Advancement Program (LEAP), the internet-based clinical trial database, has turned out to be a major effort but one that will reap huge benefits for all involved in the conduct of clinical trials within the Institute. The database has been established and although there have been expected "wrinkles" along the way; the remote data entry system is close to implementation.

Several major accomplishments were noted in 2003. Significant and broad NY-ESO-1 and MAGE-3 induced humoral and cellular immunity were demonstrated in clinical trials using NY-ESO-1 protein (Melbourne) and MAGE-3 protein (New York) when used with saponin-related adjuvants. The monoclonal antibody chimeric 806, which targets EGFR that is over-expressed in tumors has been produced by the BPF in Melbourne. A sufficient amount of antibody is available for use in the first human trial, scheduled to be initiated in Q2 2004. This is of considerable significance since the preclinical data are impressive and the Institute has full ownership of this antibody.

Eric W. Hoffman

Elke Jäger, M.D.

Krankenhaus Nordwest
Frankfurt, Germany

Research Report

These research experiments continued to be performed in conjunction with Affiliates Drs. Alexander Knuth and Dirk Jäger (Zürich Affiliate Center, Switzerland).

1. Expression analysis of NY-BR-1 in different types of cancer

RT-PCR analysis was performed on different cancer tissues to asess the frequency of NY-BR-1 expression.

Tumor	Positive	Negative	Total
Breast	77	103	180
Ovarian	3	28	31
Prostate	16	54	70

There was no obvious correlation found between NY-BR-1 expression and clinical stage of disease, metastatic patterns, treatment history, HER2 expression status, or histological subtype.

The finding that a low NY-BR-1 expression was found in ovarian cancer adds a new potential target antigen for immunotherapeutic interventions in this disease.

2. Assessment of spontaneous immunity against NY-BR- 1 in patients with NY-BR-1+ cancer

NY-BR-1 serum antibody was evaluated in 530 serum samples of patients with NY-BR-1 positive and negative breast cancer. Recombinant NY-BR-1 protein covering 50 % of the carboxy-terminal gene product was prepared and used for Western Blot analyses. Of 530 samples tested, three showed detectable NY-BR-1 antibody. Detectable NY-BR-1 antibody reactivity was restricted to patients with NY-BR-1 positive tumors, and was not found in healthy individuals.

3. Identification of new NY-BR-1 epitopes as targets for CD4 and CD8 T cell responses in cancer patients

NY-BR-1 specific CD4 and CD8 T cell responses were assessed using synthetic NY-BR-1 18-mer peptides covering aa 1004 – 1397 of the entire NY-BR-1 gene product. This region of the NY-BR-1 protein was chosen because it was indicated by different programs used for the analysis of peptide binding motifs predicted to contain the highest rate of potential MHC class I and class II binding epitopes.Thirty-nine NY-BR-1 18-mer peptides were synthetized that overlap by 10 aa..

Fourteen patients and one healthy individual were chosen for the assessment of NY-BR-1 specific CD4 and CD8 T cell reactivity. All patients had NY-BR-1 positive cancers (12 breast, two prostate). CD4 and CD8 T cells were separated from peripheral blood lymphocytes (PBL) and used for stimulation assays. T cells were presensitized using the whole panel of 39 NY-BR-1 peptides in separate wells, or by pools containing five NY-BR-1 peptides in each well. After five to six days of presensitization, CD4 or CD8 T cells were tested in ELISPOT assays to determine the effector reactivity against the whole panel of 39 single NY-BR-1 peptides, which were loaded onto autologous CD4- and CD8-depleted PBL as antigen-presenting cells (APC). In case CD4 or CD8 T cells reactive with specific NY-BR-1 peptides were detected, ELISPOT assays were repeated using single peptides to confirm the specificity of the reaction at the level of presensitization and target recognition. At this stage, MHC restriction of T cell responses has not yet been analyzed.

CD4 T cell responses were detected in six patients with NY-BR-1 positive breast (five) and prostate

(one) cancer. CD4 T cells of all six patients reacted with a total of 16 NY-BR-1 18-mer peptides in ELISPOT assays. The results were confirmed in at least one independent experiment. One of six patients also showed CD8 T cell reactivity against four different NY-BR-1 peptides. CD4 T cell reactivity was also detected in one healthy individual, who is NY-BR-1 antibody negative and does not show NY-BR-1 specific CD8 T cell reactivity.

CD8 T cell responses were generally difficult to detect following the approach described above. It is assumed that presensitization with 18-mer peptides is of low efficacy due to the length of the reagent that probably provides multiple competing binding sites to different MHC class I and class II alleles of the APC. Nevertheless, CD8 T cell reactivity was detected in two patients, against five different NY-BR-1 peptides. The analysis of the MHC class I restriction elements is ongoing using allogeneic EBV-B cell lines which share only one single MHC class I allele with the peptide-reactive effector T cells as APC.

Once the MHC classI/II restriction elements have been identified, NY-BR-1 peptide-reactive CD4 and CD8 T cells will be tested for recognition of HLA-matched NY-BR-1 positive tumor cell lines. The group recently succeeded in establishing two new breast cancer cell lines that will be evaluated in this context. Based on the findings of NY-BR-1 expression in ovarian cancer, it may be possible to analyze a much larger panel of NY-BR-1+ tumor cell lines in the near future, because ovarian cancer is significantly easier to culture.

4. Identification of new NY-ESO-1 epitopes as targets for CD4 and CD8 T cell responses in patients pre and post NY-ESO-1–specific vaccination

Patients with NY-ESO-1 positive cancer were immunized with synthetic NY-ESO-1 peptides p157-167 and p157-165 (LUD00-009), and/or with recombinant NY-ESO-1 vaccinia- or fowlpox viral constructs (LUD00-014) to generate NY-ESO-1 peptide-specific CD8 T cell responses (LUD00-009) or NY-ESO-1-specific CD4 and CD8 T cell responses (LUD00-014) in vivo. Since the studies are ongoing, this is an incomplete reporting on new NY-ESO-1 epitopes found to be recognized by post-vaccine CD4 or CD8 T cells. Non-vaccinated patients with detectable NY-ESO-1 antibody were included in the analysis.

CD4 T cell reactivity was assessed using NY-ESO-1 overlapping 18-mer pepitdes spanning the entire NY-ESO-1 gene product (total number of synthetic peptides was 28). CD4 effector T cells were presensitized for five days with each peptide in single wells and tested for recogntion of the stimulating peptide in ELISPOT assays using autologous CD4- and CD8-depleted PBL as APC. Post-vaccine CD4 T cell responses have been evaluated in eight patients so far. Four of these had detectable CD4 T cell responses against 16/28 NY-ESO-1 peptides. Three of 16 peptides represent already known target epitopes for CD4 T cell recognition in the context of HLA-DRB4*-0101-0104, 13 peptides represent new target epitopes. Studies to identify the MHC class II restriction elements are ongoing using allogeneic EBV-B cell lines matched for 1 single MHC class II allele with effector cells as APC in ELISPOT assays. Once the MHC restriction has been defined for each epitope, effector CD4 T cells will be tested for recognition of MHC class II matched NY-ESO-1 positive tumor cell lines.

NY-ESO-1 specific CD8 T cell responses were assessed after presensitization of effector cells with recombinant adeno-ESO viral constructs against the panel of synthetic NY-ESO-1 overlapping 18-mer pepitdes separately pulsed onto T2 cells as APC. Effector T cells were collected from patients who were NY-ESO-1 antibody positive, or who had received vaccination with synthetic NY-ESO-1 peptides p157-167 and p157-165. One new NY-ESO-1 epitope (p55-72) was identified that is probably presented by HLA-A3 (confirmatory studies are ongoing). NY-ESO-1 p55-72 was recognized by CD8 T cells of 4 different patients with detectable NY-ESO-1 serum antibody.

5. Clinical Trials Center Activity

Four different clinical immunotherapy studies being performed at Krankenhaus Nordwest recruited patients during the year 2003.

LUD00-009: Phase 1 study of intensive course immunization with NY-ESO-1 derived peptides presented by MHC class I molecules alone and in combination with GM-CSF in patients with advanced NY-ESO-1 or LAGE expressing tumors

Twenty patients with different types of cancer have been entered in this study; nine patients were NY-ESO-1 antibody negative, 11 were NY-ESO-1 antibody positive. Nine patients have completed three cycles of immunization, 11 discontinued the treatment because of rapid tumor progression or on their own discretion. There was no significant toxicity oserved. NY-ESO-1 specific T cell responses of high magnitude and early onset were observed in the majority of patients who completed three cycles of immunization. Peptide specific DTH reactions of the inflammatory type correlated with high magnitude CD8+ T cell responses detectable in the peripheral blood. The rapid induction of CD8+ T cell responses is believed to be of therapeutic significance, potentially preventing the development of immune escape mechanisms in vivo.

LUD00-014: Phase 1 study of recombinant vaccinia-NY-ESO-1 (rV-NY-ESO-1) and recombinant fowlpox-NY-ESO-1 (rF-NY-ESO-1) in patients with NY-ESO-1 positive cancers

Twenty-nine patients with different types of cancer have been entered in this study, 19 patients have completed at least four immunizations, and 10 patients have discontinued treatment earlier for rapid disease progression or on their own discretion. There was no significant toxicity other than skin reactions at the immunization sites observed. Fifteen patients were HLA-A2 positive, and T cell responses were monitored against the HLA-A2 restricted NY-ESO-1 peptides p157-165 and 157-167. The majority of HLA-A2 positive patients who completed at least four immunizations have developed NY-ESO-1 specific CD8+ T cell responses within two to four months after the start of immunization. Two of 23 NY-ESO-1 antibody negative patients showed a sero-conversion toward a detectable NY-ESO-1 serum antibody during vaccination. One patient developed a partial remission of subcutaneous and peritoneal melanoma metastases during the first four months of immunization along with a strong NY-ESO-1 specific CD8+ T cell response. NY-ESO-1 specific CD4+ T cell responses are being monitored. Preliminary results suggest that CD4+ T cell responses are induced in some but not all patients who completed at least four immunizations. The example of one patient with detectable NY-ESO-1 serum antibody and CD4+ and CD8+ T cell reactivity at baseline shows that CD4+ T cell responses are broadened against nine different NY-ESO-1 epitopes (compared to five epitopes recognized at baseline) after four immunizations. These finding suggest that immunization with recombinant NY-ESO-1 vaccinia- and fowlpox constructs can induce NY-ESO-1 specific humoral and cellular immune responses in vivo that may have therapeutic effects in patients with NY-ESO-1 expressing cancer.

LUD00-026: Phase 1 study of immunization with NY-ESO-1 derived peptides presented by MHC class I molecules alone or combined with Poly Arginine in patients with advanced NY-ESO-1 or LAGE expressing tumors

Seven HLA-A2 positive patients with different types of cancer have been entered to this protocol. Four patients received polyarginine as an adjuvant. There was no significant toxicity observed. Patients who received polyarginine did not develop stronger skin reactions or imune responses to the vaccine. Three patients are continuing the protocol for extended progression-free intervals during immunization.CD8+ T cell responses against the immunizing peptides were observed in five patients that correlated with detectable DTH reactions at the sites of peptide iinoculation. The best clinical response observed in this study is stabilization of disease of extended duration.

LUD01-014: Phase 1/2 study of chimeric monoclonal antibody G250 (cG250) in combination with vinblastine administered weekly by intravenous infusion to patients with advanced renal cell carcinoma

Nineteen patients with advanced renal cell carcinoma progressive before study entry have been included in this protocol, 11 have completed at least six weekly treatments. No cG250 antibody-related toxicity was observed in this study. There was no indication of HACA devopment in any of the patients treated. The best clinical response observed is stablization of disease, with extended progression-free intervals observed

in three patients of 17, 14, 9 months, respectively. The immunological monitoring of this study will include the assessment of G250 specific CD1d-restricted T cell responses in collaboration with Drs. Gerd Ritter (LICR New York Branch) and Affiliate Vincenzo Cerundolo (Oxford Affiliate Center, UK).

Publications

Primary Research Articles

1. Atanackovic D., Matsuo M., Ritter E., Mazzara G., Ritter G., Jager E., Knuth A., Old L.J., Gnjatic S. Monitoring CD4(+) T cell responses against viral and tumor antigens using T cells as novel target APC. Journal of Immunology Methods (2003) 278:57-66.
2. Cebon J., Jager E., Shackleton M.J., Gibbs P., Davis I.D., Hopkins W., Gibbs S., Chen Q., Karbach J., Jackson H., MacGregor D.P., Sturrock S., Vaughan H., Maraskovsky E., Neumann A., Hoffman E., Sherman M.L., Knuth A. Two phase I studies of low dose recombinant human IL-12 with Melan-A and influenza peptides in subjects with advanced malignant melanoma. Cancer Immunity (2003) 3:7.
3. Gnjatic S., Atanackovic D., Jager E., Matsuo M., Selvakumar A., Altorki N.K., Maki R.G., Dupont B., Ritter G., Chen Y.T., Knuth A., Old L. J. Survey of naturally occurring CD4+ T cell responses against NY-ESO-1 in cancer patients: correlation with antibody responses. Proceedings of the National Academy of Sciences USA (2003) 100:8862-7.
4. Gnjatic S., Atanackovic D., Matsuo M., Jager E., Lee S.Y., Valmori D., Chen Y.T., Ritter G., Knuth A., Old L.J. Cross-presentation of HLA class I epitopes from exogenous NY-ESO-1 polypeptides by nonprofessional APCs. Journal of Immunology (2003) 170:1191-6.
5. Jager E., Jager D., Knuth A. Antigen-specific immunotherapy and cancer vaccines. International Journal of Cancer (2003) 106:817-20.
6. Mischo A., Wadle A., Watzig K., Jager D., Stockert E., Santiago D., Ritter G., Regitz E., Jager E., Knuth A., Old L., Pfreundschuh M., Renner C. Recombinant antigen expression on yeast surface (RAYS) for the detection of serological immune responses in cancer patients. Cancer Immunity (2003) 3:5.

Ferdy J. Lejeune, M.D., Ph.D.

Centre Hospitalier Universitaire Vaudois
Lausanne, Switzerland

Research Report

Our research activity related to LICR concerns two distinct fields :

- Melanoma immunology and immunotherapy
- Anti-angiogenesis.

Dr. Olivier Michelin who received his MD/PhD degree in 2002, during his joint appointment with LICR and CePO, continued his training in clinical oncology at Centre Pluridisciplinaire d'Oncologie (CePO) together with his research at the LICR and Swiss Institute of Bioinformatics. His in silico studies focus on melanoma antigens and TCRs. It is expected that this approach will help defining optimal epitopes and effectors. Dr. Michelin also initiated a collaboration with Dr. Curzio Rüegg of CePO on in silico design of anti-integrins agents, in order to design effective antiangiogenic agents.

Melanoma immunology and immunotherapy

The Melanoma Immunology and Immunotherapy program was initiated in 1992 as a joint venture with the division of Immuno-Oncology of the LICR. This program continued in 2003 with completion of ongoing studies and new protocols. The publications focused on cytotoxic lymphocyte (CTL) phenotype in relation to melanoma immunity and vaccination.

The implementation of two new vaccination protocols at CePO:

1) "Vaccination of patients with stage III of IV Malignant Melanoma with Melanoma Antigen Peptides [Melan-A analog (ELA), NY-ESO-1b(A) and MAGE-A10] and montanide adjuvant". Principal investigator : Dr. D. Liénard.

In comparison with previous protocols, this study uses a multipeptide strategy. It was accepted by the scientific review board of the Centre Pluridisciplinaire d'Oncologie on April 24th 2003 and accepted by the Ethics Committee.

2) "Phase I study of in vivo expansion of Melan-A/MART-1 antigen-specific CD8 T lymphocytes following transient immunosuppression in patients with advanced melanoma." Principal investigator : Dr. S. Leyvraz from CePO.

This is the first protocol of adoptive immunotherapy ever initiated in Switzerland. It results from the synergy between LICR, CePO and NCCR (National Center for Competence in Research). This protocol was accepted by the scientific review board on November 20th 2003 and is being submitted to the Ethics Committee.

Since LICR studies always require blood and tumor samples from patients, we have initiated the preparation of a master protocol for blood and tissue donation. It should concern most patients who could become eligible for vaccination studies or immunology explorations. The draft is being examined by the Ethics Committee.

Antiangiogenesis

With Dr. Rüegg (a member of the LICR angiogenesis group), a large translational research program has been initiated aiming at defining cell and soluble markers of angiogenesis in cancer patients, as well as evaluating tumor vascularisation (collaboration with Dr. Meuwly, ultrasound specialist CHUV). This program is part of the NCCR in collaboration with the Swiss Institute of Experimental Cancer Research (ISREC).

Publications

Primary Research Articles

1. Ayyoub, M., Zippelius A., Pittet M.J., Rimoldi D., Valmori D., Cerottini J.C., Romero P., Lejeune F., Lienard D., Speiser D.E. Activation of Human Melanoma Reactive CD8+ T Cells by Vaccination with an Immunogenic Peptide Analog Derived from Melan-A/Melanoma Antigen Recognized by T Cells-1. Clinical Cancer Research (2003) 9(2):669-77.
2. Rimoldi, D., Lemoine R., Kurt A.M., Salvi S., Berset M., Matter M., Roche B., Cerottini J.C., Guggisberg D., Krischer J., Braun R., Willi J.P., Antonescu C., Slosman D., Lejeune F.J., Lienard D. Detection of micrometastases in sentinel lymph nodes from melanoma patients: direct comparison of multimarker molecular and immunopathological methods. Melanoma Research (2003) 13(5):511-520.
3. Rimoldi, D., Salvi S., Lienard D., Lejeune F.J., Speiser D., Zografos L., Cerottini J.C. Lack of BRAF mutations in uveal melanoma. Cancer Res. (2003) 63(18): 5712-5.
4. Speiser, D.E., Rimoldi D., Batard P., Lienard D., Lejeune F., Cerottini J.C., Romero P. Disease-driven T cell activation predicts immune responses to vaccination against melanoma. Cancer Immunity (2003) 3:12.
5. Valmori, D., Dutoit V., Ayyoub M., Rimoldi D., Guillaume P., Lienard D., Lejeune F., Cerottini J.C., Romero P., Speiser D.E. Simultaneous CD8+ T cell responses to multiple tumor antigen epitopes in a multipeptide melanoma vaccine. Cancer Immunity (2003) 3:15.

Chaitanya Divgi, M.D.

Memorial Sloan-Kettering Cancer Center
New York, USA

Research Report

In 2003, significant progress was made with regard to the clinical investigation of the antibody cG250. Clinical data was collated from patients in trials using cG250, final reports were sent to the Food and Drug Administration (FDA), and a publication has been accepted by the Journal of Nuclear Medicine regarding these data. These dosimetry data have been combined and compared with the Phase I single large dose study carried out under the direction of Dr. Wil Buijs with Dr. Egbert Oosterwijk (Nijmegen Affiliate Center). The conclusions drawn from the comparison of these studies has been submitted for publication. These studies led to the need for accurate quantitation of tumor and normal uptake of iodinated cG250 and a protocol has been drawn up accordingly (LUD 02-003). Investigational New Drug (IND) approval has been obtained from the FDA for this protocol and Memorial Sloan-Kettering Cancer Center (MSKCC) Internal Review Board (IRB) approval is imminent.

A collaboration with Dr. Peter Smith-Jones (MSKCC), resulted in the successful chelation of cG250 with DOTA (1,4,7,10-tetraazacyclododecane-N,N'-N",N"'-tetraacetic acid), and the protocol for its clinical testing has been completed and approved by the LICR Protocol Review Committee (pending revisions). We expect FDA and MSKCC approval by the end of this month.

A Phase I study evaluating the increasing amounts of huA33 in patients with advanced colon cancer was completed and results have been published. Another study evaluating the combined effects of huA33 with combination chemotherapy (BOS-STREP) was also completed and published. Both of these studies underscored the need for continued development of this unique protein including methods to obviate its immunogenicity. Production of a clinic-grade antigen binding construct, single chain Fv-A33 (sFv) was therefore begun and is expected to be completed by Q2 2004. Dr. Smith-Jones and I have successfully conjugated both ^{131}I and ^{111}In to sFv and have shown that the former is unstable *in vivo*. We will therefore utilize radiometal-DOTA-conjugated-sFv in all clinical trials. A constructed collaboration between the group at the Cornell University/LICR Biological Production Facility in Ithaca, NY (with Dr. Carl Batt), the New York Branch of the LICR (Mr. Leonard Cohen) and our group (Drs. Smith-Jones and Divgi) will optimize DOTA-conjugated sFv for clinical diagnostic and therapeutic trials.

The immunology program project grant (CA 33049), initiated by Drs. Herbert Oettgen and Lloyd Old, and now headed by Dr. Alan Houghton (MSKCC), was submitted for renewal, with the sFvA33 and related constructs, forming one project. This project will also bring together Dr. K. Dane Wittrup, a LICR collaborator from MIT, who will be responsible for the construction of bi-specific antigen constructs.

Finally, we were also part of a multi-center Phase I trial evaluating an antibody against fibroblast protein-alpha. This trial was partially supported by the immunology program project grant and has been published.

Publications

Primary Research Articles

1. Oosterwijk E., Brouwers A., Boerman O.C., Larson S.M., Old L.J., Mulders P., Divgi C.R. Monoclonal antibody therapy of kidney cancer. Cancer Treatment and Research (2003) 116:199-212.
2. Oosterwijk E., Divgi C.R., Brouwers A., Boerman O.C., Larson S.M., Mulders P., Old L.J. Monoclonal antibody-based therapy for renal cell carcinoma. Urologic Clinics of North America (2003) 30(3):623-631.

3. Burke J.M., Caron P.C., Papadopoulos E.B., Divgi C.R., Sgouros G., Panageas K.S., Finn R.D., Larson S.M., O'Reilly R.J., Scheinberg D.A., Jurcic J.G. Cytoreduction with iodine-131-anti-CD33 antibodies before bone marrow transplantation for advanced myeloid leukemias. Bone Marrow Transplant (2003) 32(6):549-556.

4. Pandit-Taskar N., Hamlin P.A., Reyes S., Larson S.M., Divgi C.R. New strategies in radioimmunotherapy for lymphoma. Current Oncology Reports (2003) 5(5):364-371.

5. Scott A.M., Wiseman G., Welt S., Adjei A., Lee F.T., Hopkins W., Divgi C.R., Hanson L.H., Mitchell P., Gansen D.N., Larson S.M., Ingle J.N., Hoffman E.W., Tanswell P., Ritter G., Cohen L.S., Bette P., Arvay L., Amelsberg A., Vlock D., Rettig W.J., Old L.J. A Phase I dose-escalation study of sibrotuzumab in patients with advanced or metastatic fibroblast activation protein-positive cancer. Clinical Cancer Research (2003) 9(5):1639-1647.

6. Sgouros G., Squeri S., Ballangrud A.M., Kolbert K.S., Teitcher J.B., Panageas K.S., Finn R.D., Divgi C.R., Larson S.M., Zelenetz A.D. Patient-specific, 3-dimensional dosimetry in non-Hodgkin's lymphoma patients treated with 131I-anti-B1 antibody: assessment of tumor dose-response. Journal of Nuclear Medicine (2003) 44(2):260-268.

Egbert Oosterwijk, Ph.D.

University Medical Center Nijmegen,
Nijmegen, The Netherlands

Research Report

Clinical studies

In 2003 we finalized a Phase I/II clinical study "Radioimmunotherapy using 131I-cG250 in Patients with Metastasized Renal Cell Carcinoma with Two Consecutive Treatments at the Maximum Tolerated Dose" with chimeric monoclonal antibody (mAb) G250, recognizing a Renal Cell Carcinoma (RCC)-associated antigen, most abundant on RCC of the clear cell type. The study was designed to define the safety and toxicity of two sequential high-dose treatments of 131I-cG250, and to assess their therapeutic efficacy. Patients (n=29) with progressive metastatic RCC received a diagnostic infusion of 131I-cG250 to assess uptake in metastatic lesions and for dosimetric analyses, followed by 131I-cG250 (n=27) at the maximum tolerated dose (MTD) determined in a previous study one week later. If no Grade 4 hematological toxicity was observed, a second diagnostic infusion of 131I-cG250 (n=20) was given after three months. In the absence of rapid blood clearance of 131I-cG250, indicating the lack of human-anti-chimeric-antibodies (HACA), a second radioimmunotherapy (RIT) of 131I-cG250, escalated from 1110 MBq/m2 (n=3) to 1665 MBq/m2 (n=16) was administered one week later. Patients were monitored for another three months and evaluated by CT at three monthly intervals. MTD of the second RIT was 1665 MBq/m2 due to dose-limiting hematological toxicity. No correlation was found between observed hematological toxicity and radiation absorbed dose to the whole-body or bone marrow, nor administered activity (MBq and MBq/kg). Sixteen patients completed the protocol at both MTDs. In seven patients disease stabilization occurred while on study, eight patients showed progressive disease and one patient was not evaluable due to HACA formation. Patients went off study because of Grade 4 hematological toxicity after the first RIT (n=3), development of HACA titers (n=4), or rapid disease progression (n=2). An inverse relation between tumor size and radiation absorbed dose was observed. Most tumor lesions received < 10 Gy, whereas only lesions < 5 g absorbed > 50 Gy.

We concluded that: [1] in this group of patients with end-stage RCC, the MTD of the 2nd treatment was 75% of the MTD of the first treatment due to dose-limiting hematological toxicity; [2] two cycles of 131I-cG250 could be safely administered without severe toxicity in the majority of patients; [3] no objective responses were observed, but in half of the patients two RIT doses resulted in stabilization of previously progressive disease while on study (six months); [4] when HACA developed, the rapid clearance of radioactivity from the blood and body prohibited further treatment; According to the calculated absorbed dose in metastatic lesions, future radioimmunotherapy studies with iodinated G250 should aim at treatment of minimal residual disease or in an adjuvant setting.

In a separate clinical trial we investigated the behavior of cG250 antibody labeled with a residualizing radionuclide (111In) or radioiodine in patients with recurrent or metastasized RCC. Since this was an intrapatient comparison we were able to assess whether, and to what extent, the radiation dose to the tumor differed when the cG250 antibody was labeled with either radioisotope. After receiving consecutive injections with 111In-DTPA-G250 (day 0) and I-131-G250 (day four), scintigraphic images were obtained and were analyzed visually and quantitatively by calculating the uptake per metastasis. In addition, the biodistribution of the two radiolabeled forms of mAb cG250 in relevant organs was assessed. In summary, substantially higher In-111-DTPA-cG250 accumulation was observed than radioiodinated cG250 accumulation, demonstrating that the uptake of a cG250 labeled with a residualizing radionuclide in RCC metastases is significantly higher in most RCC lesions as compared to the uptake of I-131-cG250. Based on these observations we are in the progress of initiating a clinical trial with cG250 labeled with the residualizing radionuclide Lu-177.

Preclinical studies

Pretargeting strategies

We have developed an efficient pretargeting strategy for renal cell carcinoma (RCC) based on biologically produced bispecific antibody (bsMAb). To determine the influence of various tumor characteristics we investigated this pretargeting strategy in three different RCC tumor models. Three different human RCC xenografts in nude mice were pretargeted with bispecific antibody anti-RCC x anti-DTPA (bsMAb G250xDTIn-1) and three days after administration of the bsMAb, mice were i.v. injected with 111In-labeled bivalent peptide diDTPA-FKYK. The three RCC tumors were characterized in vivo and in vitro for G250-antigen expression, pharmacokinetic behavior and vascularization.

Very high uptake of the radiolabel was observed in all three tumor models. Remarkably, tumor uptake and G250 antigen expression were inversely correlated in both the pretargeting approach and with directly labeled MAb. Immunohistochemical analysis of the tumor vasculature showed different vascularization patterns. The study emphasizes the influence/importance of tumor characteristics other than antigen density on tumor targeting.

Gene therapy

Adenoviral retargeting

The use of recombinant adenoviruses in cancer gene therapy is limited by the widespread expression of the coxsackievirus and adenovirus receptor on normal human cells. Targeting adenoviral vectors to renal cell carcinoma (RCC) cells may improve their potential in cancer gene therapy of patients with metastatic RCC. A recombinant bispecific single-chain antibody directed against G250 protein and the adenovirus fiber knob domain was constructed. The bispecific antibody was used to retarget recombinant adenovirus expressing the green fluorescent protein under control of the cytomegalovirus promoter. G250-positive RCC cells displayed enhanced susceptibility for transduction adenovirus complexed with the G250-directed bispecific single-chain antibody when compared with native adenovirus. This enhanced transduction was restricted to G250-positive RCC cells and could be abolished completely in the presence of excess G250 protein. The results of this study demonstrated the feasibility of immunologic retargeting of adenovirus to RCC cells with the highly tumor-specific G250 protein as the target. This strategy may provide the possibility of improving cancer gene therapy for patients with RCC.

Conditional replicating Adenovirus

After identifying the minimal promoter region still able to specifically drive the tumor-specific expression of G250, the promoter region was cloned into several adenoviral vectors to investigate whether this leads to a conditionally replicating adenovirus (CRAd). Preliminary evidence indicates that indeed, such viruses only replicate in G250-positive cells. We are now constructing viruses in which the native tropism is completely ablated and are inserting sequences encoding for bispecific single-chain antibody directed against G250 protein and the adenovirus fiber knob domain. Thus a G250-redirected CRAd will be available to test whether this leads to selective infection and replication in G250-positive cells. Once this vector becomes available, in vitro and in vivo experiments will be performed.

Publications

Primary Research Articles

1. Oosterwijk, E., Brouwers, A., Boerman, O. C., Larson, S. M., Old, L. J., Mulders, P., Divgi, C. R. Monoclonal antibody therapy of kidney cancer. Cancer Treatment and Research (2003) 116:199-212.

2. Brouwers, A. H., Buijs, W. C., Oosterwijk, E., Boerman, O. C., Mala, C., De Mulder, P. H., Corstens, F. H., Mulders, P. F., Oyen, W. J. Targeting of metastatic renal cell carcinoma with the chimeric

monoclonal antibody G250 labeled with (131)I or (111)In: an intrapatient comparison. Clinical Cancer Research (2003) 9:3953S-3960S.

3. van Schaijk, F. G., Oosterwijk, E., Soede, A. C., Oyen, W. J., McBride, W. J., Griffiths, G. L., Goldenberg, D. M., Corstens, F. H., Boerman, O. C. Pretargeting with labeled bivalent peptides allowing the use of four radionuclides: (111)In, (131)I, (99m)Tc, and (188)Re. Clinical Cancer Research (2003) 9:3880S-3885S.
4. Brouwers, A. H., Frielink, C., Oosterwijk, E., Oyen, W. J., Corstens, F. H., Boerman, O. C. Interferons can upregulate the expression of the tumor associated antigen G250-MN/CA IX, a potential target for (radio)immunotherapy of renal cell carcinoma. Cancer Biotherapy and Radiopharmacy (2003) 18:539-547.
5. Oosterwijk, E., Divgi, C. R., Brouwers, A., Boerman, O. C., Larson, S. M., Mulders, P., Old, L. J. Monoclonal antibody-based therapy for renal cell carcinoma. Urologic Clinics of North America (2003) 30:623-631.
6. Mulders, P., Bleumer, I., Oosterwijk, E. Tumor antigens and markers in renal cell carcinoma. Urologic Clinics of North America (2003) 30:455-465.
7. Jongmans, W., van den, O. K., Tiemessen, D. M., Molkenboer, J., Willemsen, R., Mulders, P. F., Oosterwijk, E. Targeting of adenovirus to human renal cell carcinoma cells. Urology (2003) 62:559-565.
8. Bleumer, I., Oosterwijk, E., De Mulder, P., Mulders, P. F. Immunotherapy for renal cell carcinoma. European Urology (2003) 44:65-75.
9. Jongmans, W., Tiemessen, D. M., Oosterwijk, E., and Mulders, P. F. Correspondence re: Y. S. Haviv, J. L. Blackwell, A. Kanerva, P. Nagi, V. Krasnykh, I. Dmitriev, M. Wang, S. Naito, X. Lei, A. Hemminki, D. Carey, D. T. Curiel, Adenoviral gene therapy for renal cancer requires retargeting to alternative cellular receptors. Cancer Research (2003) 63:1994-1995.

Vincenzo Cerundolo, M.A., M.D., Ph.D., F.R.C.Path., F.Med.Sci.

Institute of Molecular Medicine
Oxford, UK

Research Report

Research in the laboratory is mainly focused on the understanding of the mechanisms that control the cell-cell interplay required for optimal expansion and activation of tumour-specific T cell populations. The use of HLA class I tetramers has provided important insights into the analysis of tumour specific immune responses, and allowed direct demonstration of expanded populations of activated tumour specific cytotoxic T lymphocytes (CTL) in some cancer patients. The overall aim of the research programme is to build up a detailed picture of the mechanisms controlling the cellular immune response to tumour specific proteins and develop novel vaccination strategies. These studies are carried out by analysing responses restricted by HLA class I/II molecules. More recently these studied have been extended to the analysis of responses restricted by CD1 molecules. Particular attention has been focused on the cross talk between innate and adaptive immune response, because optimising tumour vaccination protocols will require a deeper understanding of the signals that the immune system coordinates in order to respond to pathogenic infection. Compounds that mimic these signals may therefore be exploited as adjuvants in current tumour vaccination strategies.

i)Development of novel vaccination strategies

Over the last year the group has carried out research that has provided insights into the dynamics of T cell expansion during vaccination strategies. The team has developed new protocols for rapid identification of high affinity human and murine T cells ex vivo, compared the ability of different dendritic cell (DC) populations to expand antigen specific T cells, and identified novel protocols to prime and boost tumour and virus specific T cells. Finally, the group has also demonstrated that *in vivo* stimulation of NKT cells enhances CD4+ and CD8+ T cell responses to soluble antigen through direct interaction with dendritic cells.

The intentions in the next few years are to continue this line of research and to extend previous results by optimising strategies capable of inducing poly-valent CTL responses. Several strategies will be pursued:

1) The assessment of whether stronger and broader priming by poly-epitope vaccines can be obtained by expressing, within poly-epitope constructs, ligands of the activatory receptor NKG2D that have previously been shown to stimulate NK and CD8+ T cell responses. Several viral vectors will be engineered including lentiviral vectors, which the group has recently shown to be capable of priming and boosting T cell responses.

2) The comparison of the ability of human and mouse myeloid and plasmacytoid DC (PDC) to boost *in vivo* and *in vitro* higher numbers of functional CTL that target multiple CTL epitopes, and boosting strategies based on the intravenous injection of recombinant viruses encoding poly-epitope constructs.

3) NKT cells are present in large numbers in the blood, liver and spleen of mice and humans. The group has recently demonstrated that injection of NKT ligand α GalactosylCeramide (α GalCer) induces maturation of DC in vivo and results in the expansion of antigen specific T cells. The analyses of the cross-talk between NKT cells, DC and T lymphocytes will be continued by assessing effects of TLR- and invariant NKT- cell ligands on DC maturation and antigen specific T cell responses *in vivo*. The team will assess whether invariant NKT cell stimulation has an impact upon cross presentation and whether this can overcome T cell tolerance. These experiments will be further extended by comparing several analogues

of α GalCer and by characterising their ability in vivo and in vitro to induce T cell responses. Generation of soluble NKT T cell receptor (TCR) will provide us with the opportunity of carrying out kinetic and structural studies, in collaboration with Dr. Yvonne Jones (Oxford). The group is also collaborating with Dr. Yoram Reiter (Haifa) who has developed protocols to generate class I/peptide specific antibodies. Using CD1d/α GalCer monomers, a panel of 20 monoclonal antibodies have been generated capable of specifically recognising CD1d molecules loaded with α-GalCer.

ii) Effect of hypoxia on DC maturation, antigen processing and expansion of tumour specific T cell responses

Very little is known on the effect of hypoxia on antigen presentation, DC maturation, and on the homing of T cells. Experiments that address these issues, using protocols developed in the laboratory, are being performed to assess the ability of mature DC to prime antigen specific T cells *in vitro*, to analyze efficiency of TAP translocation and peptide loading of class I molecules. A specific question is whether hypoxic conditions may result in the presentation of a novel pool of self peptides and priming of T cell responses specific for hypoxia inducible proteins. The reasoning is that chronic expression of hypoxic inducible proteins may be capable of breaking tolerance specific for such proteins and induce a specific response. Two complementary lines of research are currently being pursued:

a) Analysis of the immune response specific to the carbonic anhydrases IX (CA IX), which is expressed in tumour hypoxic areas and constitutively expressed in over 80% of renal cell carcinomas (RCC), due to the von Hippel Lindau (vHL) mutation. Four HLA-A*0201 restricted epitopes within CA IX have been described in healthy volunteers. The group is currently characterizing T cell responses to CA IX in peripheral blood lymphocytes and tumour infiltrating lymphocytes from RCC patients and correlating T cell frequency to CA IX expression in corresponding tumour sections. The group has begun a collaboration with Dr. Christoph Renner (Homburg Affiliate Center), who has generated a panel of monoclonal antibodies specific to A2 molecules loaded with CA IX derived peptide epitopes. BIAcore studies that measure the affinity of such antibodies are being performed, prior to their use in immunohistochemistry and functional studies.

b) Parallel experiments have been carried out in a mouse model by transfecting tumour cells (B16 cells) with cDNA encoding β-galactosidase driven by a hypoxia inducible promoter and by monitoring the frequency of β-galactosidase specific T cells and their ability to home into hypoxic areas, visualised by X-gal staining.

iii) Analysis of glycolipid T cell response in health and disease

Analysis of the role of CD1 has been hampered by the lack of reliable and sensitive techniques to isolate glycolipid specific T lymphocytes *ex vivo*. Over the last three years, the group has developed novel protocols for the generation of CD1d/glycolipid tetramers, which allow sensitive and highly specific isolation and characterisation of CD1 restricted T lymphocytes from peripheral blood and lymphocytes. More recently, these results were extended to all the members of the CD1 family by developing additional protocols to refold denatured CD1 molecules in the presence of defined lipid ligands and short mono-alkyl detergent molecules.

Application of these protocols has provided an opportunity to isolate lipid specific T cell lines upon priming of healthy donors' peripheral blood lymphocytes (PBL) with autologous DC pulsed with synthetic gangliosides. The intention is to apply these techniques to analyze the presence of glycolipid specific T cells in cancer patients and in tumour bearing mice. The group has recently generated CD1b transgenic mice and intend to use these mice to study their ability to generate CD1b restricted responses using purified glycolipids. To assess the glycolipid antigenic profile presented by CD1 molecules, a panel of lentiviral vectors that encode soluble CD1 molecules expressing Fc fragment (to ensure their purification using Protein A beads) has been generated. In preliminary experiments, it has been established that such

CD1d and CD1b lentiviruses can infect a broad range of different tumours cells *in vitro* and generate large amounts (mgs) of soluble CD1 molecules. The CD1 bound glycolipids will be eluted and identified by tandem mass spectroscopy and high performance liquid chromatography (HPLC) analysis. These experiments are carried out in collaboration with Dr. Terry Butters (Glycobiology Institute, Oxford).

iv) Clinical trial programme

An important aspect of the future work will continue to be a translational research programme based on the application of protocols derived from the above pre-clinical studies. The group has recently completed a Phase I clinical trial in melanoma patients vaccinated with plasmid DNA followed by defective pox-virus (MVA) encoding a string of melanoma epitopes. The results of this trial, which have been submitted for publication, were very informative in assessing the kinetics of T cell expansion and interplay between T cell responses.

Publications

Primary Research Articles

1. Choi E.M., Chen J., Salio M., Lissin N., Hermans I., Silk J.D., Mirza F., Palmowski M.J., Dunbar P.R., Jakobsen B.K., Sewell A.K., Cerundolo V. High avidity antigen specific CTL identified by CD8 independent tetramer staining. Journal of Immunology (2003) 171(10):5116-5123.
2. Salio M., Cella M., Facchetti F., Shepherd D., Colonna M., Cerundolo V. Plasmacytoid Dendritic cells prime IFN-γ secreting Melan-A specific CD8 lymphocytes and are found in melanoma lesions. European Journal of Immunology (2003) 33(4):1052-62.
3. Salio M, Dulphy N., Renneson J., Herbert M., McMichael A., Marchant A., Cerundolo V. Efficient priming of antigen-specific cytotoxic T lymphocytes by human cord blood dendritic cells. International Immunology (2003) 15(10):1265-73.
4. Hermans I.F., Chong T.W., Palmowski M.J., Harris A.L., Cerundolo V. Synergistic effect of metronomic dosing of cyclophosphamide with specific anti-tumour immunity in a murine melanoma model. Cancer Research (2003) 63(23):8408-13.
5. Hermans I., Silk J.D., Gileadi U., Salio M., Ritter G., Schmidt R., Harris A.L., Old L., Cerundolo V. NKT cells can modulate CD4+ and CD8+ T cell responses to soluble antigen in vivo through direct interaction with dendritic cells. Journal of Immunology (2003) 171(10):5140-5147.
6. Denkberg G., Lev A., Eisenbach L., Benhar I., Reiter Y. Selective targeting of melanoma and APCs using a recombinant antibody with TCR-like specificity directed toward a melanoma differentiation antigen. Journal of Immunology (2003) 171(5):2197-207.

Svetomir N. Markovic, M.D., Ph.D.

Mayo Clinic
Rochester, MN, USA

Research Report

As an affiliate member of LICR our group has been awarded the opportunity and privilege to collaborate with world leaders in cancer immunotherapy. The interactions that have been made possible by our membership to LICR have allowed our efforts to grow and slowly develop into a comprehensive program for translational cancer immunotherapy at the Mayo Clinic. Our group efforts are divided into two major projects: melanoma immunotherapy; and post-autologous stem cell transplant immune recovery and immunomodulation. Herein is a brief summary of our activities in both projects in 2003:

Melanoma Immunotherapy

- **Peptide vaccines:** In continuation of our efforts to improve the efficacy of peptide based vaccines for patients with melanoma, we completed a randomized phase II clinical trial evaluating the role of low dose GM-CSF as immune adjuvant co-emulsified with melanoma peptide vaccines (MART-1, tyrosinase and gp100) in Montanide ISA 51. The doses of GM-CSF used in this study were 0, 10 and 50µg. The study did not demonstrate improved efficacy of immunization using the highest dose of GM-CSF. In view of our pre-clinical data suggesting that further dose escalation of GM-CSF would be warranted, we proceeded to a follow-up trial using GM-CSF at higher doses (75 and 100µg) as well as a gp100 peptide that contained overlapping HLA class I and class II binding sites. This study is currently active and anticipated to complete accrual by late 2004.
- **Aerosol vaccines:** In close collaboration with our colleagues at the LICR New York Branch, we have developed a Phase I study evaluating the safety and immunological efficacy of aerosol NY-ESO-1 protein immunization. Pre-clinical studies demonstrate effective immunization of mice using aerosol delivery of peptide antigens. The clinical trial is an extension of our pre-clinical work to improve immunization efficacy of a cancer vaccine using aerosol delivery. The clinical trial is approaching final stages of development and we anticipate study activation by mid to late 2004.
- **Cytokine therapy:** Two projects have been in ongoing development using cytokines for the treatment of advanced stage malignant melanoma. The first project evaluates the utility of aerosolized GM-CSF delivered by inhalation to patients with metastatic melanoma in the lung. The current study (N0071) is a dose escalating trial evaluating the ability of increasing doses of aerosolized GM-CSF to induce melanoma specific cytotoxic T lymphocyte responses. The study is ongoing and preliminary evidence suggests development of systemic anti-melanoma immunity at a dose of 1250µg of GM-CSF administered twice/day for 14 days. The metastatic tumor in the lung is the source of the antigens.

  The second project is focused on the use of natural alpha interferon (Alferon N) and its ability to up-regulate NK cell cytotoxicity pre-operatively in the setting of resected stage IV melanoma. Based on our pre-clinical data we have demonstrated that effective pre-surgical resection up-regulation of NK cytotoxicity is effective in preventing systemic tumor dissemination in 70% of treated animals. The current clinical trial is attempting to translate these observations into patients that are treated with interferon prior to resection of metastatic melanoma. The first phase of the study, identifying the dose/regimen of interferon necessary to up-regulate NK cytotoxicity is complete. The phase II component, evaluating the clinical impact of this intervention, is currently awaiting activation.

Post-autologous stem cell transplant (post-ASCT) immune reconstitution/immunotherapy

In 2003, we were able to further our understanding of the role of early lymphocyte reconstitution and its

impact on clinical outcomes in patient undergoing ASCT for the treatment of hematological malignancies and breast cancer. We have been able to identify NK cells as the key element influencing the ≥80% 10 year survival of patients with non-Hodgking's lymphoma that reconstitute their lymphocyte counts by day 15 post transplant. We have also identified the autograft as the source of reconstituting NK cells having direct impact on survival. Our first clinical trial attempting to "engineer" the autograft in such a way that it would be enriched for NK cells is currently under way, and is expected to be completed by mid 2004.

Publications

Primary Research Articles

1. McWilliams R.R., Brown P.D., Buckner J.C., Link M.J., Markovic S.N. Treatment of brain metastases from melanoma. Mayo Clinic Proceedings (2003) 78(12):1529-1536.
2. Rao R.D., Anderson P.M., Arndt C.A., Wettstein P.J., Markovic S.N. Aerosolized granulocyte macrophage colony-stimulating factor (GM-CSF) therapy in metastatic cancer. American Journal of Clinical Oncology (2003) 26(5):493-498.
3. Rao R.D., Markovic S.N., Anderson P.M. Aerosol therapy for malignancy involving the lungs. Current Cancer Drug Targets (2003) 3(4):239-250.
4. Porrata L.F., Gastineau D.A., Padley D., Bundy K., Markovic S.N.. Re-infused autologous graft natural killer cells correlates with absolute lymphocyte count recovery after autologous stem cell transplantation. Leukemia & Lymphoma (2003) 44(6):997-1000.
5. Pockaj B.A., Jaroszewski D.E., DiCaudo D.J., Hentz J.G., Buchel E.W., Gray R.J., Markovic S.N., and Bite U. Changing surgical therapy for melanoma of the external ear. Annals of Surgical Oncology (2003) 10(6):689-696.

Alexander Knuth, M.D., Dirk Jäger, M.D., Alfred Zippelius, M.D.

Universitätsspital Zürich
Zürich, Switzerland

Research Report

These research experiments continued to be performed in conjunction with Affiliate Dr. Elke Jäger (Frankfurt Affiliate Center, Germany).

This year was devoted to setting up the technical and administrative basis necessary for the Cancer Antigen Discovery Program and the Clinical Vaccine Program following the group's relocation to University Hospital Zürich in April 2003. After renovation of the laboratory tract and recruiting the research group (two postdoctoral fellows, two MD research fellows, one pre-diploma student, and four technicians) the laboratory is now endowed with all the essential technical equipment. In addition, all the techniques and laboratory procedures necessary for the scientific programs have been established. Examples of these are the routine typing of all tumor tissue for the expression of differentiation and cancer testis antigens by RT-PCR, and, in collaboration with the Department of Pathology, immunohistochemical staining for NY-ESO-1 on paraffin-embedded tissue.

Having obtained approval from the local and state ethics committees for the collection of patient samples (tumor tissue and peripheral blood), we are now able to begin sample collection. We have initiated collaborations with clinical and research departments in gynecology, urology, dermatology, surgery, and pathology, to enable us to collect material from patients with different tumor types. To document the collected patient samples and the typing results, all data are deposited into an ACCESS-based database that was programmed in collaboration with the information technology service of the hospital. This database will also be a valuable tool for the monitoring of patients on study protocols.

1.) SEREX- based Antigen Discovery

Tumor systems derived from patients with favorable history of disease were chosen for SEREX analyses. First, serum from a patient with a metastatic undifferentiated carcinoma that showed spontaneous regression of multiple lesions was tested with a commercially available testis cDNA library. The screening yielded 25 sero-positive clones derived from seven different genes. Six out of these seven antigens exhibited ubiquitous expression in a normal tissue panel. The most predominant clone identified in this screening (14 out of 25 clones) was derived from RIO-10 kinase, which was shown to be variably expressed in a panel of normal and tumor tissues. The mRNA and protein expression of this antigen will be further characterized.

Secondly, a cDNA expression library from a biopsy taken from a skin metastasis was constructed. Nine positive clones were identified, three of which had already been identified in the testis library screening. We are currently analyzing the sequences of the remaining clones.

2) Characterization of new tumor antigens

In collaboration with Drs. Ivan Gout and Valeriy Filonenko in Kyiv, Institute of Molecular Biology and Genetics NAN of Ukraine (from the James R. Kerr Program), a monoclonal antibody (mAb) for NY-BR-1 was generated using a short recombinant protein fragment that has no homologies in the protein database. Four clones were generated, two of which seemed to be NY-BR-1 specific. One clone identified a protein of the estimated size in Western blots using lysates from NY-BR-1 positive and negative cell lines. In collaboration with Dr. B. Odermatt from the Department of Pathology, Zürich University Hospital, the mAb was used to stain paraffin embedded breast tissue and breast cancer samples. Very specific staining

of single cells of the normal duct epithelium was observed. The majority of breast cancer cells reveal expression of NY-BR-1, suggesting a significant up-regulation of this tumor antigen. The collection of breast cancer and prostate cancer samples is being enlarged, in order to confirm these observations. To evaluate spontaneous humoral immune responses, several recombinant NY-BR-1 protein fragments have been produced for Western Blot analyses. In addition a full-length recombinant clone of NY-BR-1 has been constructed for in vitro synthesis of the NY-BR-1 protein.

3) Characterization of Anti-tumor T cell responses

a) Assessment of spontaneous humoral immune responses

Humoral immune responses towards NY-ESO-1, RAB38 and NY-BR-1 in cancer patients are currently being evaluated. Recombinant SSX-2 and MAGE-A3 proteins have also been generated for also performing Western Blot analyses specific for these two antigens. In collaboration with Dr. Elke Jäger, 28 serum samples from patients with malignant melanoma have been analyzed, with four showed significant antibody titers (14%). The assessment of RAB38-specific T cell responses in those patients is ongoing. In collaboration with Dr. Michael Pfreundschuh (Homburg Affiliate Center, Germany), the sera from 50 patients with breast cancer was also analyzed. Using three different protein fragments covering approx. 80% of the full-length sequence, nine samples showed reactivity against at least one fragment (18%). The corresponding primary tumor was also analyzed to determine the NY-BR-1 expression, and 36 out of 50 (72%) tested positive by RT-PCR. In collaboration with the Department of Gynecology, the group is now analyzing, on a routine basis, newly diagnosed breast cancer patients that undergo primary surgery. In addition to the expression analysis using the primary tumor, the serum is tested for NY-BR-1 specific antibody responses using NY-BR-1 protein fragments. The full-length NY-BR-1 protein will be used in the future. So far, three primary breast cancers have been characterized for NY-BR-1 expression, and all were strongly positive.

b) Assessment of spontaneous T cell responses

To further study the antigenicity and immunogenicity of previously identified epitopes derived from tumor antigens, the CD8 T cell-mediated immunity towards antigens that are expressed in tumor lesions of patients was evaluated. In particular, T cell immunity in patients with multiple myeloma will be assessed; the group very recently succeeded in getting funding from the Swiss National Funding agency for this research project. To do this, the group has initiated a collaboration with Dr. Frédéric Levy from the LICR Lausanne Branch, to apply the improved reverse immunology approach in the identification of T cell epitopes from the cancer testis antigen CT7.

In addition to the assessment of CD8 T cell responses, the group is currently establishing the analysis of tumor antigen-specific CD4 T cell responses. In collaboration with Dr. Immanuel Luescher from the LICR Lausanne Branch, experiments are ongoing to evaluate novel MHC class II multimeric constructs to monitor CD4 T cell responses.

c) Identification of novel HLA-A2 restricted CD8 T cell epitopes for NY-BR-1

In recent analyses two potential HLA-A2 restricted epitopes of NY-BR-1 have been identified. To further assess the immunogenicity and tumor reactivity of T cells specific for these epitopes, peripheral blood from eight patients and five healthy individuals were used for the analysis of NY-BR-1 specific CD8 T cell responses. CD8 T cells were separated and stimulated with the two peptides. T cells were tested with multimers and ELISPOT assays. In addition, we were able to generate T cell lines and clones to analyze their lytic capacity against breast cancer cell lines. As the expression of NY-BR-1 in breast cancer cell line is low to absent, further work has to elucidate potential regulatory mechanisms of NY-BR-1 expression.

d) Identification of novel HLA-A2 restricted CD8 T cell epitopes for RAB38

Peripheral blood from 10 patients and five healthy individuals was used for the assessment of RAB38 specific CD8 T cell responses. RAB38 specific CD8 T cell responses were assessed using synthetic RAB38 18-mer peptides covering the entire RAB38 gene product. Twenty-six RAB38 18-mer peptides were

synthetized that overlap by 10 amino acids (aa). CD8 T cells were isolated and stimulated using the whole panel of RAB38 peptides, and then tested in ELISPOT assays against peptide-loaded antigen-presenting cells (APC). T cell reactivity for one 18 aa peptide was detected in two melanoma patients and one healthy individual, and this was confirmed in an independent experiment. Using an algorithm predicting HLA class I binding affinities, a nomamer peptide within the sequence of the long 18 aa peptide was identified and could reproduce T cell reactivity. T cell lines and clones to test tumor reactivity are being generated, and the analyses on the immunogenicity and antigenicity of the identified peptide are being expanded.

4) Clinical Trials Center Activity

In addition to the estaablishment of immunomonitoring tools, the group has established logistics for the realization of immunotherapy phase-I trials. This includes the organization of the data management, and the required correspondence with the ethic committees and Swiss Medic, the Swiss regulatory authority.

Two different clinical immunotherapy studies have been approved by the local ethical committee and have been initiated.

LUD01-014: Phase I/II study of chimeric monoclonal antibody G250 (cG250) in combination with vinblastine administered weekly by intravenous infusion to patients with advanced renal cell carcinoma

LUD 2003-024: Phase I study of immunization with MAGE-3 protein and NY-ESO-1 protein combined with CpG 7909 in patients with high-risk stage D1 or advanced prostate cancer expressing MAGE-3 and/or NY-ESO-1 and/or LAGE-1.

Publications

Primary Research Articles

1. Atanackovic D., Matsuo M., Ritter E., Mazzara G., Ritter G., Jager E., Knuth A., Old L.J., Gnjatic S. Monitoring CD4(+) T cell responses against viral and tumor antigens using T cells as novel target APC. Journal of Immunology Methods (2003) 278:57-66.
2. Cebon J., Jager E., Shackleton M.J., Gibbs P., Davis I.D., Hopkins W., Gibbs S., Chen Q., Karbach J., Jackson H., MacGregor D.P., Sturrock S., Vaughan H., Maraskovsky E., Neumann A., Hoffman E., Sherman M.L., Knuth A. Two phase I studies of low dose recombinant human IL-12 with Melan-A and influenza peptides in subjects with advanced malignant melanoma. Cancer Immunity (2003) 3:7.
3. Gnjatic S., Atanackovic D., Jager E., Matsuo M., Selvakumar A., Altorki N.K., Maki R.G., Dupont B., Ritter G., Chen Y.T., Knuth A., Old L. J. Survey of naturally occurring CD4+ T cell responses against NY-ESO-1 in cancer patients: correlation with antibody responses. Proceedings of the National Academy of Sciences USA (2003) 100:8862-7.
4. Gnjatic S., Atanackovic D., Matsuo M., Jager E., Lee S.Y., Valmori D., Chen Y.T., Ritter G., Knuth A., Old L.J. Cross-presentation of HLA class I epitopes from exogenous NY-ESO-1 polypeptides by nonprofessional APCs. Journal of Immunology (2003) 170:1191-6.
5. Jager E., Jager D., Knuth A. Antigen-specific immunotherapy and cancer vaccines. International Journal of Cancer (2003) 106:817-20.
6. Mischo A., Wadle A., Watzig K., Jager D., Stockert E., Santiago D., Ritter G., Regitz E., Jager E., Knuth A., Old L., Pfreundschuh M., Renner C. Recombinant antigen expression on yeast surface (RAYS) for the detection of serological immune responses in cancer patients. Cancer Immunity (2003) 3:5.

Cancer Vaccines

Active and Closed Clinical Trials – January 1st 2003 to December 31st 2003

MAGE

Trial: LUD1995-004 (Closed)

Principal Site: Clinique Universitaires Saint-Luc, Brussels, Belgium

Principal Investigator: Dr. M. Marchand

Title: Pilot Study of Immunization with the MAGE-3.A2 Peptide in Patients with Malignant Melanoma, Non Small Cell Lung Cancer, Head and Neck Squamous Cell Carcinoma, Esophageal Squamous Cell Carcinoma or Bladder Carcinoma

Trial: LUD1996-007 (Closed)

Principal Site: Clinique Universitaires Saint-Luc, Brussels, Belgium

Principal Investigator: Dr. M. Marchand

Title: Pilot Study of Immunization with the MAGE-1.A1 and MAGE-3.A1 Peptides in Patients with Malignant Melanoma, Non-Small Cell Lung Carcinoma, Head and Neck Squamous Cell Carcinoma, Esophageal Squamous Cell Carcinoma or Bladder Carcinoma

Trial: LUD1997-004

Principal Site: Clinique Universitaires Saint-Luc, Brussels, Belgium

Principal Investigator: Dr. M. Marchand

Title: Phase I/II Study of High-Frequency and Prolonged Immunization with the MAGE-3.A Peptide in Stage III/IV Tumor-Bearing Patients with Malignant Melanoma

Trial: LUD1999-003 (Closed)

Principal Site: Clinique Universitaires Saint-Luc, Brussels, Belgium

Principal Investigator: Dr. M. Marchand

Title: Phase I/II Study of Intradermal and Subcutaneous Immunization with the Recombinant MAGE-3 Protein in Patients with MAGE-3+ , Measurable non-visceral metastatic melanoma

Trial: LUD1999-010

Principal Site: Columbia-Presbyterian Medical Center, New York, USA

Principal Investigator: Dr. N. Altorki

Title: MAGE-3 Protein in Patients with Non-small Cell Lung Cancer

Trial: LUD2001-006 (Closed)

Principal Site: Clinique Universitaires Saint-Luc, Brussels, Belgium

Principal Investigator: Dr. N. VanBaren

Title: Determination of MAGE in peptide-induced immune responses in patients with resected primary or regional melanoma at high risk of relapse

Trial: LUD2002-002

Principal Site: Clinique Universitaires Saint-Luc, Brussels, Belgium

Principal Investigator: Dr. M. Marchand

Title: Phase 1/2 study of intramuscular immunization with recombinant MAGE-3 protein and peptides mixed with an immunological adjuvant containing CpG/QS21/MPL (SB AS-15) in patients with MAGE-3 positive, measurable metastatic cutaneous melanoma

Melan-A

Trial: LUD1996-010

Principal Site: Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland

Principal Investigators: Drs. Lienard, and M. Marchand

Title: Phase I Study of Immunization with Melan-A Peptide, Influenza Matrix Peptide and Adjuvant SB AS-2 in HLA-A2 + Patients with High Risk Stage III or Stage IV Malignant Melanoma

Trial: LUD1998-009

Principal Site: Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland

Principal Investigators: Drs. Lienard and D. Speiser

Title: Specific Immunotherapy of Skin Melanoma Patients with Melan-A/MART-1 and Influenza Peptides and Immunological Analysis of the Vaccine Site Draining Lymph Node (Phase I)

Trial: 2000-005 (Closed)

Principal Site: Austin Hospital, Melbourne, Australia

Principal Investigators: Drs. J. Cebon and I. Davis

Title: A Phase I Study of Immunization with melanoma antigen in conjunction with GM-CSF in patients with fully resected stage 2, 3 or 4 malignant melanom

Trial: LUD2000-018

Principal Site: Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland

Principal Investigator: Dr. D. Speiser

Title: Immunotherapy of HLA-A2 positive stage III / IV melanoma patients with CpG and Melan-A peptide (phase I study)

Trial: LUD2000-025 (Closed)

Principal Site: Weill Medical College of Cornell University, New York, USA

Principal Investigator: Dr. K. Papadopolous

Title: A phase 1 study of peptide-based vaccine therapy in patients with high-risk or metastatic melanoma

NY-ESO-1

Trial: LUD2000-014

Principal Site: Krankenhaus Nordwest, Frankfurt, Germany

Principal Investigator: Dr. A. Knuth

Title: Phase 1 study of recombinant vaccinia NY-ESO-1 (rv-NY-ESO-1) and recombinant fowlpox-NY-ESO-1 (rf-NY-ESO-1) in patients with NY-ESO-1 positive cancers.

Trial: LUD2000-024

Principal Site: Weill Medical College of Cornell University, New York, USA

Principal Investigator: Dr. K. Papadopolous

Title: NY-ESO-1 peptide immunization of patients with cancer expressing NY-ESO-1 antigen

Trial: LUD2000-026

Principal Site: Krankenhaus Nordwest, Frankfurt, Germany

Principal Investigator: Dr. A. Knuth

Title: Phase 1 study of immuniation NY-ESO-1 derived peptides presented by MHC class I molecules administered alone or combined with polyarginine in patients with advanced NY-ESO-1 or LAGE-expressing tumours

Trial: LUD2001-017

Principal Site: Austin Hospital, Melbourne, Australia

Principal Investigators: Drs. J. Cebon and I. Davis

Title: Evaluation of NY-ESO-1 immunity in patients who have previously been vaccinated with NY-ESO-1 protein.

Trial: LUD2002-004

Principal Site: Memorial Sloan-Kettering Cancer Center, New York, USA

Principal Investigators: Dr. D. Bajorin

Title: NY-ESO-1 protein immunization of post-cystectomy or post-nephroureterectomy patients with transitional cell carcinomas expressing NY-ESO-1 or LAGE-1 antigen.

Trial: LUD2002-007

Principal Site: Columbia-Presbyterian Medical Center, New York, USA and Krankenhaus Nordwest, Frankfurt, Germany

Principal Investigators: Drs. N. Altorki and E. Jäger

Title: A pilot study of NY-ESO-1b peptide plus CpG 7909 and Montanide® ISA-51 in patients with cancer expressing NY-ESO-1 or LAGE-1.

Trial: LUD2002-011

Principal Site: Roswell Park Cancer Institute, New York, USA

Principal Investigators: Dr. K. Odunsi

Title: A pilot clinical trial of NY-ESO-1DP4 p157-170 (NY-ESO-1DP4), a peptide of potentially dual MHC class I and class II specificities, in patients with epithelial ovarian, fallopian tube or primary peritoneal carcinoma whose tumors express NY-ESO-1 or LAGE-1.

Trial: LUD2002-013

Principal Site: Austin Hospital, Melbourne, Australia

Principal Investigators: Drs. J. Cebon and I. Davis

Title: A phase II study of the clinical and immunological effects of NY-ESO-1 ISCOM® vaccine in patients with measurable stage III and IV malignant melanoma.

Trial: LUD2002-014

Principal Site: Memorial Sloan-Kettering Cancer Center, New York, USA

Principal Investigators: Dr. J. Dupont

Title: Phase 1 study of NY-ESO-1b peptide plus Montanide ISA-51 in patients with ovarian, primary peritoneal, or fallopian tube cancer expressing NY-ESO-1or LAGE-1.

Multi-Antigen Vaccines

Trial: LUD2000-021 (Closed)

Principal Site: Austin Hospital, Melbourne, Australia

Principal Investigators: Drs. I. Davis and J. Cebon

Title: A phase I/II trial of FLT-3L-mobilized dendritic cells pulsed with peptides derived from cancer-testis antigens in HLA-A2 positive patients with treated cancer and minimal residual disease at high risk of relapse

CpG Oligodeoxynucleotides

Trial: LUD2000-002

Principal Site: Austin Hospital, Melbourne, Australia

Principal Investigator: Dr. I. Davis

Title: A Phase I/II Study of CpG Oligodeoxynucleotides 7909 in the Treatment of Patients with Advanced Renal Cell Carcinoma

Cancer: Renal cell carcinoma

Targeted Antibodies

Active and Closed Clinical Trials – January 1st 2003 to December 31st 2003

A33

Trial: LUD2002-017

Principal Site: Austin Hospital, Melbourne, Australia

Principal Investigator: Dr. A. Scott

Title: Phase 1 trial of oral capecitabine combined with 131I-huA33 in patients with metastic colorectal cancer.

Cancer: Colorectal cancer

G250

Trial: LUD1999-005 (Closed)

Principal Site: University Hospital Nijmegen, Nijmegen, The Netherlands

Principal Investigator: Dr. E. Oosterwijk

Title: Extended Phase I Radioimmunotherapy Trial With 131I-labeled Chimeric Monoclonal Antibody G250 in Patients with Metastatic Renal Carcinoma: Radioimmunotherapy with Two Sequential High-dose 131I-cG250 Treatments

Trial: LUD2000-010 (Closed)

Principal Site: Austin Hospital, Melbourne, Australia

Principal Investigator: Dr. A. Scott

Title: A Pilot Study of cG250 and Low Dose Subcutaneous IL-2 in Patients with Advanced Renal Cell Carcinoma

Trial: LUD2001-014

Principal Site: Krankenhaus Nordwest, Frankfurt, Germany

Principal Investigator: Dr. A. Knuth

Title: Phase 1 study of chimeric monoclonal antibody of cG250 in combination with vinblastine administered weekly by intravenous infusion to advanced renal cell carcinoma

3S193

Trial: LUD1997-010

Principal Site: Austin Hospital, Melbourne, Australia

Principal Investigator: Dr. A. Scott

Title: Phase I Trial of hu3S193 in Patients with advanced epithelial carcinoma expressing Lewis Y antigen

Trial: LUD2001-018

Principal Site: Memorial Sloan-Kettering Cancer Center, New York, USA

Principal Investigator: Dr. C. Divgi

Title: Single-dose, cohort study of increasing doses of Yttrium-90 conjugated to humanized monoclonal antibody 3S193 (90Y-hu3S193) in patients with advanced ovarian cancer

LUDWIG INSTITUTE FOR CANCER RESEARCH

Affiliate Research Reports

Peter ten Dijke, Ph.D.

TGF-β research group
Division of Cellular Biochemistry
Netherlands Cancer Institute
Amsterdam, The Netherlands

Research Report

Transforming growth factor-β (TGF-β) superfamily members regulate a broad scale of developmental processes, and perturbation of their activity has been implicated in a large variety of human diseases. The aims of our research are to elucidate the molecular mechanisms by which TGF-β family members elicit their cellular effects via their serine/threonine kinase receptors and intracellular Smad effectors, and to generate animal models with subverted TGF-β family signaling for human diseases.

TGF-β receptor signal transduction

Cancer cells have often become insensitive to TGF-β-induced growth inhibition and apoptosis because of mutation or functional pertubation of TGF-β signaling components. In late phases of tumorigenesis, TGF-β has been shown to function as a tumor promoter. Carcinomas often secrete high amounts of TGF-β, and respond to it by enhanced invasion and metastasis. TGF-β may mediate these effects directly on tumor cells via subverted Smad-dependent or Smad-independent responses. To obtain more insight into Smad-independent responses we generated a mutant TGF-β type I receptor (ALK5) that is defective in Smad activation, and characterized its signaling properties. The Smad-defective ALK5 mutant was defective in TGF-β-induced extracellular matrix induction, growth inhibition, and epithelial to mesenchymal transdifferentiation, thus these are likely to be dependent on the Smad pathway. However, JNK MAP kinase activation was activated by this mutant ALK5 receptor, and is therefore not dependent on the Smad pathway. This Smad defective mutant will be a valuable tool to further dissect the importance of Smad-independent signaling.

Regulating endothelial cell behavior by TGF-β

TGF-β has been shown to be a potent regulator of proliferation and differentiation of endothelial cells (EC). Moreover, genetic studies in humans and mice have revealed the importance of TGF-β in the regulation of vascular morphogenesis. We found that in endothelial cells, TGF-β can bind and transduce signals via two distinct type I receptors, i.e. the broadly expressed ALK5 that induces Smad2/3 phosphorylation and the endothelium-restricted ALK1 that mediates Smad1/5 activation. This is in contrast to other cells in which TGF-β signals via ALK5. Activation of TGF-β/ALK5 or TGF-β/ALK1 pathways was found to have opposite effects on endothelial behavior; ALK5 inhibits EC migration and proliferation, whereas ALK1 stimulates both processes. We have identified genes that are specifically induced by TGF-β via either the ALK5 or the ALK1 pathway. The TGF-β/ALK5 pathway can elicit an antagonistic signal via direct interaction and activation of ALK1. Activated ALK1 not only induced a biologically opposite response from ALK5, but also directly inhibited ALK5/Smad signaling. Interestingly, we also recently demonstrated that ligand binding and intracellular kinase domains of ALK5 are required for TGF-β/ALK1 activation. The requirement for ALK5 in ALK1 activation and the counteractive interplay between ALK5 and ALK1 provides the EC with an intricately regulated TGF-β controlled switch, which will determine whether its fate is quiescence or active migration and proliferation. The activation state of the endothelium may therefore depend on the level of TGF-β-induced ALK1 or ALK5 activation.

Bone Morphogenetic Protein (BMP)-induced osteoblast differentiation

BMPs have an important role in controlling mesenchymal cell fate, and mediate these effects by regulating gene expression. The critical target genes through which BMPs mediate changes in cell fate are poorly characterized. When C2C12 myoblasts are stimulated with BMPs their differentiation in osteoblast-like

cells is promoted. We performed transcriptional profiling of C2C12 cells upon ectopic expression of one of three distinct BMP type I receptors in a constitutively active mutant form. Each of the three constitutively active BMP type I receptors stimulated equal levels of both R-Smad phosphorylation and alkaline phosphatase, an early marker for osteoblast differentiation. Interestingly, all three type I receptors induced identical transcriptional profiles. Many extracellular matrix genes were upregulated, muscle-related genes downregulated and transcription factors/signaling components modulated. These target genes, some of them unexpected, may offer new insights into how BMPs elicit biological effects.

Publications

Primary Research Articles

1. Althini S., Usoskin D., Kylberg A., ten Dijke P., Ebendal T. Bone morphogenetic protein signalling in NGF-stimulated PC12 cells. Biochemical and Biophysical Research Communications (2003) 307:632-639.
2. Chuva de Sousa Lopes S.M., Carvalho R.L.C., van den Driesche S., Goumans M-J., ten Dijke P., Mummery C.L. Distribution of phosphorylated Smad2 identifies target tissues of TGF-β ligands in mouse development. Gene Expression Patterns (2003) 3:355-360.
3. Dooley, S., Hamzavi J., Breitkopf K., Wiercinska E., Said H.M., Lorenzen J., ten Dijke P., Gressner A.M. Smad7 prevents activation of hepatic stellate cells and liver fibrosis in rat. Gastroenterology (2003) 125:178-191.
4. Edlund S., Bu, S., Schuster N., Aspenstrom P., Heldin N-E., ten Dijke P., Heldin C-H., Landstrom M. TGF-β1-induced apoptosis of prostrate cancer cells involves Smad7-dependent activation of p38 by TAK1. Molecular Biological Cell (2003) 14:529-544.
5. Goumans M-J., Lebrin F., Valdimarsdottir G. Controlling the angiogenic switch: a balance between two distinct TGFβ receptor signaling pathways. Trends in Cardiovascular Medicine (2003) 13:301-307.
6. Goumans M-J., Valdimarsdottir G., Itoh S., Lebrin F., Larsson J., Mummery C., Karlsson S., ten Dijke P. Activin receptor-like kinase (ALK)1 is an antagonistic mediator of lateral TGFβ/ALK5 signaling. Molecular Cell (2003) 12: 817-828.
7. Itoh S., Thorikay M., Kowanetz M., Moustakas A., Itoh F., Heldin C-H., ten Dijke P. Elucidation of Smad requirement in transforming growth factor-β type I receptor induced responses. Journal of Biological Chemistry (2003) 278:3751-3761.
8. Janssens K., ten Dijke P., Ralston S.H., Bergmann C., van Hul W. Transforming growth factor-β1 mutations in Camurati-Engelmann disease lead to increased signaling by altering either activation or secretion of the mutant protein. Journal of Biological Chemistry (2003) 278:7718-7724.
9. Kaivo-Oja N., Bondestam J., Kamarainen M., Koskimies J., Vitt U., Cranfield M., Vuojolainen K., Kallio K.P., Olkonen V.M., Hayashi M., Moustakas A., Groome N.P., ten Dijke P., Hsueh A.J., Ritvos O. Growth differentiation factor-9 induces Smad2 activation and inhibin B production in cultured human granulosa-luteal cells. Journal of Clinical Endocrinology and Metabolism (2003) 88:755-762.
10. Korchynskyi O., Dechering K.J., Sijbers A., Olijve W., ten Dijke P. Gene array analysis of bone morphogenetic protein type I receptor-induced osteoblast differentiation. Journal of Bone and Mineral Research (2003) 18:1177-1185.
11. Kunzmann S., Wohlfahrt J.G., Itoh S., Asao H., Komada M., Akdis C.A., Blaser K., Schmidt-Weber C.B. SARA and Hgs attenuate susceptibility to TGF-β1-mediated T cell suppression. FASEB Journal (2003) 17:194-202.

12. Larsson J., Blank U., Helgadottir H., Bjornsson J.M., Ehinger M., Goumans M.J., Fan X., Leveen P., Karlsson S. TGF-β signaling-deficient hematopoietic stem cells have normal self-renewal and regenerative ability in vivo despite increased proliferative capacity in vitro. Blood (2003) 102:3129-35.
13. Liang Y.Y., Lin X., Liang M., Brunicard F.C., ten Dijke P., Chen Z. Choi K.W., Feng X.H. dSmurf selectively degrades DPP-activated MAD and its overexpression disrupts imaginal disc development. Journal of Biological Chemistry (2003) 278:26307-26310.
14. Roelen B.A.J., ten Dijke P. Controlling mesenchymal stem cell differentiation by TGF-β family members. Journal of Orthopeadic Science (2003) 8:740-748.
15. Shiraishi K., Kurisaki A., Valcourt U., Terentiev A.A., Pardali K., ten Dijke P., Heldin C-H., Ericsson J., Moustakis A. Nuclear factor YY1 inhibits transforming growth factor β and bone morphogenetic protein-induced cell differentiation. Molecular Cell Biology (2003) 23:4494-4510.
16. Suh N., Roberts A.B., Birkey Reffey S., Miyazono K., Itoh S., ten Dijke P., Heiss E.H., Place A.E., Risingsong R., Williams C.R., Honda T., Gribble G.W., Sporn M.B. Synthetic triterpenoids enhance transforming growth factor β/Smad signaling. Cancer Research (2003) 63:1371-1376.
17. Ten Dijke P., Korchynskyi O., Valdimarsdottir G., Goumans M-J. Controlling cell fate by BMP family members. Molecular and Cellular Endocrinology (2003) 211:105-113.
18. Ten Dijke P., Fu J., Schaap P., Roelen B.A.J. Signal transduction of bone morphogenetic proteins in osteoblast differentiation. Journal of Bone and Joint Surgery American Volume (2003) 85-A Suppl 3:34-38.

Reviews / Book Chapters / Commentaries

1. Ten Dijke P., Piek E. Receptor serine/threonine kinases. The encyclopedia of endocrinology and endocrine diseases. Ed. L. Martini, Academic Press 2003

V. Peter Collins, M.D., B.Ch., F.R.C.Path., F.Med. Sci.

Division of Molecular Histopathology,
Department of Pathology
University of Cambridge
Cambridge, UK

Research Report

The research aims of the group are to define the molecular mechanisms involved in the oncogenesis and progression of primary human brain tumors. These data will form the basis for the identification of prognostic markers, indicators of sensitivity and resistance to therapy, and the development of logical, innovative and specific treatment modalities. The research focuses on characterizing the genomic abnormalities and transcriptosome in a large panel of histologically well-characterized tumors from patients with careful follow up.

Correlating Genetic Findings In Gliomas With Clinical Data

The group has previously reported on a relatively comprehensive analysis of the Rb1 and the p53 pathways in a series of 190 diffuse adult astrocytic tumors. This study demonstrated that the p53 and Rb1 pathways were specifically targeted in the majority of glioblastomas, but only one gene coding for a protein component of each pathway was mutated in each tumor. In addition, the majority of the tumors were analyzed for loss of wild type PTEN and epidermal growth factor receptor (EGFR) amplification. The group has now completed a study correlating these genetic findings to survival in glioblastomas (malignancy grade IV according to the World Health Organization, WHO) and was able to identify significantly differing groups. The team found that abnormalities in any of the four genes (CDKN2A, CDKN2B, RB1, CDK4) coding for components of the Rb1 pathway were associated with shorter survival ($p=0.002$). In combination with loss of wild-type PTEN, the association was even stronger ($p<0.001$), the median survival being 166 days as compared with the group without these abnormalities. However, where the tumor showed a typical glioblastoma histology, the median post-operative survival was 437 days. The survival difference remained statistically significant in Cox Regression analysis adjusting for age ($p=0.012$). The findings indicate that knowledge of the molecular genetic abnormalities in glioblastomas provides important data in assessing individual patients. As further advances in the understanding of the molecular genetics and cell biology of gliomas are made, in addition to providing prognostic information, such data may also provide targets for innovative therapy in the individual case.

EGFR Family

The group has continued studies of 7p and the EGFR gene. The genes amplified adjacent to EGFR, without amplification of EGFR, are being investigated. The most frequently amplified and highly expressed adjacent gene has been cloned into an expression vector with and without green fluorescent protein (GFP) in order to investigate the localization of the protein in the cell and its function. The various rearrangements of the amplified EGFR gene and its amplicon in brain tumors are being documented in detail in the light of the chromosome 7 sequence data that is now available.

Expression Of Adenovirus Receptors By Astrocytic Gliomas

In collaboration with the Stockholm Branch, the group has documented the expression of the coxsackie and adenovirus receptor (CAR) expression in astrocytic tumors and xenografts. A great variation of CAR expression was also observed in primary astrocytomas of different malignancy grades. The mean value of CAR expression was significantly lower in the 22 Grade IV tumors studied as compared to the values for 6 Grade II (p 0.01) and 6 Grade III (p 0.01) tumors. When the hCAR expression in 11 xenografts derived

from Grade IV gliomas were compared to the levels detected in the original parental tumors, a mean 12-fold higher expression was seen in the xenografts (p 0.01). Two xenografts with low hCAR expression grew considerably faster than the hCAR-expressing cells. These results have relevance for the use of adenoviruses in gene therapy against astrocytomas.

Analysis Of The Genome In Gliomas.

In an attempt to clarify the chromosomal status of the uncommon but histologically well defined glioma known as Myxopappillary Ependymoma (MPE) and identify commonly aberrant regions in the genome of this tumor, the group has combined three molecular/cyto/genetic methods to study 17 MPEs. Comparative genomic hybridization of 7/17 tumors identified concurrent gain on chromosomes 9 and 18 as the most frequent finding. The majority of the 17 tumors were also studied using microsatellite analysis with markers spanning the whole chromosomes 9 and 18 and interphase-fluorescent in situ hybridization (FISH) with centromeric probes for both chromosomes. The combined results were consistent with concurrent gain in both chromosomes 9 and 18 in 11/17 cases, gain of either chromosome 9 or 18 and imbalance in other chromosome in 3/17 tumors, and allelic imbalances of chromosomes 9 or 18 in 3/17 and 1/17 tumors, respectively. Other abnormalities observed included gain of chromosomes 3, 4, 7, 8, 11, 13, 17q, 20, and X, and loss of chromosomes 10 and 22. These findings represent some steps towards understanding the molecular mechanisms involved in the development of MPE.

In the last two years the group has put much energy into developing genomic array techniques that would allow the more detailed analysis of the tumor series. This has resulted in the group now having all the techniques for the production, printing, and use of genomic arrays. The group currently has access to a tiled chromosome 1 array and has itself produced a 10Mb, and a 1Mb array, as well as tiled arrays for chromosomes 6, 10, and 22. Some of these have been in use over the last year, and the analysis of the large amounts of data that these genomic arrays provide is currently being completed. The group also spent time working on techniques to amplify the limited material from clinical samples for transcriptosome analysis. However, following many tests of available cDNA and oligonucleotide arrays, it was concluded that valuable clinical material should be used only on quality controlled manufactured arrays.

Interest in chromosome 22 and NF2 is continuing, with a number of studies being published on the group's series of schwannomas, and these have helped form the basis of a major change in technology, namely establishing the use of genomic arrays in the analysis of tumor genomes. Previous low-resolution schwannoma studies have reported diverse frequencies (30-80%) of 22q deletions, involving the neurofibromatosis-2 tumor suppressor (NF2) gene. The group studied 47 patients and detected heterozygous deletions in 21 (45%) of schwannomas, which could be classified into three profiles. The predominant profile (17/21) was a continuous deletion consistent with monosomy 22. The second profile was composed of four cases displaying interstitial deletions of various sizes. Two of these did not encompass the NF2 locus, which emphasizes the importance of other loci in schwannoma development. These findings warrant further studies of an extended tumor series, to better define additional, putative 22q-located loci important for schwannoma development.

Publications

Primary Research Articles

1. Backlund L.M., Nilsson B.R., Goike H.M., Schmidt E.E., Liu L., Ichimura K., Collins V.P. Short postoperative survival for glioblastoma patients with a dysfunctional Rb1 pathway in combination with no wild-type PTEN. Clinical Cancer Research (2003) 9(11):4151-8.

2. Fuxe J., Liu L., Malin S., Philipson L., Collins V.P., Pettersson R.F. Expression of the coxsackie and adenovirus receptor in human astrocytic tumors and xenografts. International Journal of Cancer (2003) 103(6):723-9.

3. Jungbluth A.A., Stockert E., Huang H.J., Collins V.P., Coplan K., Iversen K., Kolb D., Johns T.J., Scott A.M., Gullick W.J., Ritter G., Cohen L., Scanlan M.J., Cavenee W.K., Old L.J. A monoclonal antibody recognizing human cancers with amplification/overexpression of the human epidermal growth factor receptor. Proceedings of the National Academy of Sciences USA (2003) 100(2):639-44.

4. Mahler-Araujo M.B., Sanoudou D., Tingby O., Liu L., Coleman N., Ichimura K., Collins V.P. Structural genomic abnormalities of chromosomes 9 and 18 in myxopapillary ependymomas. Journal of Neuropathology and Experimental Neurology (2003) 62(9):927-35.

5. Mantripragada K.K., Buckley P.G., Benetkiewicz M., De Bustos C., Hirvela C., Jarbo C., Bruder C.E., Wensman H., Mathiesen T., Nyberg G., Papi L., Collins V.P., Ichimura K., Evans G., Dumanski J.P. High-resolution profiling of an 11 Mb segment of human chromosome 22 in sporadic schwannoma using array-CGH. International Journal of Oncology (2003) 22(3):615-22.

6. Petalidis L., Bhattacharyya S., Morris G.A., Collins V.P., Freeman T.C., Lyons P.A. Global amplification of mRNA by template-switching PCR: linearity and application to microarray analysis. Nucleic Acids Research (2003) 31(22):e142.

Kari Alitalo, Ph.D.

University of Helsinki,
Helsinki, Finland

Research Report

A significant achievement by the laboratory was the isolation, cloning and characterization of the first lymphangiogenic growth factor, vascular endothelial growth factor (VEGF) -C, and the isolation of lymphatic endothelial cells. These findings opened the lymphatic vascular system to molecular analysis after over a hundred years of descriptive pathology. The laboratory has also been central in the isolation and characterization of VEGF-B, VEGF-C and VEGF-D receptors and signal transduction pathways, and the function of VEGFR-3, showing that this receptor is required for angiogenesis and later in lymphangiogenesis in embryos. The group has also analyzed Vegfc deficient mice, and shown that VEGF-C is required for the initial steps in lymphatic development. Interestingly, VEGF-D does not rescue the $Vegfc^{+/-}$ phenotype. The laboratory's current studies include the analysis of Vegfc x Vegfd double knockout mice, in collaboration with Drs. Marc Achen and Steven Stacker from the LICR Melbourne Branch, which should reveal whether there are any additional ligands for VEGFR-3. To identify new genes and corresponding modifying factors, and to investigate their role in lymph/angiogenesis in vertebrate models, Dr. Alitalo's team is utilizing innovative, high throughput and large-scale functional genomics approaches.

The laboratory has demonstrated that VEGF-C is overexpressed in tumors, and its receptor VEGFR-3 is up-regulated in angiogenic tumor vasculature. These studies revealed VEGF-C associated tumor lymphangiogenesis, intralymphatic tumor growth, the association of VEGF-C with tumor metastasis, and the ability to inhibit metastasis by blocking the VEGFR-3 signal transduction pathway. The data demonstrate that during tumor lymphangiogenesis and cancer dissemination via the lymphatics, the newly formed lymphatic vessels originate from the pre-existing lymphatic network with little if any involvement of bone marrow-derived endothelial progenitor cells. Activation of the lymphatic endothelium by inducing lymphatic sprouting and dilation of pre-existing lymphatic vessels was shown to be crucial for tumor cell entry into the lymphatics and their spread to the lymph nodes. Interestingly, inhibition of VEGFR-3 signaling via an adenoviral vector mediated systemic delivery of a soluble VEGFR-3-Ig fusion protein could efficiently block lymph node metastasis. The basis of this inhibition will be analyzed in future studies. Also, the group is trying to develop preclinical models and surrogate endpoints for metastasis inhibition. During the past years the laboratory has developed a method for the isolation of human primary lymphatic and blood microvascular endothelial cells, and has shown that these cells have interesting differences in gene expression relevant for their distinct functions in vivo. These studies establish the central role of the VEGF-C gene in lymphangiogenesis and make it a key target for the inhibition of tumor lymphangiogenesis and metastasis.

Publications

Primary Research Articles

1. Abassi Y.A., Rehn M., Ekman N., Alitalo K., Vuori K. p130Cas Couples the tyrosine kinase Bmx/Etk with regulation of the actin cytoskeleton and cell migration. Journal of Biological Chemistry (2003) 278(37):35636-43.

2. Auvinen M., Jarvinen K., Hotti A., Okkeri J., Laitinen J., Janne O.A., Coffino P., Bergman M., Andersson L.C., Alitalo K., Holtta E. Transcriptional regulation of the ornithine decarboxylase gene by c-Myc/Max/Mad network and retinoblastoma protein interacting with c-Myc. International Journal of Biochemistry and Cell Biology (2003) 35(4):496-521.

3. Bhardwaj S., Roy H., Gruchala M., Viita H., Kholova I., Kokina I., Achen M.G., Stacker S.A., Hedman M., Alitalo K., Yla-Herttuala S. Angiogenic responses of vascular endothelial growth factors in periadventitial tissue. Human Gene Therapy (2003) 14(15):1451-62.

4. Dixelius J., Makinen T., Wirzenius M., Karkkainen M.J., Wernstedt C., Alitalo K., Claesson-Welsh L. Ligand-induced vascular endothelial growth factor receptor-3 (VEGFR-3 heterodimerization with VEGFR-2 in primary lymphatic endothelial cell regulates tyrosine phosphorylation sites. Journal of Biological Chemistry (2003) 278(42): 40973-9.

5. Gerhardt H., Golding M., Fruttiger M., Ruhrberg C., Lundkvist A., Abramsson A., Jeltsch M., Mitchell C., Alitalo K., Shima D., Betsholtz C. VEGF guides angiogenic sprouting utilizing endothelial tip cell filopodia. Journal of Cell Biology (2003) 161(6):1163-77.

6. Hu B., Guo P., Fang Q., Tao H.Q., Wang D., Nagane M., Huang H.J., Gunji Y., Nishikawa R., Alitalo K., Cavenee W.K., Cheng SY. Angiopoietin-2 induces human glioma invasion through the activation of matrix metalloprotease-2. Proceedings of the National Academy of Sciences USA (2003) 100(15):8904-9.

7. Iivanainen E., Nelimarkka L., Elenius V., Heikkinen S.M., Junttila T.T., Sihombing L., Sundvall M., Maatta J.A., Laine V.J., Yla-Herttuala S., Higashiyama S., Alitalo K., Elenius K. Angiopoietin-regulated recruitment of vascular smooth muscle cells by endothelial-derived heparin binding EGF-like growth factor. FASEB Journal (2003) 12:1609-21.

8. McColl B.K., Baldwin M.E., Roufail S., Freeman C., Moritz R.L., Simpson R.J., Alitalo K., Stacker S.A., Achen M.G. Plasmin activates the lymphangiogenic growth factors VEGF-C and VEGF-D. Journal of Experimental Medicine (2003) 198(6):863-8.

9. Nykanen A.I., Krebs R., Saaristo A., Turunen P., Alitalo K., Yla-Herttuala S., Koskinen P.K., Lemstrom K.B. Angiopoietin-1 protects against the development of cardiac allograft arteriosclerosis. Circulation (2003) 107(9):1308-14.

10. Rissanen T.T., Markkanen J.E., Gruchala M., Heikura T., Puranen A., Kettunen M.I., Kholova I., Kauppinen R.A., Achen M.G., Stacker S.A., Alitalo K., Yla-Herttuala S. VEGF-D is the strongest angiogenic and lymphangiogenic effector among VEGFs delivered into skeletal muscle via adenoviruses. Circulation Research (2003) 92(10):1098-106.

11. Rutanen J., Leppanen P., Tuomisto T.T., Rissanen T.T., Hiltunen M.O., Vajanto I., Niemi M., Hakkinen T., Karkola K., Stacker S.A., Achen M.G., Alitalo K., Yla-Herttuala S. Vascular endothelial growth factor-D expression in humanatherosclerotic lesions. Cardiovascular Research (2003) 59(4):971-9.

12. Salven P., Mustjoki S., Alitalo R., Alitalo K., Rafii S. VEGFR-3 and CD133 identify a population of CD34+ lymphatic/vascular endothelial precursor cells. Blood (2003) 101(1):168-72.

13. Tille J.C., Wang X., Lipson K.E., McMahon G., Ferrara N., Zhu Z., Hicklin D.J., Sleeman J.P., Eriksson U., Alitalo K., Pepper M.S. Vascular endothelial growth factor (VEGF) receptor-2 signaling mediates VEGF-C (deltaNdeltaC)- and VEGF-A-induced angiogenesis in vitro. Experimental Cell Research (2003) 285(2):286-98.

14. Veikkola T., Lohela M., Ikenberg K., Makinen T., Korff T., Saaristo A., Petrova T., Jeltsch M., Augustin H.G., Alitalo K. Intrinsic versus microenvironmental regulation of lymphatic endothelial cell phenotype and function. FASEB Journal (2003) 14:2006-13.

15. Wickstrom S.A., Alitalo K., Keski-Oja J. Endostatin associates with lipid rafts and induces reorganization of the actin cytoskeleton via down-regulation of RhoA activity. Journal of Biological Chemistry (2003) 278(39):37895-901.

16. Zhang R., Xu Y., Ekman N., Wu Z., Wu J., Alitalo K., Min W. Etk/Bmx transactivates VEGFR2 and recruits phosphatidylinositol 3-kinase to mediate TNF-induced angiogenic pathway Journal of Biological Chemistry (2003) 278(51):51267-76.

Reviews/Book Chapters/Commentaries

1. Jeltsch M., Tammela T., Alitalo K., Wilting J. Genesis and pathogenesis of lymphatic vessels. Cell Tissue Research (2003) 314:69-84.
2. Kubo H., Alitalo K. The bloody fate of endothelial stem cells. Genes & Development (2003) 17(3):322-9.
3. Lohela M., Saaristo A., Veikkola T., Alitalo K. Lymphangiogenic growth factors, receptors and therapies. Thrombosis Haemostasis (2003) 90:167-84.
4. Makinen T, Alitalo K. Molecular mechanisms of lymphangiogenesis. Cold Spring Harb Symposium on Quantitative Biology (2003) 67:189-96.
5. Saharinen P., Alitalo K. Double target for tumor mass destruction. Journal of Clinical Investigation (2003) 111(9):1277-80.
6. Wickstrom S.A., Keski-Oja J., Alitalo K. Matrix reloaded to circulation hits the tumor target. Cancer Cell (2003) 3(6):513-4.
7. Yla-Herttuala S., Alitalo K. Gene transfer as a tool to induce therapeutic vascular growth. Nature Medicine (2003) 6:694-701.

Michael Pfreundschuh, M.D. & Christoph Renner, M.D., Ph.D.

University of Saarland
Homburg, Germany

Research Report

Our group in Homburg is actively involved in two LICR programs: the identification of new tumor antigens using the SEREX methodology (Cancer Antigen Discovery Program): and the generation of new antibody constructs for the treatment of cancer (Antibody Targeting Program. Within these Programs we are attempting the following:

1. more detailed characterisation of known SEREX defined antigens (SSX-2, SCP-1) in regard to their expression pattern in different tumors and potential function
2. generation of an eukaryotic expression system either in yeast or mammlians to overcome limitations set by the conventional SEREX technology
3. identification of immunogenic peptides suitable for vaccine purposes
4. systematic serological analysis of immune responses in a broad range of tumor patients

The transfer of the SEREX technology into an eukaryotic expression system (yeast) has allowed us to identify potential candidate genes by automatic cell sorting. From our analysis of a breast cancer library, many genes known to be overexpressed in breast cancer have been isolated, and we are currently further evaluating these as potential target antigens. Using this methodology a new mucine variant has been identified. The analysis is currently being extended to cancers of the prostate, pancreas and colon, and glioblastoma and mantle cell leukemia.

Within the LICR Antibody Targeting Program we have two major goals focusing on the isolation of MHC-peptide specific antibodies, and the generation of new recombinant antibodies with enhanced biological effector function. MHC-peptide antibodies are a new class of antibodies that allow for the specific detection of antigen derived peptides presented in a predefined MHC context. As an example, we have generated antibodies that recognise specifically the NY-ESO$_{157-165}$-peptide/HLA-A0201 complex. Peptide variants such as the NY-ESO-1$_{157-167}$ peptide or the cryptic NY-ESO$_{159-167}$ peptide were not recognized. Moreover, the antibody blocked in a dose dependent fashion the recognition of NY-ESO-1/HLA-A2 positive tumor cells by NY-ESO-1$_{157-167}$ peptide specific CD8(+) T cells. Our antibody is currently the only tool that binds with TCR-like specificity to the NY-ESO-1$_{157-165}$/HLA-A2 complex and is, therefore, a prime reagent for the development and monitoring of NY-ESO-1 based vaccine trials as it distinguishes clearly between CTL responses against immunological meaningful or cryptic NY-ESO-1 derived peptides.

Clinical trials with monoclonal antibodies for the treatment of solid tumors have demonstrated in the majority of cases that the intrinsic biological acitivity of these molecules is too low to induce a significant level of tumor cell destruction. Therefore, we have adopted the technology of immunocytokines to enhance biological activity. Tumor necrosis factor (TNF) and interleukin-8 were chosen since these cytokines are very capable of recruiting polymorphic nucleated cells including macrophages which cause intensive local activation. The TNF-immunocytokine (anti-FAP-TNF) generated carries a TNF dimer that binds with moderate affinity to TNF-RI but is very efficient at activating TNF-RII when binding to tumor cells via the antibody domain. The preferential activation of TNF-RII with low affinity to TNF-RI should result in reduced systemic TNF related side-effects with strong TNF activity at the tumor site. The generation of a high level producing cell line will hopefully allow for the transition of the system into the clinic.

Publications

Primary Research Articles

1. Mischo A., Wadle A., Watzig K., Jager D., Stockert E., Santiago D., Ritter G., Regitz E., Jager E., Knuth A., Old L., Pfreundschuh M., Renner C. Recombinant antigen expression on yeast surface (RAYS) for the detection of serological immune responses in cancer patients. Cancer Immunity (2003) 3:5.
2. Deckert P.M., Renner C., Cohen L.S., Jungbluth A., Ritter G., Bertino J.R., Old L.J., Welt S. A33scFv-cytosine deaminase: a recombinant protein construct for antibody-directed enzyme-prodrug therapy. British Journal of Cancer (2003) 88(6):937-939.
3. Wagner C., Neumann F., Kubuschok B., Regitz E., Mischo A., Stevanovic S., Friedrich M., Schmidt W., Rammensee H.G., Pfreundschuh M. Identification of an HLA-A*02 restricted immunogenic peptide derived from the cancer testis antigen HOM-MEL-40/SSX2. Cancer Immunity (2003) 3:18.
4. Niemeyer P., Tureci O., Eberle T., Graf N., Pfreundschuh M., Sahin U. Expression of serologically identified tumor antigens in acute leukemias. Leukemia Research (2003) 27(7):655-60.
5. Xie X., Wacker H.H., Huang S., Regitz E., Preuss K.D., Romeike B., Parwaresch R., Tiemann M., Pfreundschuh M. Differential expression of cancer testis genes in histological subtypes of non-Hodgkin's lymphomas. Clinical Cancer Research (2003) 9(1):167-73.

Carl Batt, Ph.D.

Cornell University
Ithaca, USA

Research Report

In 2003, the Cornell University/Ludwig Institute for Cancer Research Partnership inaugurated a 1200 square foot clean room complete with all of the necessary equipment and infrastructure for the Good Manufacturing Practice (GMP) production of recombinant proteins. Two primary expression systems are typically pursued almost in parallel, *Escherichia coli* and *Pichia pastoris*. Each offers its own advantages and challenges. To support the GMP effort, a quality control laboratory has been established to insure that the product is consistent by a number of analytical measures. This GMP facility, in conjunction with the bioprocess development laboratory, enables the Partnership to offer LICR a core competence in the area of recombinant protein production under conditions that allow these therapeutic agents to be used in Phase I clinical trials.

To date the Partnership has focused on two broad areas of targets; single chain antibodies and cancer-testis (CT) antigens. The current list of targets has been formulated with input from a wide representation of the LICR, and prioritized with respect to needs and technical progress against the goals of the program. We envision a minimum of four targets being produced by the GMP in any 12 month period with a much greater number in development at any one time.

We have expressed the single chain antibody that recognizes the A33 antigen. This ScFv was developed by Dr. Christoph Rader at Scripps and was transferred to the Partnership under the auspices of the LICR. The ScFv was expressed in *Pichia pastoris* and served as a model for the development of unique methanol feeding strategies that allow for optimization of protein production. The expression of the ScFv through modeling, assembly and implementation of a PID controller was optimized to yield a final production level of more than 6 gm/L of fermentation broth. By the judicious insertion of a αMF signal sequence upstream of the ScFv, we were able to secret the targeted protein into the medium greatly simplifying down stream purification. A combination of ion exchange and hydrophobic interaction chromatography was used to purify the ScFv to near homogeneity and with overall yields of approximately 25%. This fermentation and purification process has been transferred to the GMP, and the production of the ScFv-A33 represents the inaugural product produced in the GMP.

A series of other targets, mainly CT antigens are currently under development in the Partnership bioprocess development laboratory including NY-ESO-1, MAGE A3, Melan-A SSX2, NY-BR-1 and LAGE-1. The expression of these targets is being pursued in *E. coli* and *P. pastoris* in parallel, with priority then given to the system that is most likely to succeed in the GMP. While *E. coli* is more facile to manipulate, all of the CT antigens form inclusion bodies to varying degrees which complicates purification. Therefore *P. pastoris* is the system of choice when high levels of expression and secretion of the target are observed.

NY-ESO-1 has been expressed in *E. coli* both as a native protein and tagged with a 'his tag' which facilitates subsequent purification. While NY-ESO-1 forms inclusion bodies, subsequent purification protocols have been developed and optimized that result in a moderate degree of purification. The resulting product appears to be as pure as reagents currently used in clinical trials and this process will be transferred to the GMP in the second quarter of 2004. MAGE A3, Melan-A and SSX2 are all being expressed in *E. coli* and purification protocols being developed. Expression of these CT antigens is being simultaneously pursued in *P. pastoris* with some success. NY-ESO-1 appears to be secreted as a soluble protein, but the N-terminal signal sequence is not being processed and remains on the protein found in the culture medium. Cell lysis has been largely eliminated as an explanation and this phenomenon is currently being investigated.

Richard R. Schmidt, Ph.D.

Fachbereich Chemie
Universität Konstanz
Konstanz, Germany

Research Report

Chemical Synthesis and Preparation of Glycolipids (alpha-Gal-Cer), Chelating Agents, Signal Inhibitor Mimetics and Synthetic QS 21 Adjuvant

In 2003, the group in Konstanz worked in four different areas which were partly supported by LICR:

Synthesis of α-Galactopyranosyl Ceramide and Structural Variants

CD1 proteins are a lineage of antigen-presenting molecules with unusually hydrophobic ligand-binding grooves that present glycolipid antigens for T-cells. α-Galactopyranosyl ceramide, with C18-phytoshingosine as one of the lipid moieties, is such a potent stimulator of human T-cells. Stimulation occurs through binding of the glycolipid by CD1d, presentation to T-cells, and formation of a CD1d-glycolipid-T-cell receptor complex. The demand for this compound for immunological studies in the laboratories of Drs. Vincenzo Cerundolo (Oxford Affiliate Center), and Gerd Ritter (LICR New York Branch) was reason to repeat our previously reported synthesis and to provide material to these laboratories. This work was also extended to a structural variant with a C9-phytosphingosine residue which was also employed in biological studies.

Synthesis of Saponin QS21 Analogs as Immune Adjuvants

Saponins are complex amphiphilic glycolipid conjugates which are mainly of plant origin. Many of them exhibit immunostimulatory properties. A particularly interesting class of saponins is obtained from the bark of *Quillaja Saponaria* Molina, a tree which grows mainly in South America. The most famous saponin from this source is QS 21, a bisdesmosidic triterpenoid glycoside with a very complex structure; this compound is the most potent saponin immune adjuvant known to date. Because of the complexity of this structure, about 30 to 40 structurally simpler derivatives were synthesized in the Konstanz group and provided to the LICR New York Branch for biological studies. For various reasons extension of this work to partial structures of QS21 is desirable. This work was also initiated in 2003.

Synthesis of Bifunctional Chelating Agents for Radioimmunotherapy (RIT)

Bifunctional chelating agents continue to be an interesting topic in medicinal and chemical research because of their high usefulness in the diagnosis and therapy of cancer diseases. The principal idea underlying radioimmunoimaging (RII) and RIT is to combine a molecule with a chelating functionality, which will sequester a radioactive metal ion, together with a monoclonal antibody or any other receptor-specific substrate. This approach allows it to deliver the radiopharmaceutical specifically to the malignant tissue while minimizing the risk of unspecific irradiation of sane tissue. We have investigated the synthesis of some novel bifunctional chelating agents which partly possess a carbohydrate backbone. Some target molecules have been made available for evaluation in a collaboration with Drs. Chaitanya Divgi and Peter Smith-Jones (New York (Memorial Sloan-Kettering) Affiliate Center) and Dr. Ritter. Because of the excellent results a provisional patent application was filed.

Synthesis of Carbohydrate Based VEGF Peptide Mimetics as Angiogenesis Inhibitors

Angiogenesis plays an important role in the pathogenesis of a number of diseases, in particular in promoting the growth and metastatic spread of solid tumours. Members of the vascular endothelial growth factor (VEGF) family of proteins are key mediators of angiogenesis; their biological effects are mediated through receptors (VEGFRs). Recently VEGF-D has been shown to be expressed in human tumours, to be angiogenic and lymphangiogenic. Therefore, VEGF-D may be of particular importance for

the malignancy of tumours, as metastases can spread via either blood vessels or lymphatic vessels. The goal of our work in collaboration with Dr. Richard Hughes (University of Melbourne) and Drs. Marc Achen and Steven Stacker (LICR Melbourne Branch) is to use a partial peptide structure of VEGF-D as a lead for the design of VEGFR-2/-3 inhibitors, which could be potential anti-tumour agents. In order to reduce biological degradation sugar residues are included in the synthesis design.

In 2003 we completed the synthesis of fifteen sugar amino acid containing cyclic peptides of type A and B which mimic a loop structure of VEGF-D. Preliminary biological studies with some of these compounds exhibit binding inhibition to VEGFRs.

Publications

Primary Research Articles

1. Hermans I.F., Silk J.D., Gileadi U., Salio M., Mathew B., Ritter G., Schmidt R.R., Harris A.L., Old L., Cerundolo V., Journal of Immunology (2003) 171:5140-7.

Patents

1. Arslantas E., Schmidt R.R., Smith-Jones P., Ritter G. TAME Based Chelators and Uses thereof. Patent LUD5876-NHD (10314593)

Seppo Ylä-Herttuala, M.D., Ph.D.

A.I.Virtanen Institute
University of Kuopio
Kuopio, Finland

Research Report

Angiogenesis and Lymphangiogenesis in Animal Models

During the last year we have tested the efficacy of various vascular epidermal growth factor (VEGF) family members for their ability to induce angiogenesis in a rabbit hindlimb model, using adenovirus-mediated gene transfer. VEGF-A, VEGF-B, VEGF-C long form, VEGF-C156 mutant, VEGF-D long form and mature short form of VEGF-D were compared using both histological and magnetic resonance imaging (MRI) methods and analysis of the blood flow. It was found that of all VEGFs, VEGF-A and VEGF-D mature short form were the most efficient in inducing angiogenesis and improved blood flow in rabbit hindlimbs. All VEGFs appeared to be safe and well tolerated. Thus, it is concluded that VEGF-A and VEGF-D mature form are potential drugs for therapy of muscle ischemia. VEGF-C and VEGF-D long forms only showed lymphangiogenic activity in rabbit hindlimbs. The same viruses were tested for their angiogenic and lymphangiogenic activity in the periarterial space using the collar model in rabbit carotid arteries. VEGF-C and VEGF-D long forms showed angiogenic activity, but VEGF-A and the mature short form of VEGF-D were also the most highly angiogenic growth factors in this model.

VEGF-A and mature short form of VEGF-D were also tested in a pig myocardial model using percutaneous, catheter-mediated delivery of adenoviruses. It was found that intraventricular delivery was efficient in causing capillary enlargement and improved blood flow in the pig myocardium. The effect was dose-dependent and optimal dose was established. No alterations were found in clinical chemistry parameters. Results suggest that members of the VEGF family may be useful for the treatment of myocardial ischemia.

As part of collaborative work with other LICR laboratories, we have provided several adenoviral constructs encoding members of the VEGF family and VEGF receptors to Dr. Kari Alitalo's group (Helsinki Affiliate Center) and to other LICR laboratories.

VEGF Ligand and Receptor Characterization

Expression of VEGF-A and –D and VEGF receptors 1 and 2 were analyzed in human normal and atherosclerotic arteries. It was found that VEGF-A and D were mainly expressed in medial smooth muscle cells. Macrophages also expressed both VEGF-A and VEGF-D around neovessels in atherosclerotic lesions. VEGF receptor 1 and 2 expression was found in endothelial cells both in normal and atherosclerotic arteries.

Publications

Primary Research Articles

1. Rissanen T.T., Markkanen J.E., Gruchala M., Heikura T., Puranen A., Kettunen M.I., Kholova I., Kauppinen R.A., Achen M., Stacker S.A., Alitalo K., Ylä-Herttuala S. VEGF-D is the strongest angiogenic and lymphangiogenic effector among VEGFs delivered into skeletal muscle via adenoviruses. Circulation Research (2003) 92:1098-1106.
2. Bhardwaj S., Roy H., Gruchala M., Viita H., Kholova I., Kokina I., Achen M.G., Stacker S.A., Hedman M., Alitalo K., Ylä-Herttuala S. Angiogenic responses of vascular endothelial growth factors in periadventitial tissue. Human Gene Therapy (2003) 14:1451-1462.

3. Rutanen J., Leppänen P., Tuomisto T., Rissanen T.T., Hiltunen M.O., Vajanto I., Niemi M., Häkkinen T.P., Karkola K., Stacker S.A., Achen M.G., Alitalo K., Ylä-Herttuala S. Vascular endothelial growth factor-D expression in human atherosclerotic lesions. Cardiovascula Research (2003) 59:971-979.

Reviews / Book Chapters / Commentaries

1. Ylä-Herttuala S., Alitalo K. Gene transfer as a tool to induce therapeutic vascular growth. Nature Medicine (2003) 9:694-701.

Sunil R Lakhani M.D., F.R.C. Path.

Institute for Cancer Research
London, UK

Research Report

The team based at the Breakthrough Breast Cancer Centre, ICR, London, is a translational molecular pathology group. The classical pathological data in making a cancer related diagnosis include (sub)classification, grade (differentiation), stage (extent of disease), and prognostic and predictive factors. The overall objective of the group is to utilise molecular techniques in conjunction with traditional pathological data to extend the understanding of the disease process and hence provide information to aid patient management. Questions arising from clinical practice within the Breast Unit are brought into the laboratory for investigation with the hope of translating the information back to the clinic for practical use in making patient related decisions.

The two major areas of work fall into 'Molecular Genetic Classification' of breast cancer and molecular pathology of the 'Multistep Model'. The two broad areas are however, not mutually exclusive and considerable overlaps exist between the two.

Molecular Genetic Classification of Breast Cancer

Projects within this area include the definition and molecular pathology of basal/myoepithelial lesions within the breast. The classification, behaviour, prognosis and carcinogenic pathways of these tumours are essentially unknown, making clinical management decisions of these tumours a challenge. Dr Laura Fulford (LICR) and Dr Jorge Reis-Filho have been appointed to study for PhD degrees. Both are Pathology trainees. They will extend the work on myoepithelial tumours, Dr Fulford by studying the comparative pathology of myoepithelial lesions in salivary gland, breast, skin and lung and Dr Reis-Filho will study metaplastic carcinomas of the breast.

The pathology and molecular pathology of familial breast cancers has been carried out as a European based consortium activity. This work is now being translated to clinical practice to identify high-risk women. It also links with the basal project above. Lobular carcinomas, which also have a familial association, have been investigated and this work connects the multistep model with classification studies. The management of lobular carcinoma in situ (LCIS) remains controversial and difficult due to lack of data and its relationship to other pathologies, such as ductal carcinoma in situ (DCIS) and invasive ductal carcinoma, remain unclear. The genetic basis of lobular neoplasia and its management are important issues for investigation in the context of familial cancers as well as the multistep model.

Multistep carcinogenesis

The screening program has led to an increase in the identification of early precancerous and 'borderline' lesions. This has highlighted deficiencies in the pathological classification systems, the inter-relationships between the lesions seen and their natural history, making clinical management problematic. As part of the investigations of such lesions within the breast, we have previously investigated DCIS, atypical ductal hyperplasia (ADH) and hyperplasia of usual type (HUT). We are now investigating LCIS lesions, either seen incidentally or via the screening program and another recently recognised screen-detected lesion referred to as 'Columnar Cell Hyperplasia'(CCH). CCH are increasingly seen in core biopsies but their significance and hence subsequent management (to carry out further excision or not) remains unclear. The objective is to understand the biological (clonal) nature of the lesions, their inter-relationships with other higher grade lesions such as DCIS and hence suggest possible management strategies.

We have previously demonstrated that genetic changes identified in these preinvasive lesions are also seen in apparently normal breast tissue. Using cells separation techniques, we have demonstrated that these alterations are seen in both the epithelial compartments, the luminal and myoepithelial. The

investigation of changes in the normal cell compartment also links in with basal/myoepithelial tumours and with familial cancers, as we are investigation changes in the normal cells from patients harbouring germline BRCA1/2 mutations. This work is done in collaboration with Dr. Mike O'Hare at the UCL Branch of LICR.

Pathology Facility in the Breakthrough Centre/ICR

The work in the laboratory is underpinned by a Pathology facility, which provides a service for cutting and staining tissue sections, tissue arrays, FISH and immunohistochemistry including work-up of new antibodies. This facility also provides the same service to other teams within the Breakthrough Centre, the ICR and Royal Marsden Hospital Breast Unit and the LICR.

The Pathology core facility has since 2001 cut over 33,000 sections and over 7500 blocks, have carried out 20,000 immunocytochemical procedures and have also worked up approximately 90 antibodies.

Publications

Primary Research Articles

1. Jones, C., Merrett, S., Thomas, V.A., Barker, T.H. Lakhani, S.R. Comparativ genomic hybridisation analysis of bilateral hyperplasia of usual type of the breast. Journal of Pathology (2003)199(2):152-156.
2. Jones, C., Tooze, R., Lakhani, S.R. Malignant adenomyoepithelioma of the breast metastasising to the liver. Virchows Archive (2003) 442:504-506.
3. Logarajah, S., Hunter, P., Kraman, M., Steele, D., Lakhani, S., Bobrow, L., Venkitaraman, A., Wagner, S. BCL-6 is expressed in breast cancer and prevents mammary epithelial differentiation. Oncogene (2003) 22(36):5572-8.
4. Mackay A., Jones C., Dexter T., LA Silva, R., Bulmer K., Jones A., Simpson, P., Harris, R.A., Jat, P.S., Neville, A.M., Reis, L.F.L., Lakhani, S.R., O'Hare, M.J. CDNA microarray analysis of genes associated with ERBB2 (HER2/neu) overexpression in human mammary luminal epithelial cells. Oncogene (2003) 22:2680-2688.
5. Novelli, M., Cossu, A., Oukrif, D., Quaglia, A., Lakhani, S., Poulsom, R., Sasieni, P., Carta, P., Contini M., Pasca A., Palmieri G., Bodmer W., Tanda Francesco. X-inactivation patch size in human female tissue confounds the assessment of tumor clonality. Proceedings of the National Academy of Sciences (2003) 100(6):3311-3314.
6. Reis Filho, J.S., Fulford, L.G., Lakhani, S.R., Scjmitt, F.C. Pathologic quiz case: 62-year-old female with a 4.5cm nodule in the right breast. Archives of Pathology and Laboratory Medicine (2003) 127(9): e396-8.
7. Reis Filho, J.S., Fulford, L.G., Freeman, A., Lakhani, S.R. Pathologic quiz case: a 93-year-old-female with an enlarged and tender left breast. Histiocytoid variant of lobular breast carcinoma. Archives of Pathology and Laboratory Medicine (2003) 127(12): 1626-8.
8. Timpanidis, P.C., Lakhani, S.R., Groves, R.W. Progesterone receptor-positive eruptive syringoma associated with diabetes. Journal of the American Academy of Dermatology (2003) 48(5 Suppl):S103-4.

Reviews/Book Chapters/Commentaries

1. Lakhani S.R. The diagnosis and management of pre-invasive breast disease – current challenges, future hopes. Breast Cancer Research (2003) 5:248-9.

2. Lakhani S.R. The Morphology of Breast Tumors in BRCA 1 /2 Carriers. Pathology Case Reviews (2003) 5:240-244.

3. Pia-Foschini, M., Reis Filho, J.S., Eusebi, V., Lakhani, S.R. Salivary gland-like tumours: surgical and molecular pathology. Journal of Clinical Pathology (2003) 56:497-506.

4. Reis-filho J.S., Lakhani S.R. The diagnosis and management of pre-invasive breast disease. Genetic alterations in pre-invasive lesions. Breast Cancer Research (2003) 5:313-319.

5. Simpson, P.T., Gale, T., Fulford, L.G., Reis-Filho, J, Lakhani, S.R. The diagnosis and management of pre-invasive breast disease. Pathology of atypical lobular hyperplasia and lobular carcinoma in situ. Breast Cancer Research (2003) 5:258-262.

6. Lakhani, S. R., Dilly, S.A., Finlayson, C.J., Dogan, A. Basic Pathology: An Introduction to theMechanisms of Disease. 3rd Edition. 2003, London: Arnold.

7. Lakhani, S.R., Jones, C. Molecular pathology for breast carcinoma in Early Breast Cancer from early diagnosis to multidisciplinary management, 2nd Edition, 2003. MWE Morgan, R Warren, G Querci Della Rovere (eds). Martin Dunitz Ltd.

Joseph Schlessinger, Ph.D.

Yale University
New Haven, USA

Research Report

The following studies were performed in my laboratory in 2003.

Essential role of the protein tyrosine kinase Pyk2 in the control of macrophage functions.

The biological role of the protein tyrosine kinase Pyk2 was explored by targeting the Pyk2 gene by homologous recombination. Macrophages isolated from Pyk2-/- mice failed to become polarized, to undergo membrane ruffling and to migrate in response to chemokine stimulation. It is demonstrated that the impaired function of macrophages *in vitro* and *in vivo* is caused by deficiency in cellular signaling pathways that are dependent on the integrity of Pyk2 including IP_3 production, Ca^{+2} release and PI-3 kinase activation among other activities.

Mechanism of autoinhibition and activation of FGF and EGF receptors.

Analysis of the crystal structure of the intact extracellular ligand binding domain of Fibroblast Growth Factor Recepter (FGFR) 3 in complex with FGF1 and quantitative binding experiments with FGF1, heparin and recombinant D1 have shown that the first Ig-like (D1) domain and the acid-box region are responsible for an intramolecular mechanism of autoinhibition of FGF and heparin binding to D2 and D3, the region shown to be responsible for FGF and heparin binding.

On the basis of the three-dimensional structure of the extracullular ligand binding domain of Epidermal Growth Factor Recepter (EGFR), it was proposed that the intramolecularly-tethered and autoinhibited configuration corresponds to the low affinity FGFR while the extended configuration accounts for the high affinity EGFRs on intact cells. We have tested this model experimentally by utilizing site-directed mutagenesis and theoretically by mathematical modeling. It is concluded that the extended configuration of EGFR does not account for the high affinity of EGF binding to EGFR on intact cells and that the tethered configuration provides only a limited control of EGFR functions. However, the dimerization arm in domain-II of EGFR plays a critical role in EGF-induced EGFR dimerization and activation.

Additional studies which were performed include analysis of the role of Gab1 in mediating FGFR3 signaling, the role of FRS2α in FGF dependent signaling pathways during embryonic development and description of a novel "glutamine switch" mechanism for nucleotide selectivity of phosphodiesterases.

Publications

Primary Research Articles

1. Okigaki M., Davies C., Falasca M., Harroch S., Felsenfeld D.P., Sheetz M.P., Schlessinger, J. Pyk2 regulates multiple signaling events crucial for macrophage morphology and migration. Proceedings of the National Academy of Sciences USA (2003) 100, 10740-10745.

Review/Book Chapters/Commentaries

1. Schlessinger J. Signal Transduction: Autoinhibition Control. Science (2003) 300, 750-752.
2. Schlessinger J., Lemmon M.A. SH2 and PTB domains in tyrosine kinase signaling. Science Signal Transduction Knowledge Enivronment (2003) (191) Re12 (2003).

Graham Warren, Ph.D.

Yale University
New Haven, Connecticut, USA

Research Report

The Golgi apparatus in animal cells undergoes dramatic morphological changes at the onset of mitosis. The ribbon-like structure in the juxta-nuclear region fragments yielding individual stacks of cisternae, which in turn vesiculate into clusters of vesicles and free vesicles.

This process is mediated, at least in part, by GRASP65, a protein that the laboratory originally identified as a component of the cisternal stacking mechanism. Phosphorylation during mitosis correlated with cisternal unstacking. More recently it has been implicated as a checkpoint control for entry into mitosis – if the Golgi ribbon does not break down then the cell fails to enter mitosis. This raises the question as to the precise role played by GRASP65. Does it physically link Golgi cisternae, generating the characteristic stacked appearance? Is it a signaling molecule, with downstream effectors that mediate the actual stacking process? Or are both functions incorporated into the same molecule.

To test this, the laboratory prepared recombinant forms of GRASP65 and showed that they formed homo-dimers. These were then attached to beads which aggregated extensively, showing that GRASP65 can link adjacent surfaces. Treatment of these aggregates with mitotic cytosol or recombinant CDK1/cyclin B and polo-like kinase, broke them up, and further treatment with protein phosphatase PP2A led to re-aggregation. This mimics the unstacking and re-stacking of cisternae during mitosis, further corroborating the ability of GRASP65 to link surfaces.

Interestingly, interphase cytosol increased the extent of bead aggregation suggesting that there are additional proteins that enhance the linking function of GRASP65. The group is currently trying to purify these factors in the hope that they might provide regulatory factors for the observed checkpoint control.

Publications

Primary Research Articles

1. Satoh A., Wang Y., Malsam J., Beard M.B., Warren G. Golgin-84 is a rab1 binding partner involved in Golgi structure. Traffic (2003) 4:153-161.
2. Taguchi T., Pypaert M., Warren G. Biochemical Sub-Fractionation of the Mammalian Golgi Apparatus. Traffic (2003) 4:344-352.
3. Wang Y., Seemann J., Shorter J., Warren G. A direct role for GRASP65 as a mitotically regulated Golgi stacking factor. EMBO Journal (2003) 22:3279-3290.

Ellen Puré, Ph.D.

Wistar Institute
Philadelphia, Pennsylvania

Research Report

Inflammation is typically a self-limiting response to infection or injury. Although inflammation is largely a reparative response, severe acute inflammation or persistent inflammation can lead to tissue damage and loss of organ function. In fact, inflammation is now recognized to be a critical component of a wide variety of diseases ranging from acute septic shock to chronic inflammatory diseases and in some types of cancer. The group's goal is to gain insights into the molecular and cellular basis of inflammation and how inflammation impacts on tumor cell growth and metastasis. Current studies relate to several important questions in cancer biology, for example, the study of the BCR-mediated signaling pathways responsible for the reactivation of Epstein Barr virus (EBV) and EBV-associated lymphoproliferative disorders and tumors. Furthermore, the group is investigating the mechanisms that mediate leukocyte trafficking and leukocyte and parenchymal cell growth and differentiation, and how these mechanisms regulate tumor cell growth and metastasis.

Signal Transduction in B Lymphocytes

Engagement of antigen receptors on hematopoietic cells is coupled to the activation of non-receptor protein tyrosine kinases (PTKs). BCR activation of PTKs results in the activation of multiple downstream effector molecules that regulate cytoskeletal reorganization, protein translation and, gene transcription, and drive a variety of biological responses. For the past year, Dr. Puré's group has continued its studies of: 1) BCR-mediated activation of the lytic cycle of EBV in latently infected B cells; and 2) BCR-mediated signal transduction and BCR internalization.

EBV is a ubiquitous virus carried by over 90% of the world's population and implicated in the pathogenesis of several cancers including Burkitt's Lymphoma and Hodgkins disease. In immunocompetent individuals the immune response to EBV controls the infection but does not eradicate it, as virus persistently resides in a latent state in B lymphocytes. Thus EBV can be reactivated, particularly in immunosuppressed individuals, and the switch between EBV latency and reactivation can be induced as a consequence of BCR-coupled signal transduction pathways. This switch from latent infection to virus replication is initiated by the transcriptional activation of the viral immediate-early gene BZLF1 through either of two promoters, Zp or Rp. The early signaling events involved in BCR-mediated induction of BZLF1 and EBV reactivation remain in large part to be elucidated. In collaboration with Dr. Paul Lieberman (Wistar Institute), the group has recently demonstrated that several BCR proximal tyrosine kinases, Syk, Lyn, and Btk, each play an important role in the transcriptional activation of BZLF1.

As part of the signal transduction of BCR, the underlying mechanisms of receptor signaling and endocytosis linked to cytoskeletal reorganization are also being investigated. The group has particularly studied the role of Cbl, which is phosphorylated as a result of BCR-mediated signaling, in BCR endocytosis. Several manuscripts, on the role of Cbl and its adaptor proteins in the critical link between BCR engagement and reorganization of the actin cytoskeleton, are being prepared or have been submitted.

Inflammation, Fibrosis and Tumor Growth and Metastasis

Inflammation and cancer are characterized by activation of local parenchymal cells, leukocyte infiltration and, turnover and remodeling of extracellular matrix. A major focus of the laboratory is on understanding the cell-cell and cell-matrix interactions that regulate the inflammatory response and tumor cell growth and metastasis. These studies are aimed at testing the following hypotheses: 1) Modification of extracellular matrix plays a critical role in the initiation and progression of inflammatory responses and tumor cell growth and metastasis, and; 2) Activation and differentiation of parenchymal cells contribute significantly

to the progression of inflammation and tumor cell growth and metastasis.

One focus of these studies has been on the role of the adhesion receptor CD44 and its ligands. CD44 is a widely expressed family of adhesion receptors that represent alternatively spliced and posttranslationally modified products of a single gene. CD44 is a major receptor for hyaluronan (HA), a glycosaminoglycan that is ubiquitously distributed in ECM, and in addition can bind other matrix components such as fibronectin and collagen, a matrix metalloproteinase, MMP-9, growth factors and cytokines and chemokines such as osteopontin and MIP-1β. CD44 was once thought to be confined to being a simple transmembrane adhesion molecule that also played a critical role in the metabolism of hyaluronan. Intense investigation of CD44 over the past twenty years has lead to the definition of additional functions of CD44 including its capacity to mediate signal transduction, inflammatory cell function, and tumor growth and metastasis. Importantly, CD44 does not appear to play a critical role in homeostasis making CD44 particularly attractive as a potential target for novel therapeutic interventions in inflammation and cancer. In addition to being expressed as a transmembrane receptor on many cell types, a soluble form of CD44 (sCD44) is found in serum and lymph. Interestingly, immunodeficiency correlates with low plasma levels of sCD44 whereas malignant diseases and immune activation and inflammation are often associated with increased plasma levels of CD44.

The group has been investigating the role of CD44 in several murine models of inflammatory diseases and cancer by comparing pathology in wild-type and CD44-deficient mice. The group compared the inflammatory response and pathology associated with bleomycin induced fibrotic lung injury; infection with toxoplasma gondii; allergen-induced airway inflammation and hyperreactivity (with Dr. Reynold Panettieri Jr., University of Pennsylvania), atherosclerosis; and cerebral ischemic injury. In each case we found that a deficiency in CD44 had a profound impact on the initiation and or progression of disease in mice and in each case have defined multiple potential mechanisms involving leukocytes, parenchymal cells and endothelial and epithelial cells, by which CD44 contributes to pathology. In other studies, the mechanisms of the regulation of the functional capacity of soluble CD44 were investigated. Most recently, the studies have been focused on defining the role of oxidative stress in modifying components of the extracellular matrix, including hyaluronan, and testing the hypothesis that modified components of matrix play a critical part in inflammation and tumor cell growth and metastasis. A panel of markers is being established, which will allow distinguish parenchymal cells at various stages of activation and differentiation. Additionally, the role of cell surface proteinases expressed specifically on activated parencymal cells in inflammation and surrounding tumors, and the role of matrix in maintaining parechymal cells and dormant tumor cells in a quiescent state are being investigated.

Finally, over the past several years the group has identified several novel mechanisms by which the production of the key proinflammatory mediator IL-12 is regulated. Specifically, it was demonstrated that, in addition to its proinflammatory activities, tumor necrosis factor (TNF) is also a potent inhibitor of macrophage IL-12 production. Furthermore, it was discovered that 12/15-lipoxygenase is required for the production of IL-12 by macrophages *in vitro* and in atherosclerotic lesions in mice. The group is now pursuing the molecular basis for these novel mechanisms of regulation of IL-12 gene expression during an inflammatory response.

Publications

Primary Research Articles

1. Kothapalli D. Stewart S.A., Smyth E.M., Azonobi I., Puré E., Assoian R.K. Prostacyclin receptor activation inhibits proliferation of aortic smooth muscle cells by regulating cAMP response element-binding protein- and pocket protein-dependent cyclin A gene expression. Molecular Pharmacology (2003) 64:249-258.

Reviews/Book Chapters/Commentaries

1. Cichy J., Puré E. The liberation of CD44. Journal of Cell Biology (2003) 161:839-843.

Robert D. Schreiber, Ph.D.

Department of Pathology and Immunology
Washington University School of Medicine
St. Louis, Missouri

Research Report

Identification of Tumor Antigens of Tumors From Immunodeficient Mice

This project seeks to define the molecular basis for the observation that tumors produced in immunodeficient mice are, as a group, more immunogenic than similar tumors produced in genetically similar immunocompetent mice. This observation provided, in part, functional support for the refinement of the cancer immunosurveillance hypothesis into one we termed cancer immunoediting. As this latter concept is now gaining wide spread support, our overall goal is to broaden and strengthen its foundation by defining the molecular and cellular underpinnings of the process.

In this project we have focused on testing the hypothesis that methylcholanthrene-induced fibrosarcoma cells derived from immunodeficient RAG2-/- mice (i.e. RAG2-/- regressor tumor cells, highly immunogenic "unedited" tumor cells that are rejected when transplanted into wild type mice) and similar tumors derived from immunocompetent mice (i.e. wild type tumor cells, "edited" tumor cells that grow progressively when transplanted into wild type mice) would display distinct gene expression signatures. We performed three independent Affymetrix GeneChip analyses and compared gene expression patterns of eight RAG2-/- regressor MCA-sarcomas to that of eight wild type MCA-sarcomas. Globally, the number of expressed transcripts was comparable between the two groups and no obvious sub-clustering of gene expression was observed among the tumors. However, Anova analysis at very high stringency ($p<0.001$) revealed that a limited number of genes differentially segregated the tumors into their two growth phenotype groups. Statistical analysis of the gene expression levels in RAG2-/- regressor versus wild type tumors identified 47 gene sequences that were differentially expressed between the two groups. Twenty-seven sequences were expressed selectively in RAG2-/- regressor tumors while 20 were expressed selectively in wild type tumor cells. Thus, distinctive molecular immunogenicity signatures could indeed be identified and validated that faithfully differentiated between unedited tumors that were highly immunogenic and edited tumors that displayed reduced immunogenicity.

We next focused our efforts on assessing the functional consequences of the differential gene expression we observed. Although, in the future we plan to study several of the identified genes, we focused this year on only one: Cd1d. CD1d is a nonclassical MHC class I-like protein that is thought to present glycolipid antigens to a subset of NKT cells. A limited amount of data suggests that NKT cells may play a role in initiating certain anti-tumor responses, although the mechanisms by which NKT recognition of tumors occurs has remained obscure. Thus, the observation that wild type tumors express three to five times lower levels of CD1d mRNA and protein compared either to their considerably more immunogenic RAG2-/- regressor tumor counterparts or to primary skin fibroblasts from either wild type or RAG2-/- mice, strongly supported the conclusion that reduced CD1d gene expression on tumors from immunocompetent mice was the result of a cancer immunoediting process. We are now developing RNAi based methods to specifically reduce levels of CD1d on RAG2-/- regressor tumors and to determine whether this manipulation ablates their high immunogenicity. In the meantime, we have obtained some exciting data that justifies our effort in this area. As expected, when cells derived from a RAG2-/- regressor tumor (F535) are injected into naïve wild type mice they are rejected. However, these cells will form progressively growing tumors that maintain high level CD1d expression and retain the highly immunogenic phenotype of the parent when transplanted into mice depleted of both CD4+ and CD8+ cells. In contrast, F535 tumor cells injected into mice depleted of either CD4+ or CD8+ cells grow progressively. The growing cells display significantly reduced CD1d expression and form progressively

growing tumors when re-transplanted into naïve syngeneic hosts. Reconstitution of CD1d expression in the escape variants restores their immunogenicity and the complemented tumor cells are now rejected in immunocompetent but not immunodeficient mice. These results not only suggest that CD1d may indeed be a functionally important target of the cancer immunoediting process but also help establish an experimental arena in which other differentially expressed genes can be evaluated.

Publications

Primary Research Articles

1. Dupuis S., Jouanguy E., Al-Hajjar S., Fieschi C., Al-Mohsen I.Z., Al-Jumaah S., Yang K., Chapgier A., Eidenschenk C., Eid P., Al Ghonaium A., Tufenkeji H., Frayha H., Al-Gazlan S., Al-Rayes H., Schreiber R.D., Gresser I., Casanova J-L. Impaired response to interferon-alpha/beta and lethal viral disease in human STAT1 deficiency. Nature Genetics (2003) 33:388-391.
2. Kobayashi M., Kweon M.N., Kuwata H., Schreiber R.D., Kiyono H., Takeda K., Akira S. Toll-like receptor-dependent production of IL-12p40 causes chronic enterocolitis in myeloid cell-specific Stat3-deficient mice. Journal of Clinical Investigation (2003) 111:1297-1308.
3. Novack D.V., Yin L., Hagen-Stapleton A., Schreiber R.D., Goeddel D.V., Ross F.P., Teitelbaum S.L. The IkapaB function of NF-kappaB2 p100 controls stimulated osteoclastogenesis. Journal of Experimental Medicine (2003) 198:771-781.

Reviews/Book Chapters/Commentaries

4. Uppaluri R., Dunn G.P., Old L.J., Schreiber R.D. IFN* Receptor-STAT1 Signaling and Cancer Immunoediting. In: Signal Transducers and Activators of Transcription: Activation and Biology, edited by PB Seghal, T Hirano, and GR Stark, 2003, Ch. 27.
5. Schreiber G.H., Schreiber, R.D. Interferon-*. In: The Cytokine Handbook, edited by M. Lotze and A. Thomson. Academic Press, 2003, Vol. 1, pp. 567-601.

Kohei Miyazono, M.D.

Department of Biochemistry
The Cancer Institute
Japanese Foundation for Cancer Research (JFCR)
Tokyo, Japan

Research Report

Our major research interest is to elucidate how TGF-β superfamily signaling regulates the growth, differentiation, and apoptosis of various cells. Particularly, we have studied how Smad signaling pathways are regulated by various molecules. We have found several positive and negative regulators of TGF-β signaling. Recently, a small molecular weight inhibitor for TGF-β and activin receptor kinases had been generated. Using this compound, we studied how endogenous TGF-β signaling regulates growth and differentiation of various cells.

Arkadia amplifies TGF-β superfamily signaling through degradation of Smad7

Arkadia is a RING domain containing molecule that was originally identified as a protein that enhances signaling activity of Nodal. However, the mechanisms by which Arkadia affects TGF-β superfamily signaling have not been determined. We have shown that Arkadia is broadly expressed in mammalian tissues, and that it enhances both TGF-β and bone morphogenetic protein (BMP) signaling. Arkadia physically interacts with inhibitory Smad, Smad7, and induces its ubiquitination and degradation. In contrast to a HECT type ubiquitin ligase, Smurf1, which interacts with, and degrades, TGF-β receptor complexes through Smad7, Arkadia failed to associate with TGF-β receptors. Silencing of the Arkadia gene resulted in repression of transcriptional activities induced by TGF-β and BMP, and accumulation of the Smad7 protein. Arkadia may thus play an important role as an amplifier of TGF-β superfamily signaling.

Regulation of TGF-β and BMP signaling by transcriptional coactivator GCN5

In the TGF-β superfamily signaling pathways, activated Smad complexes regulate transcriptional responses of the target genes in cooperation with transcriptional coactivators and corepressors. To identify new components of transcriptional complexes containing Smad proteins, we purified DNA-binding proteins using a Smad-binding DNA element as bait. In collaboration with Dr. Ulf Hellman at the LICR Uppsala Branch, we have identified a transcriptional coactivator GCN5 as a direct partner of activated Smad complexes. GCN5 is structurally similar to, and functions like PCAF, in that it binds to TGF-β -specific R-Smads, and enhances transcriptional activity induced by TGF-β. However, GCN5, but not PCAF, interacts with R-Smads for BMP signaling pathways, and enhances BMP-induced transcriptional activity. Thus, GCN5 and PCAF have distinct physiological functions in vivo in regulation of TGF-β and BMP signaling pathways.

Endogenous TGF-β signaling suppresses maturation of osteoblastic mesenchymal cells

BMPs induce bone formation in vitro and in vivo, and TGF-β has positive and negative effects on bone formation, although the molecular mechanisms of these effects are not fully understood. We have shown that osteoblastic differentiation of mouse C2C12 cells was greatly enhanced by the TGF-β type I receptor kinase inhibitor SB431542. Endogenous TGF-β was found to be highly active, and induced expression of inhibitory Smads during the maturation phase of osteoblastic differentiation induced by BMP-4. SB431542 suppressed endogenous TGF-β signaling and repressed the expression of inhibitory Smads during this period, leading to acceleration of BMP signaling. SB431542 also induced the production of alkaline phosphatase and matrix mineralization of human mesenchymal stem cells. Thus, TGF-β inhibitors may be invaluable for the treatment of various bone diseases by accelerating BMP-induced osteogenesis.

TGF-β receptor inhibitor enhances growth and integrity of ES cell-derived endothelial cells

Embryonic vascular progenitor cells are capable of differentiating into mural and endothelial cells;

however, the molecular mechanisms that regulate their differentiation, proliferation, and endothelial sheet formation remain to be elucidated. We have shown that members of the TGF-β superfamily play important roles during differentiation of vascular progenitor cells derived from mouse embryonic stem (ES) cells. TGF-β and activin inhibited proliferation and sheet formation of endothelial cells. Interestingly, SB-431542 facilitated proliferation and sheet formation of ES cell-derived endothelial cells. Moreover, SB-431542 up-regulated the expression of claudin-5, an endothelial specific component of tight junctions. Our findings suggest that endogenous TGF-β/activin signals play important roles in regulating vascular growth and permeability.

Publications

Primary Research Articles

1. Mizuide, M., Hara, T., Furuya, T., Takeda, M., Kusanagi, K., Inada, Y., Mori, M., Imamura, T., Miyazawa, K., and Miyazono, K. Two short segments of Smad3 are important for specific interaction of Smad3 with c-Ski and SnoN. Journal of Biological Chemistry (2003) 278:531-536.
2. Sasaki, T., Suzuki, H., Yagi, K., Furuhashi, M., Yao, R., Susa, S., Noda, T., Arai, Y., Miyazono, K., and Kato, M. Lymphoid enhancer factor 1 makes cells resistant to transforming growth factor β-induced repression of c-myc. Cancer Research (2003) 63:801-806.
3. Tajima, Y., Goto, K., Yoshida, M., Shinomiya, K., Sekimoto, T., Yoneda, Y., Miyazono, K., and Imamura, T. Chromosomal region maintenance 1 (CRM1)-dependent nuclear export of Smad ubiquitin regulatory factor 1 (Smurf1) is essential for negative regulation of transforming growth factor-β signaling by Smad7. Journal of Biological Chemistry (2003) 278:10716-10721.
4. Nakano, M., Arai, E., Nakajima, Y., Nakamura, H., Miyazono, K., and Hirose, T. Immunohistochemical study of chondrolipoma: Possible importance of transforming growth factor (TGF)-βs, latent TGF-βbinding protein-1 (LTBP-1), and bone morphogenic protein (BMP) for chondrogenesis in lipoma. Journal of Dermatology (2003) 30:189-195.
5. Nishihara, A., Fujii, M., Sampath, T.K., Miyazono, K., and Reddi, A.H. Bone morphogenetic protein signaling in articular chondrocyte differentiation. Biochemical and Biophysical Research Communications (2003) 301:617-622.
6. Murakami, G., Watabe, T., Takaoka, K., Miyazono, K., and Imamura, T. Inhibition of BMP signaling by Smurf1 and inhibitory Smads. Molecular Biology of the Cell (2003) 14:2809-2817.
7. Suh, N., Roberts, A.B., Birkey Reffey, S., Miyazono,K., Itoh,S., ten Dijke, P., Heiss, E.H., Place, A.E., Risingsong,R., Williams, C.R., Honda,T., Gribble, G.W., Sporn, M.B. Synthetic triterpenoids enhance transforming growth factor-β/Smad signaling. Cancer Research (2003) 63:1371-1376.
8. Sowa, H., Kaji, H., Canaff, L., Hendy, G.N., Tsukamoto, T., Yamaguchi, T., Miyazono, K., Sugimoto, T., and Chihara, K. Inactivation of menin, the product of the MEN1 gene, inhibits the commitment of multipotential mesenchymal stem cells into the osteoblast lineage. Journal of Biological Chemistry (2003) 278:21058-21069.
9. Takebayashi-Suzuki, K., Funami, J., Tokumori, D., Saito, A., Watabe, T., Miyazono, K., Kanda, A., and Suzuki, A. Interplay between the tumor suppressor p53 and TGF-β signaling shapes embryonic body axes in Xenopus. Development (2003) 130:3929-3939.
10. Nakayama, K., Tamura, Y., Suzawa, M., Harada, S., Fukumoto, S., Kato, M., Miyazono, K., Rodan, G.A., Takeuchi, Y., and Fujita, T. Receptor tyrosine kinases inhibit bone morphogenetic protein-Smad responsive promoter activity and differentiation of murine MC3T3-E1 osteoblast-like cells. Journal of Bone and Mineral Research (2003) 18:827-835.

11. Yoshida, Y., von Bubnoff, A., Ikematsu, N., Blitz, I.L., Tsuzuku, J.K., Yoshida, E.H., Umemori, H., Miyazono,K., Yamamoto, T., and Cho, K.W.Y. Tob proteins enhance inhibitory Smad-receptor interactions to repress BMP signaling. Mechanisms of Development (2003) 120:629-637.

12. Yamasaki, K., Toriu, N., Hanakawa, Y., Shirakata, Y., Sayama, K., Takayanagi, A., Ohtsubo, M., Gamou, S., Shimizu, N., Fujii, M., Miyazono, K., and Hashimoto, K. Keratinocyte growth inhibition by high-dose epidermal growth factor is mediated by transforming growth factor β autoinduction: A negative feedback mechanism for keratinocyte growth. Journal of Investigative Dermatology (2003) 120:1030-1037.

13. Matsuyama, S., Kondo, M., Saitoh, M., Shimizu, K., Aburatani, H., Mishima, H.K., Imamura, T., Miyazono, K., and Miyazawa, K. SB-431542 and Gleevec inhibit TGF-β-induced proliferation of human osteosarcoma cells. Cancer Research (2003) 63:7791-7798.

14. Moren, A., Hellman, U., Inada, Y., Imamura, T., Heldin, C.-H., and Moustakas, A. Differential ubiquitination defines the functional status of the tumor suppressor Smad4. Journal of Biological Chemistry (2003) 278:33571-33582.

15. Midorikawa, Y., Ishikawa, S., Iwanari, H., Imamura, T., Sakamoto, H., Miyazono, K., Kodama, T., Makuuchi, M., and Aburatani, H. Glypican-3, overexpressed in hepatocellular carcinoma, modulates FGF2 and BMP-7 signaling. International Journal of Cancer (2003) 103:455-465.

16. Watabe, T., Nishihara, A., Mishima, K., Yamashita, J., Shimizu, K., Miyazawa, K., Nishikawa, S., and Miyazono, K. TGF-β receptor kinase inhibitor enhances growth and integrity of embryonic stem cell-derived endothelial cells. Journal of Cell Biology (2003) 163:1303-1311.

17. Koinuma, D., Shinozaki, M., Komuro, A., Goto, K., Saitoh, M., Hanyu, A., Ebina, M., Nukiwa, T., Miyazawa, K., Imamura, T., and Miyazono, K. Arkadia amplifies TGF-βsuperfamily signalling through degradation of Smad7. EMBO Journal (2003) 22:6458-6470.

Reviews / Book Chapters / Commentaries

1. Ito, Y., and Miyazono, K. RUNX transcription factors as key targets of TGF-β superfamily signaling. Current Opinion in Genetics & Development (2003) 13:43-47.

LUDWIG INSTITUTE FOR CANCER RESEARCH

James R. Kerr Program

Research Report

Publications

Research Report

The James R. Kerr Program integrates the research activities of laboratories affiliated with the Institute in Russia, China, the Ukraine, South Africa and Turkey. The goal of the Program is both to stimulate cancer research on a broad international basis, and to utilize the very high level of expertise that exists in the countries where the Program is becoming established to strengthen the Institute's research activities.

Bioinformatics is a strong component of the James R. Kerr Program. Dr. Mikhail Gelfand, now at the School of Bioengineering and Bioinformatics, Moscow State University has continued to concentrate his activies in the area of alternative transcript splicing. A detailed analysis of the evolution of exon intron structure and alternative splicing in a family of human CT antigens, MAGE A has been undertaken. The evolution of this family is characterized by four processes: (1) gene duplications; (2) duplications of the initial exon; (3) point mutations and short insertions/deletions inactivating splicing sites or creating new sites; and (4) deletions removing sites and creating chimaeric exons. All this concerns the genomic regions upstream of the coding region, creating a wide diversity of isoforms with different 5' untranslated regions. The processes leading to inactivation of splicing sites or emergence of new sites, was found not to be random, indiscriminate use of pre existing cryptic sites, making it likely that most observed isoforms are functional and not the result of relaxed control in transformed cells. Several examples of chimaeric mRNAs, including the presence of exons matching intergenic regions, indicate that these mRNAs are probably produced by splicing of read through transcripts rather than by trans splicing of "normal" transcripts. In addition, the group has continued curation of the literature database on alternative splicing ASDB in collaboration with the Lawrence Berkeley Laboratory. The Web interface for the database has now been developed and the data are being merged with those obtained by automatically processing SwissProt. Currently ASDB contains the data on ~6000 isoforms and ~550 regulatory elements in ~1800 genes, derived from ~1500 articles. The creation of the related EDAS database, that contains the data on alternative splicing derived from ESTs and human genome, is now almost completed. The database has several options for filtering alternative splicing events, e.g.based on coverage by multiple ESTs from several clone libraries and has a convenient graphic interface allowing the user to zoom on regions of interest. Further plans in this project include processing of other organisms for which both genomic sequence and a large number of ESTs are available (in particular, mouse, drosophila, and C. elegans). It is planned that the ASDB and EDAS databases will be merged and linked to the genome(s).

Also at the School of Bioengineering and Bioinformatics in Moscow, the group headed by Dr. Vadim Agol focuses bioinformatic capacity on the study of protein and gene families. The on-line program 'Cluster Detector' (*http://math.belozersky.msu.ru/~mlt/HF_page*) for detecting hydrophobic clusters in 3D structures of proteins and multicomponent complexes has been developed, using an original algorithm, and successfully used for the description of conservative hydrophobic core of homeodomains, as well as for the description of hydrophobic interaction of homeodomains with their recognition DNA sites. In addition, the program 'Life Core' for the detailed comparative analysis of a given family of 3D structures has also been created. It is based on an original heuristic algorithm for the determination of the geometric core of a set of related 3D structures and allows the subclassification of families of structures. In addition, the adaptation of two programs for specific use by LICR scientists has been undertaken. These are DotHelix (*http://www.genebee.msu.su/services/dhm/advanced.html*), which undertakes homology searching, and A4 (*http://www.genebee.msu.su/services/dhm/advanced.html*). The previously created HEREBase of LTR5 elements in the human genome has now been populated with 901 entries allowing the identification of downstream genes that are potentially regulated by Class 5 LTRs. Other activities included the 3D homology modeling the critical role of a specific amino acid residue (Trp460) of mengoviral RNA dependent RNA polymerase, leading to a proposal of its inter-domain interaction, the creation of a profile for the pannexin family that includes earlier known innexins and a new class of mammalian gap junction proteins and the intiation of the annotation of CT-antigens starting with SCP1.

This annotation summarizes published data, results of database searching for orthologs, verification and improvement of the proposed earlier structure of protein domains and the prediction of functional signals.

The other component of the bioinformatics activities is that being undertaken by the most recent addition to the Program, Dr. Winston Hide at the South African Bioinformatics Institute (SANBI) in Cape Town. In this instance, an ambitious program is being initiated to provide a deep and integrated bioinformatics generated database for the known CT antigens. This analysis is including the application of standardized bioinformatics techniques to the annotation of the antigens, determination of membership in gene families, definition of chromosomal location, their representation across major taxa in order to define the ancientness of the genes, determination of their expression in the major taxa and in model organisms, a detailed analysis of their embryonic expression in the mouse model. Interesting findings are already emerging relating ancientness to embryonic expression and also relating gene silencing, X chromosome inactivitation, evolution of silenced genes and the relationship between these phenomena and cancer.

A further aspect of the work of the Program in Moscow involves the biochemical analysis of apoptotic signaling in the laboratories of Professor V.P. Skulachev and Dr. A.B. Vartapetian at the School of Bioengineering and Bioinformatics. The focus of the work in Professor Skulachev's Laboratory concerns mitochondrial amplification of the apoptotic signal of TNF and some other apoptogenic agents. A study of TNF- or staurosporine-induced bystander effects has now found that this can be mediated by hydrogen peroxide. The effect is abolished by pretreatment of cancer cells with very low amount of cationic derivative of CoQ (MitoQ), an antioxidant which electrophoretically accumulates in the negatively-charged mitochondrial matrix. In related studies, further investigation of decomposition of mitochondrial filament ("thread-grain transition") during apoptosis revealed that this event, when induced by TNF, occurs prior to collapse of the mitochondrial membrane potential. When induced by staurosporine or H_2O_2, the thread-grain transition occurs after a lag-phase but, once initiated develops in only 1-3 min. As an intermediate step in this process, formation of beads-like mitochondria takes place when several swollen parts of the mitochondrial filament are interconnected with thin thread-like mitochondrial structures. Other investigations focus on cytochrome c, which is released from mitochondria during apoptosis induced by TNF. It has now been found that this becomes competent in oxidation of superoxide to O_2 with the reduced cytochrome c, appearing as a result of superoxide oxidation, being reoxidized by cytochrome oxidase in a $\Delta\mu_{H}+$-generating fashion. In addition, it has been found that cytochrome *c* forms a complex with the oncoprotein prothymosin α (ProTα), which is assumed to prevent formation of apoptosome when small amounts of cytochrome c appear in cytosol due to mitoptosis This would thus avoid self-elimination due to the presence of small numbers of damaged mitochondria. Lastly, a hypothesis has been put forward that suggest that the anti-apoptotic, antioxidant effect of novel uncoupling proteins (UCPs 2-5) is due to their activity as carriers of fatty acid peroxides. A further role for ProTα in the apoptotic process has now been uncovered that involves its redirection from the nucleus to the cell surface. This is achieved through caspase-3-mediated truncation of ProTα resulting in the elimination of the ProTα nuclear localization signal. This is an extremely early event in the process preceding externalization of phosphatidylserine, an established surface marker of apoptotic cells. Based on this process, a non-animal caspase has been identified, the first from a plant. The plant caspase is a protease with substrate specificity similar to that of human caspase-3 and is activated in the course of plant apoptosis. A specific inhibitor of the plant caspase has been designed which was also capable of inhibiting human caspase-3 and which counteracted plant apoptosis.

Using novel knockout models, the laboratory of Professor Sergei Nedospasov continued in the analysis of the physiological function of TNF *in vivo*. Novel TNF knockout mice generated with the use of of Cre-loxP technology were compared with TNF knockout mice that were generated at the New York and Melbourne branches using conventional technology. Peyer's Patches were found to be completely absent in the mice generated with the Cre-loxP technology but not in the conventionally generated knockouts. The results, implicated the TNF-TNFRp55 axis in the control of Peyer's Patch organogenesis. The results

also indicated that the design of the targeting strategy might have an effect on the phenotype, presumably through the expression of closely linked lymphotoxin genes.

The balance of the work undertaken thus far in the James R. Kerr Program is focused on cancer immunology. Sergei Nedospasov's group at the Belozersky Institute has continued cancer antigen discovery efforts using SEREX. Several novel genes were cloned from a human testis cDNA library using colon cancer patients' sera as screening reagent. These new clones were combined with previously characterized antigens, such as histone deacetylases in prototype miniarrays for high-throughput serological analysis of the sera samples from various cancers. The comparison with the collection of SEREX clones identified by M. Scanlan and colleagues at the New York branch indicated that the Moscow and the New York antigens are recognized by only partially overlapping sets of patients' sera. Efforts to extend the collections of matching tumor/benign tissues and sera from colon cancer patients for molecular analysis are being continued. In the Ukraine at the laboratory of Dr. V. Filonenko and Dr. I. Gout at the Institute of Molecular Biology and Genetics in Kyiv, the group has now extended its isolation of novel cancer antigens using SEREX to achieve a total of 124 positive clones from melanoma, thyroid and colon cancer which continue to be actively characterized and the data made available through the Cancer Immunome Database. To facilitate such characterization a bank of sera from 320 healthy donors and patients with various types of cancer has been established.

The Ukraine group has continued to make valuable contributions to the generation of immunological reagents resulting in the cloning, expression and affinity purification of a total of 39 recombinant proteins, which are then used for the generation of monoclonal antibodies either in China of the Ukraine. In this regard, seven valuable monoclonal antibodies to important cancer related molecules have now been produced in collaboration with other members of the LICR Institute. Functional characterization of well-characterized cancer antigens is also ongoing and a search for binding partners by a yeast two-hybrid approach to A33, PTEN and S6 kinase has been successfully completed. For A33 these include the cytoskeletal proteins filamin 2C and dynein, which may provide a functional link between A33 and the cytoskeleton, and basigin an extracellular matrix metalloproteinase inducer.

The major effort for the generation of monoclonal antiobodies, a key component in the James R. Kerr Program, is at the Fourth Military Medical University in Xi'an, China under the direction of Professor Boquan Jin. Investigators throughout the LICR use this center by submitting proteins and other immunogens to the Xi'an laboratory, where immunizations and fusions are carried out. Intial screening of the resulting antibodies is undertaken by ELISA and western blot analyses. Postivie clones are then shipped back to the original researcher who requested their production. Activities have been intense in 2003 with 273 antibodies being produced against 42 different antigens. In some cases, particularly in the area of cancer testis antigens, the group in Xi'an is also now undertaking initial immunohistochemical analysis to further enhance the utility of the collaboration. One such study in 2003 resulted in the characterization of excellent monoclonal antibodies for the analysis of the tissue distribution of the important cancer testis antigen CT10. The cooperation and integration between Xi'an and the branches is becoming more effective and fruitful through the increasing utilization of antibobodies produced in Xi'an in Institute projects.

The work of Professor Wei-Feng Chen and his group at Peking University in Beijing, concentrates on cancer antigens presented by hepatocellular carcinoma (HCC) and has lead to the identification of important novel CT antigens including CP1, a novel cancer-placental antigen cloned and identified by suppression subtraction hybridization (SSH) from human hepatocellular carcinoma (HCC) tissues. The CP1 gene is not expressed in normal tissues except in placenta. However, CP1 mRNA was expressed in the HCC (50%, 18/36), gastric (40%, 4/10), colon (36%, 5/14) and lung cancers (42%, 6/14) tested. A further novel gene cloned from HCC by SSH is a liver-specific gene that has been denmoniated HCC-C11. The expression of this gene is inversely correlated to the progression and differentiation of HCC and appears to be a hepatocyte differentiation factor. In addition, CAGE has also been now demonstrated to

be expressed in a high percentage of HCC tissues and to be recoqnized by antibodies in the sera of 4/79 HCC patients. In the follow up of previously identified HCC antigens, HCA 587 protein expression in the spermatogonia in the testis and HCC cells has now been demonstrated by immunohistochemical (IHC) staining. In normal tissues stained, all were negative except Purkingji cells in the cerebellum. In HCC specimens, HCA587 protein was present in 37.1% (26/70) samples being localized in both cytoplasm and nucleus. In addition, BJ-HCC-2 protein expression in testis and HCC cells has also been identified by IHC staining. In testis, the BJ-HCC-2 protein was expressed in germ cells and Sertoli cells. In HCC, the protein was expressed in cytoplasm. The protein expression rate was 25% (5/20) in HCC samples. Functional analyses of CT HCC derived CT antigens has concentrated on HCA520, a novel calcineurin protein B-subunit, which is not expressed in normal hepatocytes but which is overexpressed in HCC. Trasfection of 293 cells with HCA 520 cDNA considerably enhanced proliferation suggesting its active role in tumorigenesis. In the analysis of cellular responses to defined antigen peptides, the response of CD8+T cells derived from PBMCs of HCC patients to NY-ESO-1b was assessed by both IFN-γ release ELISPOT and tetramer-peptide assays in 3 cases. While, the tetramer-peptide assay was all positive in these 3 cases with the CD8+T cell frequency of 3.4%, 0.32% and 0.37%, respectively. However, the ELISPOT assay was only positive in the 2 cases. To test the specificity of CD8+T cell response to NY-ESO-1 in ELISPOT assay, the anti-HLA class I antibody was added to the culture system. The capacity to form spots from effector cells CD8+T cells was significantly blocked by anti-HLA class I Abs. The functional supertype of HLA-A2 molecules was elucidated for the activation of NY-ESO-1b responsive CD8+T cells and was found to include HLA-A*0203, *0207, *0206, in addition to *0201. In addition, CD8+ T cell responses to HCA587. However, among eight samples tested, only one gave a positive response to both HCA587 protein (spot forming cells: $48/2.5x10^4$) and the HLA-A2 restricted peptide FLAKLNNTV peptide (spot forming cells: $23/2.5x10^4$). Expression was inversely correlated to the differentiation status of the HCC. Stimulated by the clear immunogenicity of NY-ESO-1 in hepatocellular carcinoma a Phase 1 clinical vaccine trial is planned using NY-EO-1b peptide, which is in the process of regulatory approval, by the Chinese authorities.

Also in Beijing, Dr. Yu Wang and her colleagues at the Beijing Cancer Hospital are studying gastric cancer, which has the highest mortality rate and ranks second in morbidity among all malignant tumors in China. In a study of 101 gastric cancer samples analyzed for the presence of mRNA of NY-ESO-1 and a further 10 CT genes. Twelve out of 101 samples (11.9%) were found to be NY-ESO-1 mRNA positive, 11 of which were from advanced stage patients. In seven of the 12 NY-ESO-1 mRNA positive samples, the NY-ESO-1 protein was also detected by immunohistochemistry. An autologous humoral immune response to NY-ESO-1 was detected in six of 12 advanced stage NY-ESO-1 mRNA positive patients, indicating that NY-ESO-1 is highly immunogenic in advanced stage gastric cancer. One serum from a patient with a NY-ESO-1 negative, but LAGE-1 positive tumor was also found to be NY-ESO-1 antibody positive, possibly due to cross-reactivity between NY-ESO-1 and LAGE-1. All NY-ESO-1 mRNA positive gastric cancer samples also expressed one to seven additional CT genes, showing a tendency toward clustered expression pattern of CT genes, irrespective of disease stages. About 74% of the samples expressed at least one CT antigen, the most frequently expressed being MAGE-3 (41.6%) follwed by SSX4 (26.7%) and MAGE-1 (23.8%) etc. NY-ESO-1 and MAGE-3, among others, are thus potential targets for a multivalent CT antigen vaccine in this patient group, especially for patients with advanced gastric cancers.

The remaining two projects concern the very strong antibody responses to some tumor components that can occur in patients with lung cancer. In Moscow in the laboratory of Prof. Pavel P. Philippov, at the Belozersky Institute, analysis has focused on the antigenic protein recoverin. In an analysis of 279 serum samples from individuals with small cell lung cancer SCLC (99 patients), non-small cell lung cancer NSCLC (44 patients), and non-malignant pulmonary disorders (86 patients) as well as sera from 50 healthy donors, anti-Rc were detected in sera from 15 patients with SCLC (15% of cases) and from 9 patients with NSCLC (about 20% of cases). Only 2 anti-Rc positive cases were detected in patients with non-malignant pulmonary disorders, while no such cases were found in healthy individuals. Calculated

values of sensitivity and specificity, characterizing the diagnostic value of anti-Rc in lung cancer (SCLC+NSCLC), are equal to about 20% and 98%, respectively. In this context a more general analysis of the possible use of anti-tumor antibodies for early diagnosis of lung cancer is being initiated using sera of patients with various pulmonological disorders, as well as individuals exposed to the consequences of the Chernobyl nuclear power plant mishap (a high risk group in lung cancer). They have found that SCLC sera contain a pattern of anti-tumor antibodies, which are rarely detected in sera of patients with cancers other than SCLC and very rarely, if ever, present in sera of healthy individuals. It is of particular interest to note that in the high-risk group in lung cancer, the frequencies of the antibodies held an intermediate position between sera of SCLC patients and healthy individuals. This group of patients continues to be under medical observation to answer the question as to whether the pattern can predict the consequent development of lung cancer. Further focused studies on recoverin included an immunohistochemical investigation of paraffin sections of 44 SCLC tumors and 40 NSCLC tumors revealed 30 SCLC and 34 NSCLC recoverin-positive sections, 68% and 85%, respectively. A survey of 16 SCLC and 12 NSCLC cell lines revealed that none were capable of expressing recoverin. However, the group has been been able to stimulate recoverin expression in the SCLC cells lines NCI-H69 and NCI-H82 by butyrate treatment which will open the way to investigate whether antibodies against recoverin cause apoptotic death of recoverin-positive cancer cells.

In Istanbul, in the laboratory of Dr. Ugur Ozbek at the Genetics Department of Istanbul University, Institute for Experimental Medicine (DETAE), in collaboration with the New York Branch, the seroreactivity to the neuro-embryonic SOX Group B (1, 2 and 3) and ZIC2 has now been studied in a total of 70 small cell lung cancer patients. Antibody positivity ranged from 25.7% for SOX1 to 15.7% for ZIC2. All SOX3 patients were also seropositive for SOX1 and/or SOX2 reflecting the fact that SOX3 seroreactivity was an indication of high-titered anti-SOX reactivity. Seropositivity was observed throughout the chemotherapy treatment and followup period, indicating the antibody titers to be stable during the course of the disease, a period of about six months. None of the patients that had anti SOX or ZIC2 antibodies had evidence of paraneoplastic disease and patients with high titer anti-SOX antibody titers were found to correlate with a better prognosis and to be associated with disease parameters indicative of a more indolent course, such as limited disease, smaller tumor size and lower LDH levels.

A.J.G. Simpson

Publications

Primary Research Papers

1. Dong X-Y., Su Y-R., Xiao X-P., Yang X-A., Pang X-W., Wu H-Y., Chen W-F. Identification of two novel CT antigens and their capacity to elicit antibody response in hepatocellular carcinoma patients. British Journal of Cancer (2003) 89:291-297.
2. Evstafieva A.G., Belov G.A., Rubtsov Y.P., Kalkum M., Joseph B., Chichkova N.V., Sukhacheva E.A., Bogdanov A.A., Pettersson R.F., Agol V.I., Vartapetian A.B. Apoptosis-related fragmentation, translocation, and properties of human prothymosin alpha. Experimental Cell Research (2003) 284:209-221.
3. Goglia F., Skulachev V.P. A function for novel uncoupling proteins: antioxidant defense of mitochondrial matrix by translocating fatty acid peroxides from the inner to the outer membrane leaflet. FASEB Journal (2003) 17:1585-1591.
4. Kriventseva E.V., Koch I., Apweiler R., Vingron M., Bork P., Gelfand M.S., Sunyaev S. Increase of

functional diversity by alternative splicing. Trends in Genetics (2003) 19:124-128.

5. Li B., Qian X-P., Pang X-W., Zou W-Z., Wang Y-P., Wu H-Y., Chen W-F. HCA587 antigen expression in normal tissues and cancers: correlation with tumor differentiation in hepatocelluar carcinoma. Laboratory Investigations (2003) 83:1185-1192.
6. Li B., Wu H-Y., Qian X-P., Li Y., Chen W-F. Expression, purification and serological analysis of hepatocellular carcinoma associated antigen HCA587 in insect cells. World Journal of Gastroenterology (2003) 9(4):678-682.
7. Liu X.S., Zhu Y., Han W.N., Li Y.N., Chen L.H., Jia W., Song C.J., Liu F., Yang K., Li Q., Jin B.Q. Preparation and characterization of a set of monoclonal antibodies to TRAIL and TRAIL receptors DR4, DR5, DcR1 and DcR2. Hybrid Hybridomics (2003) 22(2):121-125.
8. Markova O.V., Evstafieva A.G., Mansurova S.E., Moussine S.S., Palamarchuk L.A., Pereverzev M.O., Vartapetian A.B., Skulachev V.P. Cytochrome c is transformed from anti- to pro-oxidant when interacting with truncated oncoprotein prothymosin α. Biochimica et Biophysica Acta (2003) 1557:109-117.
9. Nurtdinov R.N., Artamonova I.I., Mironov A.A., Gelfand M.S. Low conservation of alternative splicing patterns in the human and mouse genomes. Human Molecular Genetics (2003) 12:1313-1320.
10. Pereverzev M.O., Vygodina T.V., Konstantinov A.A., Skulachev V.P. Cytochrome c, an ideal antioxidant. Biochemical Society Transactions (2003) 31:1312-1315.
11. Zhang H-G., Pang X-W., Shang X-Y., Xing Q., Chen W-F. Functional supertype of HLA-A2 in the presentation of flu matrix p58-66 to induce CD8+ T-cell response in a Northern Chinese population. Tissue Antigens (2003) 62: 1-11.

Reviews / Book Chapters / Commentaries

1. Skulachev V.P. Aging and the programmed death phenomena. In: Topics in Current Cenetics, Vol. 3 Eds: T. Nystrom and H.D. Osiewacz, Model systems in ageing. Springer-Verlag Berlin Heidelberg, pp. 191-238, 2003.
2. Skulachev V.P. Programmed death phenomena at various levels of development of the living systems. In: Formal descriptions of developing systems. Eds: J. Nation et al. Kluwer Publishers, Netherlands, pp. 61-86, 2003.
3. Vartapetian A.B., Uversky V.N. Prothymosin alpha: a simple yet mysterious protein. In: Protein Structures: Kaleidoscope of Structural Properties and Functions Eds: V.N.Uversky, Research Signpost, Kerala, pp. 223-237, 2003.