Leading Edge Research in Cancer Biology, Protein Structure & Bioinformatics

Graduate studies in Medical Biophysics at the University of Toronto
# Table of Contents

Chairman’s Message ....................................................... p. 1
Our Graduate Program .................................................... p. 1
Cell & Molecular Biology Stream ........................................ p. 1
The Current Faculty ....................................................... p. 2
Structural Biology Stream ................................................ p. 3
Research Themes .......................................................... p. 4
Landmark Publications ................................................... p. 5
Major Funding ............................................................. p. 6
Graduate Admissions ...................................................... p. 6
Where are they now? Recent Ph.D. Graduates ......................... p. 7
Faculty & Project Descriptions - Table of Contents ................. p. 8
Faculty & Project Descriptions ........................................... p. 9
Department of Medical Biophysics

Cell, Molecular & Structural Biology

Chairman's Message

Welcome to the Department of Medical Biophysics at the University of Toronto. Within these pages you will find information describing the Department, its research and graduate programmes as well as its history, along with profiles of the Faculty and their specific interests. This booklet covers our streams in Cell, Molecular and Structural Biology; another describes the Imaging Physics stream. You will find information here on applying to our Department for graduate studies: we encourage you to go to our website at http://medbio.utoronto.ca and to contact the Department Office or the Faculty themselves with questions you may have.

For many undergraduates the first question will be, what is Medical Biophysics? The name reflects our central focus, which is the application of research disciplines spanning through biological and physical science to the problems of medicine. Our approach is unique in many respects and has been driven throughout our 50-year history by our principal research challenge: cancer. In fact, our Department originated with the Ontario Cancer Institute’s original Biology and Physics Divisions and has retained much of these roots: almost all of our laboratories are in hospital-based institutes and translation of our work into clinical medicine is our shared goal. Our programmes embrace students from backgrounds in Molecular and Cell Biology, Physiology, Biochemistry and Chemistry as well as Physics, Mathematics, Engineering, Computer Science and beyond. The diversity of our Faculty and the preponderance of multi-disciplinary projects reflects these backgrounds. In the descriptions of our laboratories and their projects in this and the companion booklet you will find projects in tumour biology, radiobiology, membrane function, molecular interactions, gene expression, cell differentiation and growth control, viral and chemical carcinogenesis, cellular and molecular immunology, hematopoiesis, macromolecular structure, the physics of radiation therapy and diagnostic imaging, the development of imaging systems involving ultrasound, x-ray, nuclear magnetic resonance and electron optics, and more. We hope that you will find them as exciting as we do.

Peter N Burns
Chairman of Medical Biophysics

Our Graduate Student Programme

The Department of Medical Biophysics graduate student training programme is a vital and dynamic component of our research environment and dates from the Department’s earliest origins. All of the core scientists hold their primary academic appointment in the Department of Medical Biophysics, which has no undergraduate programme; thus, faculty teaching is devoted entirely to graduate courses and thesis supervision.

Graduate students are selected primarily on the basis of their academic record and for their potential to become research scientists. The M.Sc. program is a common entry point but the emphasis is on reclassification into the Ph.D. programme. Course work is included in the first two years of graduate study and is intended to broaden the backgrounds of all students, most of whom enter with a degree in one of the basic sciences of biomedicine. All Medical Biophysics graduate students are supported by stipends which are reviewed annually to track inflation, including that of the graduate tuition fees. The approximately 40% of students who receive competitive scholarships from granting agencies receive a “top-up” which helps offset the increased cost of living in downtown Toronto. Our graduate students offer an exemplary record of continued achievement after they leave us: a partial list of PhD graduates and their current positions can be found on page 7.

The Cell & Molecular Biology Stream

The University of Toronto is an international leader in the field of cancer biology. The molecular and cellular biology division of the Department of Medical Biophysics is at the heart of this strength. The division derives from the earliest days of the Ontario Cancer Institute/Princess Margaret Hospital when the biological research group had a strong focus on studies of leukemia and normal bone marrow, microbial genetics, oncogenic viruses and the effects of radiation and chemotherapeutic drugs on cancers and normal tissues. Studies of the molecular mechanisms responsible for the cellular effects studied in those earlier days still represent some of the research themes running through the department and motivating clinical studies within the hospital. The MCB stream of the department has spread out over the years to have members in many of the biomedical research institutes in Toronto; in particular there are major groups of division members located at Sunnybrook Health Sciences Centre and in the Research Institute of the Hospital for Sick Children.
as well as the Ontario Cancer Institute/Princess Margaret Hospital. The current faculty in the molecular and cellular biology stream number more than 100, their research spanning a wide range of cancer biology topics from basic genetics to cell signaling, to studies in experimental animal models involving genetically modified mice, to innovative clinical studies of new drugs and new approaches to assessing treatment efficacy. The research summaries for the faculty members which follow provide more information about these topics and illustrate the opportunity for students within the department to benefit from the breadth of expertise within just one division of the Department.

Over the 50 years since the department was founded in 1958, members of the faculty, postdoctoral fellows and students within the cell and molecular biology division of the department have made many seminal contributions to our current understanding of the biology of cancer and its treatment including: the pioneering work in the laboratories of Drs Till and McCulloch on bone marrow stem cells; the early studies of somatic cell genetics in the laboratories of Drs Siminovitch, Till and Whitmore that have subsequently led to the identification of genes involved in the repair of DNA damage following irradiation or drug treatment; the identification of the first known drug efflux membrane protein involved in cellular drug resistance (P-glycoprotein) by Dr Ling’s laboratory; the identification of the T-cell receptor by Dr Mak’s laboratory; the identification of mutations in the Rb protein responsible for retinoblastoma in the laboratories of Drs Phillips and Gallie; the first demonstration that exposure to hypoxia can enhance the metastatic potential of cancer cells in the laboratory of Dr Hill; the demonstration in clinical studies that a low fat diet can reduce the onset of breast cancer and that high breast density on mammograms is a poor prognostic factors for breast cancer by the group led by Dr Boyd; and the initiation of studies of metronomic chemotherapy based on studies of the anti-angiogenic effects of chemotherapeutic drugs in the laboratory of Dr Kerbel.

Divisional faculty or trainees also play or have played major roles in the leadership of biomedical research across Canada. Dr Bernstein is the recently retired president of CIHR, Drs Branton and Aubin are the current heads of the CIHR Institutes of Cancer Research and Musculoskeletal Health and Arthritis respectively; Dr Hudson is the President and Scientific Director of the new Ontario Institute of Cancer Research and Dr Philips (a past Chair of Medical Biophysics) its Deputy Director.
Dr Ling has recently resigned as the Research Director of the BC Cancer Agency in Vancouver to take up a position as the head of the new Terry Fox Cancer Institute; Dr Carlsen is Vice-President for research at the Saskatoon Cancer Agency; Dr Paige is the Vice-President for research of the University Health Network in Toronto; Dr Julius is the Vice-President for research at Sunnybrook Health Sciences Centre; Dr Mak is the head of the Campbell Family Institute for Breast Cancer Research affiliated with the University Health Network/Princess Margaret Hospital in Toronto; Dr Siminovitch was the founding chair of the Department of Medical Genetics at the University of Toronto; Dr McCulloch was the founding chair of the Institute of Medical Science at the University of Toronto; Dr Woodgett is the current head of the Lunenfeld Research Institute at the Mt Sinai Hospital in Toronto and Dr Wu was until recently Dean of Science at York University in Toronto.

The Structural Biology Stream

Scientists in The Department of Medical Biophysics at the University of Toronto are among the pioneers of the study of the structure and functional analysis of macromolecules. Even before the field was firmly established by several key recruitments in the early 1990s, Peter Ottensmeyer, Professor Emeritus and former Chair of the Department, was a pioneer in the use of the electron microscope to reconstruct three-dimensional macromolecular structures from images of individual particles, a technique that is at the forefront of Structural Biology in the 21st century. Between 1990 and 1995, a joint recruitment between the University and several Research Institutes resulted in the hiring of a significant core of Structural Biologists using the major techniques of X-ray crystallography and nuclear magnetic resonance spectroscopy. These included Medical Biophysics faculty Drs Arrowsmith, Ikura, Rose and Privé, who joined Drs Ottensmeyer, Gariepy and, later, Chakrabarty and Pai, to form a group with interests in macromolecular structures and their role in diseases. A separate Departmental Stream, closely related to Cell and Molecular Biology but with a distinct flavour, was formed in the late 1990s. This was followed by the formation of an interdepartmental graduate program in Biomolecular Structure, of which Medical Biophysics is a founding member, signifying the close collaboration among members of the Toronto Structural community.

MBP has also played a founding role in another relevant interdepartmental graduate program in Proteomics and Bioinformatics. Paul Fraser, a structural neurobiologist at the Tanz Institute on campus, and an international authority on the molecular basis of Alzheimer’s Disease, is also a MBP Faculty member. With the recruitment of Aled Edwards and participation of Cheryl Arrowsmith, Toronto, and Medical Biophysics specifically, have been at the forefront of the international effort in Structural Genomics, the high-throughput determination of macromolecular structures that followed the sequencing of the human genome. Edwards and Arrowsmith have been instrumental in several initiatives in this field, including the founding of the highly successful Structural Genomics Consortium.

In 2006, most of the Structural Biology group relocated to the new TMDT/MaRS complex on College Street, to be joined by Thomas Kislinger and Brian Raught, with expertise in mass spectrometric analysis of protein structure, and Elizabeth Tillier, who uses computational approaches to understand macromolecular interactions and evolution. Although Structural Biology research and training exists in several Departments at the University of Toronto, Medical Biophysics is unique in three major respects. The first is that Structural research is embedded in the Research Institutes (specifically the Ontario Cancer Institute) and, as such, is within the context of a program emphasizing the application of research to human health and diseases. Secondly, and related, is the constant interaction with colleagues in MBP who are involved in disparate areas of research, from Medical Imaging and Physics through to Cancer and Cell Biology. Thirdly, Structural Biology research in MBP spans almost the complete breadth of modern techniques in this field, including three-dimensional structural analysis, protein chemistry, mass spectrometry and computational biology.
Research Themes

CANCER BIOLOGY
- The molecular basis underlying lung, pancreatic and head & neck cancer
- Models of human leukemias in mice
- The role of hypoxia in solid tumour progression

BREAST CANCER
- The role of inherited breast cancer susceptibility genes
- Tumour immunology as a novel modality in the treatment of breast cancer
- Epidemiology of breast cancer

STEM CELLS
- Self-renewal properties of hematopoietic stem cells
- Characterization of stem cells in neural and pancreatic development

ONCOGENES
- Functional role of oncogenes in cancer

TUMOR SUPPRESSORS
- The role of PTEN in cancer development

SIGNAL TRANSDUCTION
- Activation and regulation of serine/threonine and tyrosine kinases
- Tyrosine phosphatases in signal termination and human disease
- Role of adaptor proteins in mediating intracellular signalling
- Signalling pathways involved in stem cell, B cell, T cell and red cell development

APOPTOSIS
- The role of Myc in biology
- Intrinsic and extrinsic pathways of apoptosis

DNA STRUCTURE AND REPAIR
- Basic investigations into chromatin structure and regulation of the histone code
- Mouse models to examine the role of DNA repair pathways in mouse development

IMMUNOLOGY
- B cell development
- T cell tolerance

DEVELOPMENTAL BIOLOGY
- Protein Kinase B regulation in Drosophila
- Sea Urchin as a model to study aging and immunity
- Targeted and random mutagenesis to inactive genes in the mouse genome

EXPERIMENTAL THERAPEUTICS
- Animal models to test novel therapeutic agents

GENE THERAPY
- Gene therapy approaches to treat rare metabolic disorders

CHEMICAL BIOLOGY
- High throughput screens to identify novel inhibitors that can be utilized in cancer treatment
- Screens to identify off-patent compounds that may have novel inhibitory functions in cancer

BIOINFORMATICS
- Cancer informatics
- Novel software to analyze protein-protein interaction databases
- Bioinformatics strategies to characterize domain families

STRUCTURAL BIOLOGY
- Three dimensional structural analysis of glycolytic enzymes, transcription factors and signalling proteins
- High throughput proteomics

PEPTIDE CHEMISTRY
- Peptides as novel therapeutic agents
- Role of peptide biology in neurodegenerative disorders such as ALS and Alzheimer’s disease
Some Landmark MBP Publications ...

DISCOVERY OF THE STEM CELL - Till & McCulloch Labs

THE SEPARATION OF CELLS - Phillips Lab

DISCOVERY OF P-GLYCOPROTEIN - Ling Lab

A MODEL FOR GASTRIC CANCER - Archer Lab

CLONING OF THE T-CELL RECEPTOR - Mak Lab

DISCOVERY THAT P53 IS A TUMOUR SUPPRESSOR - Benchimol Lab

ASSOCIATION BETWEEN HYPOXIA AND METASTASIS IN SOLID TUMOURS - Hill Lab

DISCOVERY OF FLI2 - Ben-David Lab

DISCOVERY OF SAP/JNK KINASES - Woodgett Lab

INFLUENCE OF PePTIDE CONCENTRATIONS ON T-CELL RECEPTOR FUNCTION - Ohashi Lab

DISCOVERY THAT LITHIUM IS AN INHIBITOR OF GSK-3 - Woodgett Lab

IDENTIFICATION OF TEP1 AS PART OF THE TELOMERASE HOLOENZYME COMPLEX - Harrington Lab

FUNCTION OF PTEN TUMOUR SUPPRESSOR - Mak Lab

DELETION OF CASPASE 9 IN MICE - Mak Lab

ROLE OF FADD IN REGULATING APOPTOSIS - Mak Lab

IN VIVO FUNCTION OF VEGF RECEPTOR-3 - Dumont Lab

RECONSTITUTION OF TELOMERASE - Benchimol Lab

SUPPRESSOR OF CYTOKINE SIGNALLING BINDS TO C-KIT RECEPTOR - Rottapel Lab

DISCOVERY THAT THE ADAPTOR PROTEIN GADS PARTICIPATES IN T CELL SIGNALING - McGlade Lab

MOUSE MODEL OF SIMPSON-GOLABI-BEHMEL SYNDROME - Filmus Lab

METRONOMIC THERAPY - Kerbel Lab

STRUCTURAL PROTEOMICS OF ARCHAEBACTERIA, METHANOBACTERIUM THERMOAUTOTROPHICUM - Arrowsmith, Edwards & Pai Labs

GENE THERAPY TO CORRECT FABRY DISEASE IN A MOUSE MODEL - Medin Lab

GLYCOGEN SYNTHASE KINASE 3-BETA'S ROLE IN CELL SURVIVAL - Woodgett Lab

ROLE OF TIMP-3 IN LUNG DEVELOPMENT AND MAMMARY GLAND APOPTOSIS - Ksokha Lab

STRUCTURE OF A GLYCOLYTIC ENZYME - Rose Lab

THE IMPORTANCE OF DENSITY AS A RISK FACTOR IN BREAST CANCER - Boyd Lab

ACTIVATED RETINOblastOMA IN BREAST CANCER - Zacksenhaus Lab

T CELL PHOSPHATASE SELECTS JAK1 AND JAK3 AS SUBSTRATES - McGlade & Barber Labs

BCL6-SMRT STRUCTURE - Prive Lab

CONDITIONAL INACTIVATION OF CASPASE 8 IN T CELLS - Hakem Lab

QUANTITATIVE ANALYSIS OF STEM CELL ENGRAFTMENT - Iscove Lab

STAT1 IS A NEGATIVE REGULATOR OF RED BLOOD CELL PRODUCTION - Barber Lab
CRYSSTALIZATION OF A MEMBRANE PROTEIN
- Pai Lab

RELATIONSHIP OF AGE AND TELOMERE LENGTH IN LI-FRAUMENI SYNDROME - Malkin Lab

ROLE OF GATA-6 IN ASTROCYTOMA - Gusha Lab

ROLE OF INORGANIC PHOSPHATE IN BONE FORMATION - Aubin Lab

HOMING OF STEM CELLS AFTER RADIATION
- F-F Liu Lab

ROLE OF PEPTIDE FOLDING IN AMYOTROPHIC LATERAL SCLEROSIS - Chakrabartty Lab

ROLE OF H2A.Z IN CHROMATIN STRUCTURE
- Cheung Lab

ROLE OF SIRPA IN STEM CELL ENGRAFTMENT
- Danska Lab

Major Funding

Research in the Department of Medical Biophysics is supported through numerous peer-reviewed grants held by Faculty from many agencies, including:

- Canadian Institutes of Health Research
- National Cancer Institute of Canada
- Ontario Institute of Cancer Research
- Natural Sciences and Engineering Research Council of Canada
- National Institutes of Health, US
- Cancer Research Society
- Canadian Breast Cancer Research Alliance
- Prostate Cancer Research Foundation of Canada
- Leukemia and Lymphoma Society
- Leukemia and Lymphoma Society of Canada
- Kidney Foundation of Canada
- Foundation Fighting Blindness
- Heart and Stroke Foundation

Graduate Admission: How to apply

The Department welcomes applications from graduates in any of the biological or physical sciences including chemistry, biology, genetics, immunology and biochemistry, or from medicine, engineering, computer science or related sciences. Applicants are evaluated on both their academic record and potential for creative research.

To be considered for admission, the minimum requirement is an appropriate four-year bachelor’s degree from a recognized university in Canada, or its equivalent from another institution. Most students must have at least an A- average in the final two years, but this condition is flexible, especially for applicants who have demonstrated exceptional aptitude for research. To have the best chances for acceptance and for receiving University of Toronto Fellowships or Connaught Scholarships, applications should be received by February 1.

In general, there is no direct entry into the Ph.D. programme except for an applicant who already holds a Master’s degree from a Canadian university. Students who are accepted into our Master’s programme may reclassify, if eligible, into the Ph.D. programme within 18 months of their registration. Students who have been accepted directly into the Ph.D. programme must pass a qualifying examination within 15 months of registration.

For more information on graduate admissions please contact Justin Thielman in the Medical Biophysics Office at (416) 946-2819. Application packages are downloadable from our website at http://medbio.utoronto.ca.
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<tr>
<th>Name</th>
<th>Position/Location</th>
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<tbody>
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<td>Dr. Jian Payandeh</td>
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<td>Assistant Professor, MBP, University of Toronto</td>
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<td>Dr. Valerie Wallace</td>
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<td>Associate Professor, Department of Immunology, U of Toronto</td>
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<td>Dr. Phil Branton</td>
<td>Professor, McGill University</td>
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<tr>
<td>Faculty Name</td>
<td>Page</td>
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</tr>
<tr>
<td>Dr. Michael Archer</td>
<td>9</td>
</tr>
<tr>
<td>Dr. Cheryl Arrowsmith</td>
<td>9</td>
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<tr>
<td>Dr. Jane Aubin</td>
<td>10</td>
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<tr>
<td>Dr. Dwayne Barber</td>
<td>11</td>
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<tr>
<td>Dr. Yaacov Ben-David</td>
<td>12</td>
</tr>
<tr>
<td>Dr. Norman Boyd</td>
<td>13</td>
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<td>Dr. Robert Bristow</td>
<td>14</td>
</tr>
<tr>
<td>Dr. Peter Cheung</td>
<td>15</td>
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<td>Dr. Gregory Czarnota</td>
<td>16</td>
</tr>
<tr>
<td>Dr. Jayne Danska</td>
<td>17</td>
</tr>
<tr>
<td>Dr. Daniel Dumont</td>
<td>17</td>
</tr>
<tr>
<td>Dr. Jorge Filmus</td>
<td>18</td>
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<tr>
<td>Dr. Paul Fraser</td>
<td>19</td>
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<td>Dr. Jean Gariépy</td>
<td>19</td>
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<tr>
<td>Dr. Abhijit Guha</td>
<td>20</td>
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<tr>
<td>Dr. Razquallah Hakem</td>
<td>21</td>
</tr>
<tr>
<td>Dr. David Hedley</td>
<td>22</td>
</tr>
<tr>
<td>Dr. Richard Hill</td>
<td>23</td>
</tr>
<tr>
<td>Dr. Mitsuhiko Ikura</td>
<td>24</td>
</tr>
<tr>
<td>Dr. Norman Iscove</td>
<td>25</td>
</tr>
<tr>
<td>Dr. Michael Julius</td>
<td>25</td>
</tr>
<tr>
<td>Dr. Igor Jurisica</td>
<td>26</td>
</tr>
<tr>
<td>Dr. Suzanne Kamel-Reid</td>
<td>27</td>
</tr>
<tr>
<td>Dr. Gordon Keller</td>
<td>28</td>
</tr>
<tr>
<td>Dr. Robert Kerbel</td>
<td>29</td>
</tr>
<tr>
<td>Dr. Rama Khokha</td>
<td>30</td>
</tr>
<tr>
<td>Dr. Thomas Kislinger</td>
<td>31</td>
</tr>
<tr>
<td>Dr. Anne Koch</td>
<td>31</td>
</tr>
<tr>
<td>Dr. Michelle Letarte</td>
<td>32</td>
</tr>
<tr>
<td>Dr. Fei-Fei Liu</td>
<td>33</td>
</tr>
<tr>
<td>Dr. Geoffrey Liu</td>
<td>34</td>
</tr>
<tr>
<td>Dr. David Malkin</td>
<td>34</td>
</tr>
<tr>
<td>Dr. Armen Manoukian</td>
<td>35</td>
</tr>
<tr>
<td>Dr. Philip Marsden</td>
<td>36</td>
</tr>
<tr>
<td>Dr. Lisa Martin</td>
<td>37</td>
</tr>
<tr>
<td>Dr. Jane McGlade</td>
<td>37</td>
</tr>
<tr>
<td>Dr. Jeffrey Medin</td>
<td>38</td>
</tr>
<tr>
<td>Dr. Benjamin Neel</td>
<td>39</td>
</tr>
<tr>
<td>Dr. Pamela Ohashi</td>
<td>39</td>
</tr>
<tr>
<td>Dr. Hitoshi Okada</td>
<td>40</td>
</tr>
<tr>
<td>Dr. Emil Pai</td>
<td>40</td>
</tr>
<tr>
<td>Dr. Christopher Paige</td>
<td>41</td>
</tr>
<tr>
<td>Dr. Linda Penn</td>
<td>42</td>
</tr>
<tr>
<td>Dr. Gilbert Privé</td>
<td>42</td>
</tr>
<tr>
<td>Dr. Mira Puri</td>
<td>43</td>
</tr>
<tr>
<td>Dr. Jonathan Rast</td>
<td>44</td>
</tr>
<tr>
<td>Dr. Brian Raught</td>
<td>45</td>
</tr>
<tr>
<td>Dr. David Rose</td>
<td>45</td>
</tr>
<tr>
<td>Dr. Aaron Schimmer</td>
<td>46</td>
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<tr>
<td>Dr. David Spaner</td>
<td>47</td>
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<tr>
<td>Dr. Vuk Stambolic</td>
<td>47</td>
</tr>
<tr>
<td>Dr. Ian Tannock</td>
<td>48</td>
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<tr>
<td>Dr. Elisabeth Tillier</td>
<td>49</td>
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<tr>
<td>Dr. Suzanne Trudel</td>
<td>49</td>
</tr>
<tr>
<td>Dr. Ming-Sound Tsao</td>
<td>50</td>
</tr>
<tr>
<td>Dr. Derek van der Kooy</td>
<td>50</td>
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<tr>
<td>Dr. Richard Wells</td>
<td>51</td>
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<tr>
<td>Dr. Shun Wong</td>
<td>52</td>
</tr>
<tr>
<td>Dr. Minna Woo</td>
<td>52</td>
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<tr>
<td>Dr. James Woodgett</td>
<td>53</td>
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<tr>
<td>Dr. Eldad Zacksenhaus</td>
<td>54</td>
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</tbody>
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Michael Archer, Ph.D.
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Role of dietary factors and susceptibility genes in cancer development

One objective of our research program is to identify molecular targets of dietary factors involved in breast and colon cancer development. Current research focuses on HMG-CoA reductase and the mevalonate pathway, cyclooxygenase-2, fatty acid synthase, and insulin, IGF-I, and other obesity-related factors. A second objective is to understand the genetic basis for the differences in susceptibility of rat strains to breast and liver cancer induction.

Selected Publications:
• Ealey, K.N., Lu, S., and Archer, M.C. Development of aberrant crypt foci in the colons of ob/ob and db/db mice: evidence that leptin is not a promoter. Molecular Carcinogenesis (in press).

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p53 related chromatin and ubiquitylation pathways

Our research focuses on the structural and biochemical characterization of proteins involved in cancer pathways, especially that of the tumor suppressor, p53. The goals of our research are to understand how p53 and related proteins communicate and signal in complex processes such as DNA damage recognition and repair, transcription regulation, and ubiquitin-mediated degradation. We take a genome-wide approach, studying whole families of related proteins that may have an impact on these pathways in order to understand how proteins selectively interact with specific members of a sequence-related family. We use Nuclear Magnetic Resonance (NMR) Spectroscopy and x-ray crystallography in conjunction with other physical and biochemical techniques to study the three-dimensional (3D) structure, dynamics and biochemical properties of proteins, protein-DNA and protein-protein complexes.

p53 is a central integrator of signaling pathways that guard genomic integrity, thereby preventing tumorigenesis. The cellular levels of p53 are tightly regulated by several ubiquitin E3 ligases that promote ubiquitylation and target p53 for 26S proteasome-dependent protein degradation. We are studying proteins that both add and remove ubiquitin from p53. We are also investigating other post-translational modifications of p53 (phosphorylation, methylation and acetylation), and how these modifications may act as signals by altering p53’s interactions with other proteins, DNA and/or chromatin. Other systems under study in the lab include the breast cancer susceptibility gene, BRCA1, the human ubiquitylation system, and proteins that “read” and “write” histone marks such as methyl-lysine recognizing proteins such as MBT repeat proteins, Chromodomains and methyl- and acetyl-transferases.

Selected Publications:
Mesenchymal stem cells and skeletal development

Research in our lab is focused on development and postnatal activity of the skeleton. Osteogenic, chondrogenic, adipogenic and myogenic cells develop from a pool of mesenchymal stem cells and primitive progenitor populations in bone and bone marrow. One aspect of our work addresses both how to characterize and how to control self-renewal, fate choice, proliferation and differentiation of the stem, progenitor and more mature precursor populations so as to map the developmental hierarchies underlying mesenchymal lineages. To do this, we are assessing the ability of endogenously produced or exogenously supplied hormones, cytokines, and growth factors to influence progenitor proliferation, self-renewal and differentiation to dissect deterministic versus stochastic pathways of mesenchymal cell development. We have acquired evidence for differentiation stage-specific regulatory pathways and for dose- and time-dependent biphasic effects of many regulators of interest. We are also interested in the regulatory and developmental basis of the phenotypic heterogeneity we have documented in post-proliferative mature cell phenotypes in the developing and mature skeleton.

Amongst regulators of the highly dynamic postnatal skeleton, we are currently studying the family of estrogen receptor-related orphan nuclear receptors, and have developed novel gain-of-function and loss-of-function transgenic mice to delineate the functional role of these transcription factors in health and disease of bones and joints. We are also using genome-wide chemical (N-ethyl-N-nitrosourea; ENU) mutagenesis in mice to identify novel genes and mutations that regulate formation and turnover of the skeleton and are characterizing their cellular and molecular modes of action in the developing and postnatal skeleton.

My laboratory has developed many transgenic and knockout mouse models with skeletal anomalies, primary cell culture models for mesenchymal cell differentiation, assays for enriching stem and progenitor populations and dissecting developmental transition points, and tools for analysing skeletal cells and their fate including fluorescence-activated cell sorting (FACS), colony assays, gene expression profiling, immunocytochemistry and immunohistochemistry, in situ hybridization of embryonic and adult tissues and cells, Western blotting, single cell assays and other state-of-the-art cell and molecular biology techniques. By this multi-faceted model organism, developmental, cell and molecular biological approach, we are advancing understanding of normal development and diseases of the skeleton.

Selected Publications:

A mouse model for human osteogenesis imperfecta (OI). Micro-computed tomography (microCT) images through the distal femur. The image on the left is from a wild type mouse; the image on the right is from a mutant mouse with a low bone mass phenotype. The low bone mass results from a point mutation, created by chemical mutagenesis, in the gene for collagen type I alpha chain, which results in reduced osteoblast differentiation and reduced bone matrix production.
Signalling Mechanisms in Hematopoietic Cells

Cells within the bone marrow respond to a host of growth factors that promote their growth, survival and differentiation. Our laboratory studies signal transduction in normal and leukemogenic hematopoiesis. Current studies are focused in three specific areas: understanding the role of Erythropoietin (EPO) in red blood cell production, characterization of the signal transduction pathways activated downstream of BCR-ABL and delineation of the molecular mechanism underlying the poor prognosis of Chronic Myeloid Leukemia patients harbouring deletions at Chromosome 9q34.

EPO is the major cytokine regulator of red blood cell production. This cytokine binds to its cognate receptor (EPO-R) and initiates signal transduction through activation of the JAK2 cytoplasmic tyrosine kinase. JAK2 subsequently phosphorylates the EPO-R on several cytoplasmic tyrosine residues, which facilitates recruitment of several SH2 domain-containing proteins. Genetic evidence suggests that deletion of EPO, EPO-R or JAK2 in mice results in embryonic lethality due to a fatal anemia that develops during embryogenesis. This implies that critical signals emanate downstream of the EPO-R and/or JAK2.

Our approaches to dissect EPO-mediated signalling are to utilize knockout mice devoid of expression of critical downstream players. In addition, we have used microarray to examine gene regulation mediated by EPO. We are most interested in characterizing the transcriptome in primary cells under conditions that support normal and stress erythropoiesis.

Approximately one-half of leukemias arise from chromosomal translocations. Novel fusion proteins are generated which deliver a growth advantage to the stem cell in which they reside in the bone marrow. We are interested in the tyrosine kinase subclass of chromosomal translocations including TEL-JAK2 and BCR-ABL. Our goal is to identify the critical downstream signalling pathways that contribute to leukemogenesis mediated by BCR-ABL. We are utilizing retroviral transduction/bone marrow transplantation technology to approach this problem.

The causative agent of Chronic Myeloid Leukemia (CML) is the BCR-ABL chromosomal translocation. Dr. Jeremy Squire has demonstrated that a subset of CML patients with poor prognosis harbour deletions centromeric to the ABL gene at Chromosome 9q34. His laboratory has mapped a minimal deleted region of 120 kb extending from the Philadelphia chromosome ABL translocation breakpoint in chronic myeloid leukemia with poor outcome. Leukemia. 17: 1313-23, 2003.

Selected Publications:

- Kolomietz E, Marrano P, Yee K, Thai B, Braude I, Kolomietz A, Chun K, Minkin S, Kamel-Reid S, Minden M, Squire JA. Quantitative PCR identifies a minimal deleted region of 120 kb from wild type and Ship1-/- mice.

Cytospin of cells isolated from Phenylhydrazine-primed spleens located in the deleted region to determine whether BCR-ABL causes a more aggressive disease in a bone marrow transplant model.
Molecular analysis of genes involved in Leukemia

It is now widely accepted that cancer is the result of a multistage process involving the activation of dominant acting oncogenes and the inactivation of tumor suppressor genes. The erytholeukemias induced by Friend virus are amongst the most thoroughly studied experimental models of the multistage nature of cancer. The distinct early and late stages of Friend leukemia, the rapid and efficient induction of disease by a single injection of virus, and the identification of a number of unique host genes that control susceptibility to leukemia induction, provide a unique model for the identification and analysis of genes involved in this multistage malignancy.

The research focus in our laboratory is to understand the molecular mechanisms underlying the multistage malignancy induced by Friend virus. This disease is initiated with a preleukemic and non-tumorigenic stage that is associated with a marked polyclonal increase in the number of erythroid progenitors. The next stage involves the appearance of clonal leukemia cells in the spleens of infected mice. With respect to the genes that are responsible for the transition of polyclonal preleukemic infected erythroid progenitors to clonal leukemic cells, our work and that of others, have shown that the evolution of clonal erytholeukemia by various strains of Friend virus is associated with the inactivation of either of the tumor suppressor genes p53 and p45 NFE2 as well as the activation of either dominant acting oncogenes Fli-1 or Spi-1. The latter two genes encode transcription factors, which belong to the family of ets oncogenes, and are activated as a result of proviral integration in the majority of Friend erytholeukemia cell lines. p53, which is also a transcription factor, is inactivated in the majority of the Friend erytholeukemia cell clones as a result of deletions, proviral insertions and mutations. In addition, we have recently identified another common site for retroviral integration, named Fli-3, from DNA of erytholeukemia cell lines and shown that the coding sequence within this locus is identical to a cluster of microRNAs, a new class of gene that is involved in regulation of other genes. Recent studies in my laboratory demonstrated that activation of this gene accelerates the progression of Friend erythroleukemia.

My laboratory is currently investigating the molecular and cellular function of Fli-1, p45 NFE2 and p53. Since Fli-1, p45 NFE2, and p53 are shown to be transcription factors, their function is assumed to regulate the expression of cellular gene(s) that are involved in cell growth and differentiation. Thus, the identity of target genes whose expression are regulated by these proteins may eventually address the broad roles played by these genes in oncogenesis. For example, target genes for Fli-1 have been implicated in erythropoietin signal transduction pathway in erythrocytes. Therefore, study of these genes could increase important insight into normal process of erythropoiesis and malignant transformation. Furthermore we are also studying the possible involvement of Fli-1, Fli-3 and p45 NFE2 in human hematopoietic malignancies.

Selected Publications:

Epidemiology and Prevention of Breast Cancer

Our research is concerned with the development of strategies to prevent breast cancer. Breast cancer is the most common cause of death from any cancer in women in most of the Western world, and the leading cause of death from all causes among women aged less than 50. Several factors have been identified that influence risk of the disease, including the characteristics of breast tissue on either mammography or histology, the number of pregnancies, alcohol, and body weight.

Mammographic density has consistently been shown to be one of the strongest known risk factors for breast cancer. Research is designed to improve our understanding of this risk factor, its measurement, its causes, and its significance as a biomarker of breast cancer risk. Current methods of measuring mammographic density are based on the 2-dimensional image. In collaboration with Dr M Yaffe (Medical Biophysics and Imaging Research, Sunnybrook Health Sciences Centre) we are now evaluating novel methods of measurement that assess the volume of the tissues in the breast that contribute to radiological density. Data collection has been completed and final calibration of the measurements and data analysis is in progress.

The breast is especially susceptible to carcinogenic events at early ages. In collaboration with Dr M Bronskill (Imaging Research, Sunnybrook Health Sciences Centre), The goal of this research is to use magnetic resonance (MR) imaging to generate quantitative estimates of breast tissue composition in young women, and to examine factors likely to be associated with variation in these measurements, in particular the relationship between lifestyle factors and genes, hormones and growth factors that influence the growth and development of breast tissue.

Striking differences exist between countries in the incidence of breast cancer. The causes of these differences are unknown, but because incidence rates change in migrants, they are thought to be due to differences in lifestyle. The goal of this research is to identify the factors responsible for international differences in breast cancer risk, and to determine whether differences in breast tissue composition, and the hormonal and growth factors associated with them contribute to differences in risk.

Previous twin studies have shown that mammographic density is highly heritable. In collaboration with Drs Rommens and Paterson at the Hospital for Sick Children, we are now carrying a large scale multicentre family-based linkage study with whole genome scanning to identify the genetic variants that influence density.

In collaboration with Dr L Martin, we have recently completed a multicentre randomized trial in women with high risk mammographic changes that tests the hypothesis that a 25% dietary reduction of calories from fat, with isocaloric replacement of carbohydrate, will reduce the incidence of breast cancer by approximately 35% over 10 years. The trial is now under analysis. Additional studies will examine the effect of this dietary intervention on plasma hormones and growth factors that may mediate environmental influences on risk of breast cancer.

Selected Publications:

DNA Repair and Genetic Instability in Solid Tumours

Cells have developed a sophisticated approach to the initial sensing and subsequent repair of DNA damage to preserve genetic stability. The objective of our clinico-translational laboratory is to understand the effect of the tumour microenvironment on the ATM-p53-53BP1 DNA damage signaling pathway and DNA double-strand break (DNA-dsb) repair. Our studies suggest that hypoxic tumour cells can have decreased DNA-dsb repair (e.g. decreased homologous recombination) and an aggressive “mutator” phenotype. We are therefore tracking DNA damage responses and repair within normal and tumour tissues to develop novel diagnostics and molecular-targeted therapies.

We interrogate protein-protein interactions during DNA-dsb repair and cell-cycle checkpoints using: siRNA knock-downs, DNA-rejoining assays (comet and CFGE assays), chromatin immunoprecipitation (ChIP), biochemical fractionation, fluorescently-tagged proteins and quantitative confocal microscopy with UV-microbeams (www.sttarr.ca).

(i) p53 and DNA repair: Mutations in the p53 tumour suppressor protein are common in many human cancers. We are interested in certain MTp53 proteins that have acquired novel properties or “gain of function” in their ability to detect DNA-dsbs, but over-ride DNA damage cell cycle checkpoints. This can lead to therapy resistance. We are tracking the sub-cellular location and function of ATM-dependent p53 phosphoforms and 53BP1 (a p53-binding protein) in response to DNA breaks and evaluating new therapies that target MTp53.

(ii) Hypoxia, DNA repair and prostate cancer: Many prostate cancer patients die each year solely from the failure of radical radiotherapy to control the primary tumour. We are interested in developing genomic (SNP, CGH) and proteomic (serum, plasma or urine) biomarkers to predict cancer therapy cure and toxicity. This includes the assessment of tissue microarrays (TMAs) for novel protein expression in patients who fail therapy. For example, we are investigating the role of hypoxia as a negative prognostic factor in prostate and other cancers. We believe that novel cancer therapies can target these resistant hypoxic cells by taking advantage of DNA repair defects. We therefore hope to select the most effective treatment for individual patients based on individual biology.

For more information, see: http://www.radiationatpmh.com/body.php?id=165

Selected Publications:

Cancer is a disease often caused by dysregulated expression of oncogenes or tumour suppressor genes. One of the fundamental mechanisms that regulate global gene expression is through post-translational modification of histone proteins. In the context of the human genome, DNA is complexed with histone to form nucleosomes and chromatin. This functional organization of the genome is tightly regulated such that only appropriate genes are maintained in an open context that is accessible by transcription factors and RNA polymerases. In contrast, inactive genes are sequestered into compact structures and are transcriptionally silenced. Histone modifications regulate the balance between these open and closed chromatin states and define the gene expression profile of the functional genome.

Our lab utilizes a combination of molecular biology and biochemistry techniques to dissect the mechanisms of how histones and histone modifications regulate gene functions. Our projects are broadly divided into 2 main interests: 1) how signal transduction pathways converge onto histones to regulate gene functions, and 2) how histone variants function in the epigenetic regulation of gene expression.

For the first interest, we focus on the role of histone H3 phosphorylation in gene activation. Upon growth factor stimulation, the activated MAP kinase pathway ultimately converges onto histones to regulate gene functions, and results in the phosphorylation of H3 at two specific serine resides (S10 and S28). The phosphorylation events are rapid and transient, and mirror the transcriptional activation of genes such as c-fos and c-jun. We are currently examining how H3 phosphorylation leads to transcriptional activation and we are testing whether these modifications recruit or repel regulatory factors to the c-fos and c-jun promoters. Our previous work also showed that H3 phosphorylation promotes acetylation on the same histone and these modifications function together to activate gene expression. The idea that histone modifications work in combinations was formally described as the Histone Code Hypothesis, and we are currently developing new techniques to dissect the intricate interplay between histone modifications in vivo and to test the validity of this hypothesis.

For the second interest, we are studying the functional role of an H2A variant, H2A.Z, in the regulation of gene expression. H2A.Z is essential for cell viability in that loss of H2A.Z function in mammalian cells leads to cell death or senescence. H2A.Z is localized to the transcription start sites of genes, and cumulative evidence suggests that this variant has both positive and negative regulatory functions in the gene expression process. We have found that a fraction of H2A.Z is modified by the addition of a single ubiquitin group and such modified H2A.Z is associated with transcriptionally silenced heterochromatin. We are currently testing how addition or removal of the ubiquitin modification on H2A.Z regulates transcription and gene expression.

Selected Publications:
Ultrasound in Cancer Treatment: New Therapies and Methods of Therapy Evaluation

Our research is centered on understanding how radiation affects blood vessels and how this contributes to tumour death. We have developed a number of small animal micro-ultrasound methods in our laboratory to detect vascular changes and separately apoptotic cell death in vivo. A number of different research projects are underway to study the basic science behind vascular responses to radiation and to therapeutically exploit this in order to radiosensitize tumours. In that regard our research interests include the novel use of pharmacologic anti-angiogenic agents and new ultrasound-activated anti-angiogenic agents.

Other projects include:
(A) Cell and Molecular Biology
Despite the use of medical ultrasound for decades the features inside cells that contribute to ultrasound backscatter at conventional- and high-frequencies remain unknown. We are systematically probing how subcellular constituents such as DNA, RNA, protein and lipids contribute to backscatter. In particular we are interested in how nuclear and chromatin structure affects ultrasound signals since we have found it to be a dominant structure in the formation of backscatter signals.

(B) Image and Spectroscopic Analysis
We are collaboratively investigating a number of spectroscopic parameters for characterizing tumours and tumour responses to chemotherapy and radiation therapy at conventional and high-frequencies. We are developing these methods to generate colour-coded ultrasound parameteric maps to aid in assessing tumour responses to therapy. Since these spectroscopic signals are potentially linked to nuclear structure and chromatin structure which differs between normal and neoplastic tissue there is potential to develop our spectroscopic methods not only into a method to track tumour responses but a potentially important diagnostic tool.

(C) Clinical Evaluation of Ultrasound Imaging & Spectroscopy
We are instituting a number of clinical evaluations of our spectroscopic detection of cell death. Our main investigational site is breast cancer patients with large ‘locally-advanced’ breast cancers who receive neoadjuvant combined chemotherapy and radiation therapy. We hope to be able to rapidly ascertain responding tumours from those that are non-responding so that the latter may be treated with different chemotherapy regimens or with radiation sensitizers in order to hopefully improve outcomes.

Selected Publications:

Results of ultrasound imaging of apoptotic cells. Each panel is a representative ultrasound scan of a pellet of acute myeloid leukaemia cells. The bottom of each ultrasound scan is at the bottom of each frame. Pellets are immersed in buffered saline. From left to right, panels correspond to cells treated with cisplatinum for 0, 6, 12, 24 and 48 h to induce varying degrees of apoptosis. A bar at the bottom right of the figure indicates the colour map used in this image, the left of the bar indicating the colour that corresponds to pixel values of 0 and the right giving the colour that corresponds to a pixel value of 256. At 0, 6, 12, 24 and 48 h histological analysis indicated that 1.6, 2, 36, 87 and 93% of all cells showed nuclear fragmentation, respectively. At the 6-h time point, 72% of the cells exhibited prominent nuclear condensation changing from a nuclear diameter 70% of the cellular diameter before addition of the drug, to a diameter 40% of the cellular diameter at 6 hours. After the 6-h time point, 95% of all cells exhibited nuclear condensation or fragmentation. The speckle pattern is characteristic of ultrasound images. The scale bar indicates 1 mm. From Czarnota et al, 1999.
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Functional Genomics of Type 1 Diabetes

Type 1 diabetes (T1D) is caused by autoimmune destruction of insulin-producing islet cells and afflicts 0.3-0.6 percent of North Americans. The disease is multifactorial caused by genetic variation at multiple loci, modified by poorly defined environmental factors. The objective of our research program is to 1) identify genes that control this process in mouse models and in human patients, 2) understand the autoimmune response that results in islet cell death, and 3) to test the idea that early immune system exposures to microbes significantly modifies genetic risk for developing T1D. We use positional cloning, gene expression microarray analysis and immunological analyses to identify the pathways controlled by T1D risk genes and understand how they are modified by exposure to intestinal commensal bacteria. We are also engaged in high-throughput human gene association studies of T1D.

Molecular pathways of Acute Lymphoblastic Leukemia

Our research program is designed to probe the developmental steps and mechanisms of leukemia in mouse models and in human patients. The objective of this program is to translate knowledge gained in mouse models and primary human leukemia, to improve the diagnosis and treatment of leukemia. Our specific areas of interest are: 1) signalling pathways that govern cell survival, differentiation or death fate decisions in normal and neoplastic lymphoid cells, 2) identification and analysis of the cells that initiate and sustain the leukemic clone (cancer stem cells) to understand molecular pathways underlying leukemia initiation and progression, and 3) how leukemic blasts expand in the central nervous system (CNS) causing a major clinical complication of leukemia and lymphomas, and 4) features of the immune system that determine engraftment of normal human blood stem cells in settings of clinical transplantation.

Selected Publications:


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Endothelial Cell Growth & Signalling

The receptor tyrosine kinase (RTK) family of cell surface proteins is known to play key roles in cell-cell communication in multi-cellular metazoan organisms. Genetic and biochemical studies on this large family of proteins have shown that different RTKs are responsible for transducing important developmental, proliferative, cell survival and migratory signals from the outside to the inside of the cell. The development and proper functioning of cell systems as diverse as the compound eye in the fly, the vulva in the nematode and hematopoiesis and endothelial growth in the mouse all depend on intact signalling pathways that are controlled by different members of the RTK family.

Our lab is investigating the signal transduction pathways of different RTKs during vascular development in the mouse and during tumour formation. Vascular endothelial cells constitute an unusually quiescent epithelial cell population. The turnover rate of both large and small vessel endothelium is very low. The mechanisms that underlie this growth control are not well understood, and the factors which initiate and control subsequent proliferation are unknown. It is clear, however, that vascular growth occurs under nonpathological conditions (e.g. wound healing, corpus luteum formation, and development) and that this growth is somehow terminated at the correct time. In contrast, uncontrolled vessel growth (including tumour vascularization, diabetic retinopathy, and arthritis) are associated with many different diseases states. The determinants which control these processes remain unknown; however the study of peptide growth factors, their receptors and their downstream substrates will provide both a biochemical and genetic entry point into the elucidation of the underlying controls of these processes.

Our group uses gene-targeting in embryonic stem cells, transgenic mice, proteomics and receptor biochemistry to attempt to address the importance of these different RTKs and their related signal transduction pathways during vascular growth in development and in disease.
Our research has the potential to impact numerous diseases where aberrant vessel growth or vessel stability lead to progression of disease or increased complications, such as diabetes, heart disease and cancer. Furthermore, his group is also using this knowledge to in fact also augment vessel growth for applications in regenerative medicine.

Selected Publications:

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Role of Amyloid & Presenelin Proteins in Alzheimer’s Disease

Research in our laboratory focuses on the biochemistry and biophysics of amyloid plaques and their relationship to sporadic and familial forms of Alzheimer’s disease. Plaques are a principal pathological feature of Alzheimer’s disease and appear as abnormal accumulations of fibrous or thread-like structures within the brain. These plaques are assembled by the misfolding and aggregation of the amyloid-β (Aβ) protein. We have been studying its properties with an emphasis on the factors responsible for the transition from the normal to diseased fibrous state, the ability of aggregated Aβ to kill nerve cells in culture, and the mechanism by which this is accomplished. Considerable advances have been made in this area and we are expanding our efforts to look for modulators of plaque formation as well as the cellular receptors which we feel are responsible for amyloid’s “killer” action. Our ultimate goal is to understand the events that culminate in these abnormal and detrimental proteins and the development of drugs capable of controlling these processes. These investigations are relevant to both the sporadic and familial forms of Alzheimer’s disease which exhibit identical amyloid pathology but differ only in their rate of progression.

In conjunction with our amyloid research and drug development, our group has been concentrating its efforts on understanding the biochemistry and structural biology of the presenelin family of proteins. Mutations in the presenelin genes are the major cause of inherited forms of Alzheimer’s disease and we have been examining the location and expression of these proteins in neuronal cells and their relationship to the pathology of Alzheimer’s disease. This biochemical and molecular biological work is complemented by our examination of the three-dimensional organization of particular regions of the presenelin protein using a variety of biophysical techniques. Through these two approaches, we will be able to provide details on presenelin function and the molecular mechanism by which this is achieved. The importance of these studies is that they enable us to understand the earliest events in Alzheimer’s disease and allow us to develop novel approaches to the treatment of a principal cause of Alzheimer’s disease.

Selected Publications:


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Molecular Engineering and the development of targeted biological therapies

Our laboratory is interested in developing both in vivo imaging modalities and targeted therapies directed at epithelial cancers (breast, colon, prostate, ovarian, lung, pancreas). Our research program is a fine balance of both basic and applied research projects aimed at understanding how peptides, proteins and oligonucleotide templates work and how they can be engineered or utilized in developing directed therapies against tumor cells (either through the design of cancer vaccines, RNA/DNA/protein therapeutics or delivery vectors).

The spectrum of approaches taken by our group to address our design and discovery programs ranges from peptide synthesis, combinatorial protein or DNA library design/screening, cell biology/microscopy techniques to yeast genetics and mouse models.

We presently are designing and screening protein libraries as well as SELEX/DNA aptamer libraries with a view to discover new surface markers on epithelial cancer cells and in designing tumor-specific agents. We are also closely involved in constructing new imaging agents for the in vivo detection of millimeter–size tumor masses. Since marker discovery is a major part of our future work and is based on no a priori knowledge of such molecules (we are scanning the surface of cancer cells), we are developing strategies, in collaboration with mass spectrometrists at UHN to identify such novel markers.

Because of the broad nature of our molecular engineering efforts, projects in our laboratory are usually tailored to a student’s expectations and aptitudes. For more information please visit http://www.jeangariepy.com.
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Biology of Central Nervous System Tumours

Our research interests centre on studying signaling complexes relevant to nervous system tumours with the aim of undertaking pre-clinical proof-of-principle experiments, which may translate into effective clinical therapies. We study two tumors: Astrocytomas- The most common primary central nervous system (CNS) tumour (studies described below); Neurofibromas- The most common peripheral nervous system (PNS) tumors.

Our interests are on aberrant signaling pathways involving growth factors, growth factor receptors and downstream signaling pathways that contribute to growth of these tumors. Specifically we are interested in mutant EGFR and aberrant p21-Ras and PI3Kinase mediated signaling complexes. Towards these studies we are using proteomic and transgenic mouse model technologies. These mouse models are used to explore genetic interactions, their role in tumor initiation and progression, discover novel genetic alterations and potential pre-clinical models to test therapies. Variations in the molecular profile of these tumors, as a reflection of the tumor microenvironment and epigenetic regulation, are another area of interest. Specifically, we are interested in variations in regulators of angiogenesis (VEGF, Angiopoietins), invasion (Npn1, Semaphorins, Plexins) and apoptosis, between the central hypoxic and more peripheral normoxic regions of these tumors. We believe this heterogeneity underlies differences in their biological and therapeutic behaviour.

In summary, our research program takes clinical

Genetically Engineered Mouse Astrocytoma Model: Sagittal section of E16.5 transgenic mouse embryo expressing activated 12V-HaRas; IRES-LacZ under control of astrocyte specific Glial Fibrillary Acidic Protein (GFAP) promoter. Blue LacZ staining, denoting expression of the transgene, is restricted to the Central Nervous System (CNS-brain and spinal cord). These transgenic mice are born normally but develop and die over a period of 3-4 months from malignant astrocytomas, which share many of the pathological and molecular similarities found in the most common of all human CNS tumors, the malignant astrocytoma.
neurooncological problems to the laboratory, where a variety of molecular investigations are undertaken to decipher the pathogenesis and function, with research ultimately aimed at developing novel therapies to help patients afflicted with these nervous system tumours.

Selected Publications:

• Kamnasaran, D, Hawkins, C, Guha, A: Characterization and transformation potential of synthetic astrocytes differentiated from murine embryonic stem cells (Glia-in press)
• Kamnasaran, D, Qian, B, Hawkins, C, Stanford, W, Guha, A. GATA6, a Novel Astrocytoma Tumor Suppressor Gene Identified by Gene Trapping from a Genetically Engineered Mouse Model of Astrocytoma: PNAS. 2007 May8;104(19):8053-8058
• Shannon, P, Sabha, N, Lau, N, Gutmann, DH, Guha, A: Pathological and Molecular Progression of Astrocytomas in a GFAP:12V-Ha-Ras Mouse Astrocytoma Model: Am J. of Pathology: Sep 167(3): 859-867, 2005

Molecular Mechanisms Of DNA Damage Repair And Apoptosis And Their Role In Cancer

The main aspect of our research focuses on studying the underlying mechanisms behind cancer development, including the influence of impaired DNA repair responses and/or apoptosis, on the onset of the disease. Although a tremendous amount of information has been gathered on the disease, there still exists a variety of questions that remain unanswered, including the mechanisms behind cancer initiation, progression and tissue specificity. Moreover, although several genes have been identified as either tumor suppressors or oncogenes, there likely exist other cancer genes that have not been identified; be it a cancer suppressing or cancer promoting function.

In our ongoing efforts to study the link between defective DNA damage repair/response and cancer our laboratory has studied both known, as well as novel tumor suppressors, including BRCA1 and Mus81, respectively. Mutations of BRCA1 increase the risk for breast, ovarian and other forms of cancer. Using mouse models, our laboratory has demonstrated that in the absence of Brca1, cells accumulate DNA damage that activates the Chk2-p53 pathway, leading to cell cycle arrest and apoptosis. We have also demonstrated essential roles for the Chk2-p53 pathway in suppressing Brca1- associated cancer. Currently we are investigating the role that apoptosis plays in preventing Brca1-associated cancer.

Mus81, in association with Emel, constitutes an endonuclease that cleaves branched DNA structures such as replication forks (RFs). We demonstrated that cells deficient for
Mus81 or Eme1, exhibit elevated genomic instability and enhanced sensitivity to the chemotherapeutic drug, mitomycin (MMC). Mus81 mutants were susceptible to developing spontaneous lymphomas, and drastically modified the tumor spectrum of p53 mutant mice. Other ongoing studies for Mus81 include the analysis of its functional interactions with Chk2, Rad54, or Blm, and the effects of dual mutations of these genes on DNA damage repair and cancer. We are also currently studying the in vivo function of Pirh2, an E3 ubiquitin ligase that interacts and ubiquitinates p53, leading to its degradation. We have generated Pirh2-deficient mice and are currently investigating the role Pirh2 plays in the regulation of p53, the DNA damage response, and cancer.

Our laboratory also focuses on identifying the mechanisms behind apoptosis. Two major apoptotic pathways exist in mammalian cells: the death receptor pathway and the mitochondrial-mediated apoptotic pathway. We are currently assessing the effect of dual inhibition of the death receptor pathway of apoptosis and inhibition of the mitochondrial-mediated apoptotic pathway on apoptotic responses, development and diseases including cancer.

The overall goal of our lab is to contribute to a better understanding of the mechanisms behind cancer, which can then be translated to improving the response of tumors to radio and chemotherapies.

Selected Publications:


David Hedley, M.D., Ph.D.
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Understanding Molecular Cancer Therapeutics

The development of molecular cancer therapeutics is a complex process that starts with the identification and validation of potential drug targets based on understanding of the molecular mechanisms of human cancers, and then proceeds through the development of novel agents that are tested initially in the laboratory, and eventually in human clinical trials. In many instances the results treating cancer patients are less dramatic than those seen in experimental models. This likely comes about due to a combination of circumstances including low drug target expression in some patients, the presence of additional oncogenic mutations that render the target non-critical for cancer growth, and suboptimal drug dosing. Our laboratory aims to understand the mechanisms of molecular cancer therapeutics action using xenograft models, linked to the analysis of samples obtained during early clinical trials. The eventual goal is a science-driven process that can identify optimum treatment schedules of molecu-
ular targeted agents for individual cancer patients.

The laboratory has a particular interest in pancreatic cancers, which are highly resistant to standard cancer treatments. These cancers have a distinctive set of molecular features including K-ras mutations and overexpression of multiple growth factor receptors that explain their aggressive biology, and provide clues for novel drug targets. Currently we are working on novel drugs targeting PI3-kinase, MEK, Src, FAK, and GSK3β. As well as standard laboratory models, we use primary xenografts derived from pancreas cancer patients. As shown in the figure, when grown in the pancreas of SCID mice these closely simulate the actual clinical situation, and allow detailed study of the effects of novel agents, linked to the underlying genetics of each tumour, in a realistic clinical setting. For monitoring in vivo drug effects, we make extensive use of sophisticated microscopy and flow cytometry methods to study complex cellular processes including effects of microenvironmental factors on tumour response, as well as the microCT, MRI, and PET imaging facilities in the STTARR Program.

Important features of our laboratory are its extensive network of interactions with the clinical trials program as well as basic and translational scientists in the University of Toronto, the application of a wide range of analytical methods, and access to exciting new drugs that are just entering human clinical trials.

Selected Publications


Richard Hill, Ph.D.
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Role of Hypoxia in Solid Tumour Progression & Metastasis

Radiation is one of the primary modalities for the treatment of localized cancer and a number of factors can influence the response of tumours and surrounding normal tissues to such treatment. These factors, which can be specific to the individual tumour or normal tissue and to their environment, can vary from patient to patient. One part of the research in our laboratory focuses on understanding how these factors influence tumour and normal tissue response to radiation treatment in individual patients. Our current work involves:

1) Examination of hypoxia and high interstitial fluid pressure (IFP) in animal models of human tumours with a focus on cancer of the cervix. In these studies we are collaborating with the clinical groups at the Princess Margaret Hospital in examining methods to exploit these factors to predict and improve treatment outcome.

2) Studies of the radiosensitivity of normal dermal fibroblasts in vivo. These studies are focusing on measuring DNA damage as a biological dosimeter for potential use in individuals who have been accidentally exposed to irradiation or for assessment of differences between in vivo and in vitro radiosensitivities of fibroblasts from patients with soft tissue sarcoma who demonstrate significant complications following combined radiation therapy and surgery.

3) Examination of the sensitivity of lung tissue to different volumes of irradiation. These studies in rat and mouse lung are investigating mechanisms associated with the response of the lung to radiation damage and drugs that may be useful to mitigate the long term effects of this damage on lung function, when applied after the radiation exposure.

The second major focus of our research is the spread of...
Fluorescent imaging of tumour growth and spread. Fluorescent cells can be used to study the spread of disease to organs, such as lung (left). Cells that emit light of different colours can be used to track specific populations of cells. The image shows a mouse lung bearing tumour colonies 2 weeks after an intravenous injection of red and green cells. Hoechst (blood perfusion) is in blue.

cancer from its initial site of growth to other locations in the body (metastasis), which is a major factor influencing the likelihood of successful treatment. The formation of metastasis by tumour cells is thought to be dependent on the expression of specific phenotypes by individual tumour cells. Our research is examining metastatic phenotypes that are expressed only transiently and that may be induced by exposure of tumour cells to conditions, such as hypoxia, which occur in the tumour microenvironment. Recent clinical results have suggested that tumours that contain substantial hypoxic regions may be more likely to form metastases. We have found in animal model systems that exposure to hypoxia, both in vitro and in vivo, can cause transient increases in the metastatic potential of tumour cells and that exposure to transient hypoxic episodes may be particularly important for this increased metastatic potential. We are examining the effect of different levels of hypoxic exposure and of exposure to intermittent hypoxia in modifying the expression of genes likely to be associated with metastasis and tumour progression in xenograft models of human cervix carcinoma and soft tissue sarcoma. We are also initiating studies to examine the role of hypoxia in modifying or maintaining the aggressive phenotype of tumour stem cells.

**Selected Publications:**


**Mitsuhiko Ikura, Ph.D.**

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**Structural Biology and Cell Signalling**

Creating and maintaining tissue organization requires strict control of three processes governed by cellular signalling: cell division, differentiation and growth. Cells which escape from “normal” controls, and proceed along a path of uncontrolled growth and migration, often lead to cancer. To this end, we are investigating the structure-function relationships of key signalling proteins by various biochemical and biophysical methods, including nuclear magnetic resonance (NMR) spectroscopy, X-ray crystallography, and fluorescence resonance energy transfer (FRET) imaging microscopy. NMR and X-ray crystallography enable us to determine the three-dimensional atomic structures of proteins and protein complexes. FRET allows for visualization of the dynamic behavior of signalling proteins in living cells.

Currently our research focuses on proteins involved in calcium signalling, cell adhesion, transcriptional regulation and bacterial signal transduction. We are part of the newly funded research program on Genomic Instability and Cancer Survival at the Advanced Medical Discovery Institute within OCI. This program will enhance our research activities in the area of cancer. In 2006 a state-of-art Untra-Shielded 800-MHz NMR instrument has been installed, which is equipped with a high-sensitivity triple-resonance cryogenic probe. This instrument is crucial for the investigation of larger proteins which are soluble only at low protein concentrations. Many cancer-related proteins fall into this category of targets, including transcriptional regulators, metabolic enzymes and regulators, and various cancer signaling proteins. Linking detailed structural analysis with molecular and cellular functional analyses of cancer proteins, we hope to make marked contributions to the fight against cancer.

3D structural information of proteins can also provide useful clues in designing new therapeutic compounds to specifically inhibit certain protein activities. In the coming years, we hope to determine 3D structures of more protein-protein complexes by NMR or X-ray crystallography to visualize, via FRET imaging microscopy, specific protein-protein interactions in various cells including tumour cells.

For further information, visit our laboratory website at:  
http://nmr.uhnres.utoronto.ca/ikura

**Selected Publications:**

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Specification of Hematopoietic Stem Cell Identity

Adult stem cells maintain proliferating cellular systems for life. They do this by generating not only cells primed to enter differentiation pathways, but also cells that instead retain the undifferentiated stem cell characteristics of the parent.

Generation of new stem cells is called "self-renewal". We are interested in two aspects of self-renewal. The first concerns the genes that come into play when stem cells undergo self-renewal divisions. The second concerns the means by which some initially renewing daughter cells lose self-renewal capacity while others continue to retain it through multiple cell divisions. We use the hematopoietic system to model these processes. Our findings have led us to focus increasingly on particular homeobox genes as central effectors of self-renewal divisions, and on the genes that control their expression as specifiers of stable stem cell identity in this system.

Selected Publications:

Michael Julius, Ph.D.
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Signal Transduction in Lymphocyte Activation

Our work over the last decade has demonstrated that the modifications of signals emanating from the T cell antigen receptor complex (TcR/CD3) mediated by accessory activation molecules (AAM) including CD4/8, CD45, CD28 and glycosylphosphatidylinositol anchored proteins (GPI-AP) can be extreme, resulting in perturbation of cell growth control, anergy, or death. Our global objectives are to: characterize the ordered interaction of AAM with TcR/CD3; define the sequence and temporal engagement of the second messenger generating systems engaged; and to characterize the TcR/CD3/AAM induced spatial re-distribution of second messenger generating systems supporting cellular activation and growth. Our approach utilizes primary T cells; T cell clonal variants and transgenesis: enabling a genetic analysis and facilitating the biochemical characterization underpinning the signaling phenotype.

The AAMs CD4 and CD8 play fundamental roles in the initiation of the earliest signals induced upon TcR/CD3 engagement through their regulation of activity and delivery of function of Src family PTK. We have discovered the interdependent temporal and spatial regulation of Lck and Fyn during proximal TcR signaling. Specifically, in primary CD4+ peripheral lymph node T cells lipid rafts (LR) function to segregate Lck and Fyn. Upon antigen receptor engagement, Lck is activated within seconds outside LR, followed by its translocation into LR and the ensuing activation of co-localized Fyn.

Genetic models and structure function analyses have enabled the formal demonstration that Fyn activation is predicated by its physical interaction with kinase active Lck within LR, and revealed a c-terminal sequence common to all members of the Lck subfamily of Src family PTK that predicates their partitioning to LR.

Our current working model proposes a biological framework based on structural distinctions amongst Src family subfamilies that rationalizes the involvement and regulation of function of multiple Src-family PTK reported to be critically involved in a variety of receptor-mediated signaling pathways.

Selected Publications:
Merely coping with the deluge of data is no longer an option; their systematic analysis is a necessity in biomedical research.

Computational biology is concerned with developing and using techniques from computer science, informatics, mathematics, and statistics to solve biological problems. Analyzing biomedical data requires robust approaches that deal with high dimensionality, multi-modal and rapidly evolving representations, missing information, ambiguity and uncertainty, noise, and incompleteness of domain theories.

Our research is focussed on integrative computational biology, and representation, analysis and visualization of high dimensional data generated by high-throughput biology experiments, in the context of cancer informatics. Of particular interest is the use of comparative analysis for the mining of integrated different datasets such as protein-protein interaction, gene expression profiling, and high-throughput screens for protein crystallization. We integrate multiple data sources and database, develop and apply combination of diverse algorithms in a systematic manner. Besides performance, our focus is on scalable algorithms with easy use by non-experts.

Truly understanding biological systems requires the integration of data across multiple high-throughput platforms. It has been established that despite inherent noise present in protein-protein interaction data sets, systematic analysis of resulting networks uncovers biologically relevant information, such as lethality, functional organization, hierarchical structure, dynamic modularity and network-building motifs. These results suggest that protein interaction networks have a strong structure-function relationship, which we use to help interpret integrated cancer profile data. Focusing on network analysis and modeling, integrated with cancer profiles will enable us to identify diagnostic and prognostic biomarkers, understand disease initiation and progression, which will lead to improving cancer treatment. Tools, such as BTSVQ, I2D (http://ophid.utoronto.ca/i2d), and NAViGaTOR (http://ophid.utoronto.ca/navigator) enable users to interpret integrated cancer profiles, and create relevant models dynamically. Many targets discovered using approaches described above will lead to uncharacterized proteins. To further our understanding of their function, we may use X-ray crystallography or NMR to determine their 3D structure.

Protein crystallization is a major bottleneck in high-throughput structure determination, partially due to many parameters affecting the crystallization outcome (e.g., purity of proteins, intrinsic physico-chemical, biochemical, biophysical and biological parameters), and the unknown correlations between the parameter and the propensity for a given protein to crystallize. Protein crystallization has two phases: search and optimization. High-throughput screening (HTS) can speed up the search phase, and has the potential to increase process quality. To achieve these benefits, we work on developing a sophisticated automated image classification algorithms, data mining and reasoning approaches to optimize protein crystallization plans, improve our understanding of the crystallization process, and increase a number of determined disease-related protein structures (http://www.cs.toronto.edu/~juris/WCG/).
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Identifying Biomarkers of Cancer Initiation, Progression and Recurrence

Work in our laboratory focuses on the application of high throughput technologies – for example, gene expression microarrays, tissue arrays, ChIP-on-chip, microRNA arrays and protein arrays – to enhance our understanding of cancer biology. Specifically, we are interested in identifying biomarkers of cancer initiation, progression and recurrence in two types of human cancer: Head and Neck Squamous Cell Carcinoma (HNSCC) and Acute Promyelocytic Leukemia (APL).

**Head and Neck Squamous Cell Carcinoma**

In this arm of our research laboratory, we are specifically interested in the etiology of oral cancers (OSCCs). The molecular genetic changes involved in oral cancer development are poorly understood. Our work focuses on three major questions:

1. **What are the gene(s) involved in recurrence of oral cancer?**
   Approximately 50% of OSCCs recur after surgery. Our current approaches involve analyzing OSCC tumour samples, as well as samples taken from the regions immediately surrounding the tumours, at time of surgery (“surgical resection margins”), in order to identify genes deregulated in the tumour and in the surrounding margin(s), which may be predictive of tumour recurrence.

2. **What are the steps involved in the development of oral cancer?**
   A significant fraction of OSCCs arise from precursor lesions called leukoplakias. In these studies, we are interested in profiling the genetic changes – specifically, changes in microRNA expression – in leukoplakias and comparing them to genetic changes found in tumours.

3. **What are the underlying genetic differences between OSCCs in young patients (< 45 yrs of age) and older patients (> 45 yrs of age)?**
   OSCCs are strongly associated with the risk factors of alcohol consumption and smoking tobacco. However, younger patients who do not exhibit exposure to either of these two risk factors are still diagnosed with OSCC. We have observed defective DNA mismatch repair and differential gene expression in young patients compared to older patients. This line of study is aimed at further understanding the genetic differences between young and older patients, and at understanding the role of defective DNA repair in OSCCs.

**Acute Promyelocytic Leukemia**

Leukemias are often associated with chromosomal translocations that give rise to fusion proteins which may deregulate cellular signaling or transcription. Our group cloned and characterized two variant fusion proteins involved in APL, NPM-RARα and NuMA-RARα, which are both aberrant transcription factors; we are especially interested in understanding the roles of these fusion proteins in the cell. Specifically, we use cell lines,
mouse models and human patient samples in order to understand leukemia biology. The questions we are most interested in include:
1.) **What are the common downstream genetic targets of the APL fusion proteins?**

APL is associated with seven known fusion proteins, all of which involve the RARα transcription factor. Yet, all fusion proteins give rise to the same disease. We hypothesize that this is because all fusion proteins have a common set of direct transcriptional targets, and are utilizing gene expression arrays and ChIP-on-chip technology to address this issue.

2.) **What are the cell biology effects of the APL fusion proteins?**

While most studies have focused on the APL fusions as transcription factors, more recent evidence has suggested that these proteins behave distinctly and have effects through protein-protein interactions on other signaling pathways within the leukemic cell. We are applying the same concept as above, and hypothesizing that there may be a common set of interactions and/or deregulated pathways shared by all APL fusion proteins, and are characterizing the protein-protein interactions of the fusions in order to find this out.

3.) **What are the necessary secondary events in leukemia development?**

Previous studies have indicated that the APL fusions are necessary, but insufficient, for the development of leukemia in mice, suggested that additional genetic “events” may cooperate with the fusions in leukemia. We are addressing this question through the above studies, and also through genetic studies of the hCG-NuMA-RARα mouse model that we previously developed and characterized.

**Selected Publications:**


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**Gordon Keller, Ph.D.**

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**Lineage Specific Differentiation of Embryonic Stem Cells**

The overall goals of our research program are to understand the mechanisms that control mesoderm and endoderm induction and specification in mouse and human embryonic stem (ES) cell cultures. Our studies focus specifically on the generation of the following derivative lineages: hematopoietic, vascular, cardiac, hepatic and pancreatic. Research projects include:

1.) characterization of the earliest developmental stages of lineage commitment  
2.) identification of the signaling pathways regulating lineage specification and maturation, and  
3.) generation of functional cell populations for transplantation in preclinical models of human disease.

For the mesoderm-derived lineages we have demonstrated that the earliest stages of hematopoietic and cardiac development in ES cell cultures are defined by the appearance of progenitors that display both tissue specific and vascular potential. Within the hematopoietic system, these progenitors are known as hemangioblasts whereas those that define the onset of cardiac development are known as cardiovascular progenitors. Our current studies are aimed at defining the mechanisms that control the proliferation and differentiation of these progenitor populations. The long-term goal of these transplantation studies is to develop new therapies for the treatment of hematopoietic and cardiovascular diseases. With respect to endoderm derivatives, we have shown that the combination of activin A, BMP-4 and bFGF will lead to the efficient generation of cells with characteristics of immature hepatocytes. Modification of this induction protocol results in the development of pancreatic progenitors. Our current research projects in this area are focused on understanding the pathways that regulate the maturation of these populations into functional hepatocytes or insulin-secreting pancreatic beta cells. Access to ES cell-derived hepatocytes and beta cells will provide a novel source of these cells for cell based therapy approaches for the treatment of diabetes and certain types of liver disease.

**Selected Publications:**


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**Dept Medical Biophysics, University of Toronto**  
Cell, Molecular and Structural Biology
Perturbing Angiogenic Pathways as a Novel Therapeutic Modality

Our research is focused in the area of tumor angiogenesis and antiangiogenic therapy. In this regard, one of the most significant developments in medical oncology over the last five years has been the approval of three different antiangiogenic drugs for the treatment of a number of human cancers, e.g. breast, colorectal, renal, and hepatocellular carcinoma. This includes bevacizumab (Avastin), the humanized anti-VEGF monoclonal antibody which is now undergoing evaluation in almost 400 clinical trials around the world. Despite the successes of antiangiogenic drugs, including bevacizumab, remarkably little is known about how these drugs actually work as anti-cancer agents. The reason for the counterintuitive property of an antiangiogenic drug enhancing the efficacy of chemotherapy is unknown and is a subject of investigation in our laboratory as well as a number of others around the world. In addition, the best way to administer chemotherapy with an antiangiogenic drug remains to be determined. Another major question in the field is how to determine the optimal biologic dose for antiangiogenic drugs and to monitor their activity in vivo.

The Kerbel group is studying the hypothesis that conventional chemotherapy can induce remarkably rapid and marked mobilizations of a proangiogenic cell population from the bone marrow compartment, known as endothelial progenitor cells, which can subsequently home to drug treated tumors and accelerate their recovery from the initial chemotherapy treatment. This reactive host response can be blocked using certain antiangiogenic drugs. The molecular pathways by which these processes occur is under intensive investigation in Dr. Kerbel’s laboratory.

Dr. Kerbel is also pioneering a concept known as ‘metronomic’ chemotherapy (where chemotherapy is given at close regular intervals at low doses with no prolonged drug-free breaks) as an exciting and novel new way to combine chemotherapy with a targeted antiangiogenic drug such as bevacizumab, for the treatment of advanced metastatic disease. We have developed a number of new models of advanced metastatic disease in immune deficient mice involving human tumor cell lines, including breast, ovarian, and hepatocellular carcinoma, as well as malignant melanoma. These studies have evolved in a new research initiative, namely, the development of models of spontaneous brain metastases and their use to study the biology and treatment of such lesions. Finally, our group is at the forefront of studying circulating cellular and molecular surrogate biomarkers in peripheral blood for antiangiogenic drugs and/or metronomic low-dose chemotherapy which can help determine the optimal biologic dose to be used for such drugs/treatments.

Selected Publications

Tissue Homeostasis and Cancer Development

The complex microenvironment contains distinct entities including the structural extracellular matrix, anchored and soluble growth factors and cytokines, and a variety of immune and inflammatory cells. Signals generated within this space dictate cell fate by influencing cell proliferation, differentiation, motility, and cell death. We aim to understand how proteolysis (MMP/TIMP/ADAM), specific growth factors (IGF-II, HGF, TNF), and tumor suppressors and oncogenes impact these signals in tissue homeostasis and tumorigenesis. We use genetic mouse models of human cancers and disease to study the cellular and molecular basis of breast, liver, lung, and bone cancers, and also investigate the role of proteolytic systems in tissue homeostasis in models of heart disease, inflammation and tissue regeneration. A central hypothesis for our work is that cellular microenvironment converges at the cell surface to trigger intracellular signaling pathways and influence cell fate.

In the past two years, we identified TIMP3 as an innate negative regulator of systemic inflammation. Its loss not only increases the proteolytic activity of ADAM17, but also of MT1-MMP known to be central to cell motility and invasion. Multiple organs of timp3-/- mice exhibit greater metastatic dissemination to several cancer cell types. We have also found that Rho C and RANKL are critical for metastasis. Using primary cultures derived from timp3 deficient mice we have shown dysregulated -catenin signaling with specific effects on its target genes (cyclin D1, MMP7) in mammary epithelial and embryonic fibroblasts; and with primary cultures of neonatal cardiac myocyte and fibroblast we have identified a novel concurrent amplification of TGF and TNF signaling due to increased proteolytic activity.

Our ongoing work on TIMP4 in heart disease reveals a differential function for this TIMP following myocardial infarction and pressure overload. Some of our recent discoveries in mouse models are particularly exciting as they hold a high potential for clinical impact, both in breast and bone tumors. A new avenue of our research involves the development of innovative imaging techniques to probe and visualize collagen changes in vivo in mouse models of human diseases.

Selected Publications:

- Murphy G, Murthy A, Khokha R. Clipping, shedding and RIPping keep immunity on cue. Trends Immunology, in press.
Thomas Kislinger, Ph.D.
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Proteome Research

The human genome has been sequenced and now sophisticated technologies are used to characterize the proteome, the set of proteins generated by that genome, as a function of developmental stage, environment, tissue-type, and pathology. The goal of our research is to understand, on a systems level, the complex biological processes that are fundamental to physiology and disease. A particular focus of our laboratory is cancer research (ovarian and breast cancer) and vascular/cardiovascular biology.

Our laboratory develops and applies powerful tools of proteomics and bioinformatics to analyze the protein complement in protein complexes, cells, tissues and organisms. Our goal is not only to determine the level of proteins but also their localization, dynamics, and interaction partners. In recent years we have developed sensitive methodologies to isolate specific subcellular fractions from mammalian cell culture or tissues.

Currently our focus is on optimizing technologies that allow for the sensitive and selective isolation and characterization of surface, plasma membrane proteins from cell culture (in vitro) or directly from the surface of microvascular endothelial cells (in vivo). The ultimate goal of these studies is to investigate the proteome dynamics of surface membrane proteins in the vasculature of disease models or in cell culture in response to specific treatments. Additionally, we are interested in dynamics of protein-protein interactions. To accomplish this goal we are applying tandem affinity purifications (TAP-tagging) in combination with mass spectrometry. Briefly, the protein of interest (bait) is expressed as a fusion protein with a dual affinity tag. This allows for mild and efficient purification of this protein and most of its interacting partners.

Our laboratory is well equipped and currently has full-time access to two latest generation mass spectrometers, a linear ion-trap (Thermo Scientific LTQ) and the new Thermo Scientific LTQ-Orbitrap XL, a high resolution, high mass accuracy hybrid mass spectrometer. These mass spectrometers are currently the standard for global, high-throughput proteomics. Additionally, we have access to a new triple quadrupole mass spectrometer for target driven proteomics and accurate quantification.

Selected Publications:


Anne Koch, M.D., Ph.D.
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DNA damage signaling, repair and cancer

DNA double-strand breaks (DSBs) represent the most lethal form of DNA damage. DSBs can result from exposure to exogenous genotoxins, such as ionizing radiation (IR) and chemotherapeutic drugs, or as a consequence of intrinsic cellular processes such as DNA replication and metabolic processes that generate DNA-damaging free radicals. If left unrepaired, DSBs can result in the loss of genetic material and DSBs are an obligate intermediate of numerous types of gross genetic aberrations such as chromosome translocations. Given the critical importance of DSBs in cancer etiology and therapy, the study of the cellular response to DSBs is of utmost importance to cancer biology. In vertebrates, there are two principal DSB repair pathways: nonhomologous end joining (NHEJ) and homologous recombination (HR). HR acts primarily after DNA replication while DSBs are an obligate intermediate of numerous types of gross genetic aberrations such as chromosome translocations. Given the critical importance of DSBs in cancer etiology and therapy, the study of the cellular response to DSBs is of utmost importance to cancer biology. In vertebrates, there are two principal DSB repair pathways: nonhomologous end joining (NHEJ) and homologous recombination (HR). HR acts primarily after DNA replication where it accurately repairs the DSB using the undamaged sister-chromatid as a DNA template. On the other hand, NHEJ is active.
throughout the cell cycle, predominating during G0 and G1, is error-prone and uses little or no sequence homology to repair DSBs. NHEJ is the major pathway involved in the repair of DSBs induced by DNA damaging agents, such as IR or radio-mimetic drugs. By virtue of its importance in promoting repair of DSBs caused by therapeutic IR and anti-cancer drugs, the study of NHEJ is germane for the development novel anti-cancer therapies.

Our laboratory is focused on studies examining the complex regulation of the DNA damage response and how its deregulation relates to carcinogenesis. Some of our specific research interests include the identification and characterization of novel DNA damage signalling pathways and repair factors involved in the cellular response to IR-induced DNA damage. We are also interested in the detailed examination of these pathways, and how they may be perturbed, in the context of human breast cancer.

Selected Publications:

Our current aim is to determine how these novel interacting proteins interact with the receptors, mediate signaling and engage in cross-talk with known pathways. We have generated mouse models of HHT and pulmonary arterial hypertension, which are useful in determining the protein interactions and pathways affected in these diseases. Since TGF-ß is a critical contributor to several cancers, we are studying the possible role of some of the novel receptor interacting proteins in ovarian cancer. Our primary focus is on molecular changes associated with early events in the disease. We are comparing ovarian epithelial cells derived from women with BRCA mutations to those of normal women in an attempt to delineate early changes caused by these mutations and potentially contributing to cell transformation. Our work may lead to the identification of early markers needed for the early diagnostic of this devastating cancer.

Selected Publications:


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Translational Research on Breast & Head & Neck Cancer

Our lab is focussed on several different aspects of translational molecular oncology, ranging from high-throughput screens to identify novel anti-cancer therapeutics, to developing predictive gene sets for human breast and head/neck cancers, to cancer stem cell investigations. Using robotic high-throughput screens, we have identified novel anti-cancer activities of existing antimicrobials. We are now pursuing the identification of novel radiosensitizers to enhance the anti-cancer properties of ionizing radiation. Using diagnostic human cancer samples, and extracting RNA, we are in the process of conducting global expression analysis of micro-RNA, a novel genetic species, which appears to be a powerful modulator of gene and protein expression in biological systems. Preliminary data are very promising, and in addition to identifying predictive signatures for improving cancer patient management, we are also studying their biological function in preclinical models of human cancers. Finally, we are also interested in understanding how hematopoietic stem cells traffic in response to ionizing radiation, in order to better understand how local radiation therapy contributes to secondary acute myeloid leukemia, a fatal iatrogenic complication in cancer patients.

Selected Publications:


Our laboratory is studying the behaviour of cancers of the aerodigestive tract (esophageal cancer, head and neck cancer, lung cancer) as well as orphan thoracic tumours such as thymoma and mesothelioma. As a unique translational epidemiologic laboratory within MBP, we are focused on discovering novel molecularly-based methods for risk stratification, prognostication, and treatment of patients.

Genetic and environmental influences are interwoven into the fabric of cancer prognosis. Our laboratory performs human population-based research, using candidate gene approaches, pathway-based approaches, and high density SNP technology to identify the potential biologic pathways involved in esophageal cancer development. Discovery of putative pathways becomes the first step in narrowing the list of potential environmental carcinogens. We are currently developing customized high density SNP-chips for assessment in aerodigestive cancers, and collaborating with computational biologists on their analysis.

These same genetic and environmental factors can play critical roles in cancer behaviour after diagnosis, and in response to therapy. Our laboratory is studying pharmacogenomic factors in cancer treatment, both in terms of traditional chemotherapeutic agents as well as molecular targeted agents. We recently assessed the role of several functional polymorphic variants of EGFR in lung cancer patients treated with an EGFR-tyrosine kinase inhibitor, and found that these polymorphic variants predict survival and treatment response. Another focus in our laboratory is the assessment of putative serum biomarkers to help monitor patient therapy and response. In addition to human population studies, pre-clinical models of esophageal and gastric cancers are being developed in our laboratory. These resources are being used to assess genotype-phenotype correlations, testing of molecular targeted drugs and novel therapeutics, and for pre-clinical testing of biomarkers.

Currently, we are assessing biomarkers and genetic variations in biologic samples obtained from the following large scale studies: Lung Cancer Screening Study; Mesothelioma Screening Study; Molecular Epidemiology of Lung, Esophageal Cancer, Thymoma and Mesothelioma, a series of US-based studies of esophageal, lung and pancreatic cancer), and Head and Neck Cancers from PMH. In addition, our laboratory is involved in the biologic assessments of a number of Phase I/II/III cancer treatment studies. We are and have been CIHR, NCI (US), NCIC-CTG, and Doris Duke funded, with close collaborations with Harvard, Laval, and Seattle.

Selected Publications:


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Genetic Determinants of Childhood Cancer Predisposition

Almost every type of cancer has been reported to occur in a familial form. Li-Fraumeni syndrome (LFS) is a paradigm cancer predisposition syndrome in which affected family members develop a wide spectrum of cancers including sarcomas, breast cancer, brain tumors and adrenocortical carcinomas at very young ages. Many affected individuals develop multiple cancers during their lifetime and these cancers frequently occur at ever younger ages with each subsequent generation. We have previously found that constitutional (inherited or de novo) mutations of the p53 tumour suppressor gene are associated with cancer predisposition in many of these families, as well as in patients with multiple primary cancers, and children with osteosarcoma, rhabdomyosarcoma, choroid plexus carcinoma or adrenocortical carcinoma in the absence of a family history of cancer.

Our research focuses on the genetic basis of LFS, and particularly on the epigenetic and molecular cellular events that modify the underlying constitutional mutant p53 genotype in LFS families. We have recently demonstrated that accelerated telomere attrition measured in peripheral blood lymphocytes is associated with accelerated tumor onset in successive generations, and that this early onset is further influenced by the existence of a variable single nucleotide polymorphism (SNP) in the hMDM2 gene. We are now examining the role of telomere dysfunction and telomerase function in the context of constitutional p53 genotypes, as well as in specific subsets of LFS tumors. We are also
utilizing high-throughput genome-wide array analyses to explore the interaction of other elements of the human genome in modulating the phenotypic variations found in LFS in the context of distinct underlying p53 alterations. Our goal is to develop an understanding of the underlying genetic basis of cancer predisposition in these cancer-prone individuals, and to eventually develop a molecular metric that may inform clinical surveillance opportunities for early tumor detection.

To complement our genetic approach to LFS, we are exploring the cell signaling mechanisms involved in the initiation and progression of rhabdomyosarcoma (RMS) – a tumor derived from immature skeletal muscle elements that represents the most common childhood cancer in LFS, and the most common sporadic soft tissue sarcoma of childhood. We have previously demonstrated that RMS cells express vascular endothelial growth factor receptor (VEGFR) and are responsive to VEGF ligand. Inhibition of this ligand-receptor interaction leads to growth inhibition of RMS cells. We are interested in determining the functional interaction of this kinase pathway with other p53-dependent and independent signaling mechanisms in RMS. Characterization of these functional pathways may lead to the identification of novel molecular therapeutic targets for these aggressive malignancies.

Selected Publications:

- Strahm D, Durbin AD, Sexsmith E, Malkin D. The CXCR4-SDF1 axis is a critical mediator of rhabdomyosarcoma metastatic signaling induced by bone marrow stroma. Clin Exp Metastasis Sep 1, 2007. [epub ahead of print]

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Understanding PKB Function via Drosophila Genetics

Our laboratory is interested in signal transduction pathways which regulate Protein Kinase B (PKB) and Glycogen Synthase Kinase-3 (GSK-3). These serine/threonine kinases are involved in multiple signaling pathways and have been shown to be linked to a variety of human diseases. For example, the hyperactivation of PKB has been shown to be involved in all stages of tumorigenesis. We use a combination of genetic, biochemical, cell biological and pharmacological approaches to the study of the cellular processes which require the function of these kinases. In order to gain insight into the mechanism of action of these enzymes, we use Drosophila melanogaster as a genetic model system. Our laboratory initiated these studies via identification of “loss of function” alleles of Drosophila PKB. We have used this allele as a tool for second site genetic modifier screens to identify many novel components of PKB function as well as identifying novel roles for PKB during development. Recently, we have used Drosophila as a drug discovery model in the identification and design of novel small molecule therapeutics using genetic tools in Drosophila. Our goal is to combine the powerful genetics of Drosophila with pharmacology to fast track the process of drug discovery. Using our approach, we have also linked GSK-3 to the regulation of the circadian clock in Drosophila and in mammalian cells. This work has provided provocative links between GSK-3 function and bipolar therapeutics including lithium. We will be using our genetic and pharmacogenetic tools in Drosophila to further explore the “mechanism of action” of these therapeutics in the design and identification of novel therapeutics.

Selected Publications:

Regulation of gene expression in endothelial cells

Current work in our lab focuses upon newer aspects of the transcriptional and post-transcriptional regulation of endothelial gene expression in health and disease. Exciting new information indicates that chromatin structure and DNA methylation play a key role in the cell-restricted expression of important endothelial genes. Furthermore, RNA binding proteins and antisense RNA interactions also play an unexpected role in regulation of endothelial gene expression, in part, through modulation of RNA stability and translational efficiency. We suspect that these pathways may involve RNA interference. Epigenetic pathways and RNA interference are newer concepts in the regulation of genes in the cardiovascular system.

Models of Endothelial Activation

The study of endothelial cells has provided unique insight into important cardiovascular diseases and the control of angiogenesis during tumour development. The control of new blood vessel formation, or angiogenesis, is orchestrated by vascular endothelium and endothelial cells respond to unique signals in their environment with a repertoire of cellular and molecular responses. Studies directed towards dissecting the molecular mechanisms underlying alterations in genotype and phenotype are underway using prototypic endothelial cell gene products (e.g. endothelin-1, eNOS, CXCR4, adhesion molecules such as VCAM-1 or ICAM-1) and exciting models of cellular activation (hypoxia, shear stress and epigenetic modifiers). An excellent example of the applicability of this approach is our recent finding that verotoxins, bacterial-derived exotoxins that cause severe inflammation of capillary beds in patients with E coli 0157:H7, regulate the expression of genes in vascular endothelium at the post-transcriptional level through AUF1 and proteasomal pathways.

Regulated expression of nitric oxide (NO) in vascular tissue - role of epigenetics.

Release of NO from the vascular endothelium represents a sensitive and highly effective local system for maintaining local blood flow to an organ. This research program played a major role in the cloning and functional characterization of endothelial NO synthase (eNOS) cDNAs and in the demonstration that expression of this endothelial gene is perturbed in atherosclerosis. Recent studies seek to define the structure and organization of the eNOS gene and examine mechanisms of regulation that are relevant to the pathobiology of endothelial cells. Current work focuses upon newer aspects of the transcriptional and post-transcriptional regulation of eNOS mRNA in development and disease.

Selected Publications:

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Etiology and Prevention of Breast Cancer

Our research focuses on the etiology and prevention of breast cancer with an emphasis on mammographic density. Mammographic density refers to a pattern of breast tissue composition (as seen on a mammogram) that is strongly associated with the risk of developing breast cancer, is common in the population, and is highly heritable. An understanding of the etiology of mammographic density, and how it is related to breast cancer risk, is likely to lead to effective preventative interventions for breast cancer. Our research uses an epidemiological approach that includes the study of diet and lifestyle factors, as well as biochemical, genetic and molecular factors measured in a variety of biological samples.

We have recently completed a long-term, multi-centre clinical trial to determine if a low-fat diet will reduce the incidence of breast cancer in women with extensive mammographic density (in collaboration with Dr. Boyd, Principal Investigator). The analysis of the data from this trial is currently underway. Future work will include an examination of the effects of diet on mammographic density, blood hormones and growth factors as well as breast tumour characteristics.

Additional studies are investigating the relationship of mammographic density with markers of oxidative stress/inflammation and with blood telomere length. We have previously shown that increased urinary malondialdehyde, a marker of lipid peroxidation, is associated with increased mammographic density. We will extend this observation by studying more specific measures of lipid peroxidation (eg. urinary isoprostanest) and markers of inflammation produced from arachidonic acid via the COX-2 pathway (eg. urinary prostaglandin E2).

Telomeres, which cap the ends of chromosomes and promote their stability, become shorter with cell replication and exposure to oxidative stress. Shorter blood telomere length has been associated with higher risk of some types of cancer. Currently we are examining whether blood telomere length is associated with mammographic density and other risk factors for breast cancer. We have set up a quantitative PCR technique to facilitate measurement of blood telomere length in large epidemiological studies. This work may suggest a novel way in which mammographic density is related to breast cancer and motivate future studies to examine whether blood telomere length is a marker of breast cancer risk.

Selected Publications:


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Signal Transduction in Normal Development and Disease.

Our research focus is on the protein networks and signal-transduction pathways that control normal cellular function and human disease. Through identification and functional characterization of intracellular adapter proteins we study the molecular mechanisms that control how signal transduction pathways are integrated, localized, and down regulated. We have discovered and characterized new adapters such as Gads and SLAP/SLAP2 that function in growth factor and antigen-receptor signaling, as well as the adaptors NUMB, EHD and Lulu/Ymo1, which regulate the activity of trans-membrane receptors that determine cell fate and polarity during development. Our lab is also studying the role of ubiquitin dependent regulation of signaling pathways. We have identified a family of novel E3 ubiquitin ligases, including LNX, and our current focus is to discover the role of LNX activity.

Variations in mammographic density
in cell fate determination and the establishment of cell polarity during embryonic development. As part of both of these projects we are developing high throughput assays of signal transduction and protein degradation pathways using robotics, protein arrays and high throughput imaging systems (www.sickkids.ca/sidnet). More information can be found at http://www.sickkids.ca/mcglade

Selected Publications:


Phosphorylation controls asymmetric localization of the cell fate determinant Numb. Numb protein (green) lacking two protein kinase C phosphorylation sites (Numb2A) is symmetrically rather than asymmetrically localized in polarized epithelial cells as shown above in optical sections taken in the z-axis. Polarized MDCK cells were fixed and co-stained with anti-aPKC (red) and anti-ZO-1 (blue) which mark the sub-apical region and the tight junction respectively. Confocal x-y sections show (I) the sub-apical localization of aPKC and (II) the basal-lateral accumulation of Numb. Size bar indicate 10 microns.

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Gene Transfer/Therapy projects to generate specific immune responses against cancers and to correct inherited / acquired diseases

We employ the latest-generation of recombinant lentiviral gene transfer vectors to deliver important transgenes into target hematopoietic cells and other tissues. We also have generated novel vectors that incorporate advanced ‘suicide’ safety systems based on modified human enzymes and unique prodrug combinations that can be used to control the fate of any vector-transduced cell in order to maximize safety after transplantation into both usual and innovative niches. Our work thus involves the full-spectrum from biochemistry to subcloning/site-directed mutation of interesting factors to construction of novel recombinant gene transfer vectors to testing outcomes in vitro to testing outcomes in vivo in small and large animal models. In addition, we are licensing vectors we have generated to a company for preparation of clinical-grade supernatants for Phase I trials.

Selected Publications:


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Signal transduction in stems cells and disease

Our laboratory studies cell signaling, with a particular emphasis on protein-tyrosine phosphatases (PTPs). We also have a developing interest in normal and tumor stem cell signaling. The roles of the SH2 domain-containing phosphatase Shp2 and its binding proteins in several human diseases are a major focus. Shp2 is expressed ubiquitously and is a positive signal transducer, required for Ras/Erk activation downstream of most receptor tyrosine kinases, cytokine receptors, and integrins. Shp2 is required for a variety of developmental processes, including the survival of trophoblast stem cells. As a consequence of loss of the latter function, Shp2-null embryos die peri-implantation. In the absence of an appropriate phosphotyrosyl peptide, Shp2 is inactive, because the N-SH2 domain is inserted into the catalytic cleft of the phosphatase (PTP) domain. We showed earlier that mutations in the SH2/PTP interface can yield "activated mutants". Analogous mutations in humans cause 50% of cases of Noonan Syndrome (NS). Interestingly, another autosomal dominant disorder, LEOPARD syndrome, is also caused by Shp2 mutations, but surprisingly, we showed recently that such mutations are catalytically inactive/impaired and act as dominant negative mutants in transfection assays. We have generated an allelic series of inducible Shp2 knockout mice and cell lines.

Current work is aimed at elucidating key Shp2 substrates using both candidate and unbiased proteomic approaches, delineating its role in specific tissues using the inducible knockouts, determining the cellular and molecular basis of how Shp2 mutations cause NS and hematopoietic disease, and generating mouse models of NS. We have also recently identified another major NS gene, and are studying the biochemical and cellular effects of these mutations, as well as the potential involvement of this gene in human tumors. Finally, we are testing the effects of specific oncogene/tumor suppressor gene mutations on prospectively purified mammary stem and progenitor cell populations, and attempting to identify/purify and culture cancer stem cells from several types of solid tumors.

Selected Publications:


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T Cell Tolerance

The focus of our lab is to investigate the mechanisms that maintain tolerance or promote T cell activation, leading to the induction of immunity, autoimmunity or potentially tumor immunity. We have used a combination of transgenic models that allow us to follow a responding T cell population specific for a defined self antigen or tumor associated antigen. In many studies we have also combined these models with various gene deficient mice to examine the importance of different molecules on the induction of T cell tolerance or activation. Our lab pursues three basic directions:

1.) Investigating the role of survival versus apoptosis on tolerance and autoimmune.
2.) Investigating signaling pathways that control T cell tolerance, activation, immunity, autoimmunity or tumor immunity.
3.) Examining the potential for immune surveillance and tumor immunity.

Selected Publications:

Regulation of Cell Cycle Progression

Our laboratory primarily is interested in the signaling pathways regulating the entry into and the progression of the cell cycle - factors which are crucial to the maintenance of genomic stability. The pathways regulating such processes also have a direct link to cellular proliferation, differentiation and survival. In fact, dysregulation of such function results in numerous adverse pathological conditions, including cancer.

By developing comprehensive data sets, we endeavor to understand how normal signaling pathways are regulated and how such functional characteristics differ in the presence of disease. In order to achieve this goal, we are currently investigating several aspects of the regulatory pathways of proliferation and survival. Firstly, we are studying the p53 signaling pathway which is essential for cell cycle arrest and apoptotic induction which occurs during numerous stresses. We have recently identified Bat3 as a novel positive regulator of p53 transactivation in DNA damage response and we are now in the process of investigating the biological activity, tissue specific function and interacting molecules of Bat3. Incidentally, the existence of bi-allelic inactivating mutation of Bat3 has recently been identified in a colon cancer cell line. Secondly, we are focusing on one of the heat shock proteins, HSP70-2. HSP70-2 is essential for meiotic cell division in male germ cells and cell proliferation of advanced human cancers, but is not required for proliferation of primary cells. Further investigation has suggested that HSP70-2 may regulate histone modifications. However, the precise molecular mechanism of just how HSP70-2 regulates cell survival and proliferation remains unclear and we therefore continue to study the signaling pathways and interacting proteins relevant to its function. Of significance, HSP70-2 was previously identified as a functional tumor antigen. We firmly believe that dissection of the mechanisms of action of cell signaling pathways holds great promise for identification of new potential therapeutic targets.

Selected Publications:


Structure and Function of Proteins

Knowledge of the three-dimensional structure of a given protein is an absolutely essential prerequisite for the full understanding of the chemical basis of an enzyme’s catalytic mechanism, for interpreting the way structural proteins like e.g. actin interact with each other or how lead compounds have to be modified to improve affinity and specificity of drug candidates. In my lab, we use X-ray crystallographic techniques to establish the molecular architectures of proteins. We integrate our structural results with biochemical and molecular-biological as well as computational data, either collected in our laboratory or available through collaborations with specialists in the respective fields.

**Orotidine 5’-phosphate decarboxylase from archaea, plasmodia and man**

ODCase is one of the most proficient enzymes known. It catalyzes the conversion of orotidine-5’-mono-phosphate (OMP) to UMP, the last step in the de novo biosynthesis of pyrimidine nucleotides enhancing the decarboxylation rate by 17 orders of magnitude without the help of any cofactors or metal ions. We have determined more than 50 structures of the various enzymes, apo-forms as well as complexes with substrate, product and a large number of inhibitors synthesized and tested in animals as anti-malaria and anti-cancer agents by our collaborators in medicinal chemistry, parasitology and cancer research.

**CorA -- a Mg2+ transporter from T. maritima**

Represents the lab’s first membrane protein structure, a pentameric protein that imports Mg2+-ions into the cell. While our lab pursues the structures of the “open” state of the molecule and structurally characterizes a large number of mutants, we also collaborate with colleagues specializing in electrophysiology, AFM and computational approaches to elucidate the details of the protein’s workings.

**Antibodies as tools in structure research**

Although the three-dimensional structure of antibodies has been known for a long time, they still represent interesting research targets as well as unique tools for stabilizing transitional states. We
are interested in the structural basis of antibody maturation, cross-reactivity, and the recognition of epitopes on HIV proteins. We also make use of antibodies in trapping intermediates of the PrPC-PrPSc transition of prion proteins for structural characterization.

**Selected Publications:**


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**Cellular Interactions and Biochemical Mediators that Promote Development**

B lymphocytes produce antibodies and present antigen. Failure to regulate the growth, development, and response of B cells can lead to malignancy, immunodeficiency and autoimmunity. Our research efforts are directed towards a better understanding of the process of B cell development and their role in disease.

B cell development is dependent on growth and differentiation factors. In some cases, such factors are produced by stromal cells. We are determining the biochemical nature of the cell-bound and secreted molecules that mediate these interactions. We have also identified interactions that occur between developing B cells themselves. These homotypic interactions appear to be critical for progression from the pre-B to the B cell stage of development. Finally, we are undertaking signal transduction experiments that seek to determine the interactions between molecular pathways regulated by critical cell surface receptors such as the pre B Cell Receptor and the IL-7 Receptor as well as growth and differentiation factors such as IL-7 and IL 21. We have cloned and characterized a novel bioactive peptide, Hemokin -1, that may also play a role in the early development of B cell progenitors.

**Immune System and Disease**

We also study B cell malignancies in both mouse and human models. Of specific interest is determining the mechanisms of action of the immune system in recognizing and destroying tumor cells. We have found that IL-12 can play a key role in promoting the ability of the immune system to recognize and eliminate the leukemia cell. We have also found that there are important differences between T cell mediated immune responses elicited by systemic administration of IL-12 compared to IL-12 gene therapy in which the leukemia cells are infected with lentiviral vectors that produce IL-12.

**Selected Publications:**

Cancer occurs when a single cell acquires multiple genetic mutations that result in uncontrolled growth and survival. The tumor cell becomes addicted to these mutations and their activated signaling pathways. Our goal is to understand, exploit and target these tumor-specific vulnerabilities. To this end we have two working groups in the lab, Tumors’ n Targets and Death n Drugs. Our ideas are simple and relevant, and our approach is novel and achievable. We focus on two major areas.

The first area of focus is the regulation and function of the Myc oncogene. Myc is deregulated in >50% of human cancers and functions as a regulator of gene transcription. We are identifying the target genes regulated by Myc, the co-factors recruited by Myc and the post-translational modifications regulating these processes. Targeting Myc’s potent transforming activity through the development of novel inhibitors is a major area of investigation. Interestingly, for a non-transformed cell with deregulated Myc expression to develop into a fully malignant cancer, it is essential that Myc’s ability to potentiate apoptosis be overcome. We aim to understand the molecular mechanism of Myc-induced apoptosis and identify genetic events that can block this programmed cell death, to cooperate with Myc in tumour progression. To achieve these goals we use state of the art technologies, including ChIP-chip, high-throughput FRET/FLIM as well as functional cloning, and have established several tissue culture and animal models for our work, including leukemia, breast, neuroblastoma, and lung cancers.

The other major area of focus is in the use of statins as anti-cancer agents. Statins are a family of drugs used routinely in the control of hypercholesterolemia that we and others, have shown can trigger tumor-specific apoptosis. Statins block HMG-CoA reductase, the rate-limiting enzyme of the mevalonate pathway. This is a basic biochemical pathway essential for cellular metabolism. Because statins are an approved drug for use in humans, we can immediately fast-track statins to impact patient care. To this end, our research goals are to understand why certain tumor-types are highly sensitive to statin-induced apoptosis. Moreover, we aim to determine how best to use statins in treatment regimes in combination with traditional and molecular targeted therapeutic options. Recent evidence in the lab suggests that statins can also sensitize tumor cells to undergo apoptosis in a mevalonate-independent mechanism. Biomarkers of sensitivity and response are also a major focus. The role of statins and the sensitivity of the tumor stem cell is also a key area of investigation. Tumor types under study include leukemia, multiple myeloma and ovarian cancer, however, several others neoplasias are also potential targets.

Selected Publications:


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Transcriptional repressor complexes and membrane proteins

Our research centers on the study of protein structure and molecular recognition, with an emphasis on understanding protein-protein, protein-peptide and protein-lipid interactions. We use a variety of biophysical, bioinformatic and biochemical techniques to address these questions, including x-ray crystallography, spectroscopy and thermodynamic methods.

Structure and function of the BTB domain
Several human BTB-zinc finger proteins, including PLZF and BCL6, are transcriptional repressors that are implicated in development and/or in cancer. The BTB domain in these proteins represses the expression of target genes by the BTB-mediated recruitment of HDAC/corepressor complexes to promoter sites recognized by the C-terminal zinc-finger regions. We are studying the interactions of BTB domains with corepressor and cullin-based ubiquitin ligase complexes with the objective of developing protein-protein interaction inhibitors to reverse the biological activities of these oncogene products.

Structural biology of membrane proteins
It is widely recognized that new methods are needed for the structural study of membrane proteins, and we are addressing the problem of membrane protein crystallization by developing lipopeptide detergents (LPDs), a fundamentally different class of amphiphile designed specifically for the crystallization of membrane proteins. We are also studying the thermodynamics of
membrane protein folding, and the fundamental properties of protein-lipid and protein detergent interactions. We have interests in several specific membrane and membrane-associated proteins, including transporters, the PagP acyl transferase, and the saposin proteins.

More information can be found at http://xtal.ubnres.utoronto.ca/prive.

Selected Publications:

• Parekh S, Privé GG and Melnick A. Therapeutic targeting of the BCL6 oncogene for diffuse large B-cell lymphomas. Leukemia and Lymphoma (2008).

Crystal structure of a complex between the BCL6 BTB domain and a peptide from the BCOR transcriptional corepressor

Research in my laboratory focuses on the cellular and molecular mechanisms that lead to the formation and stability of the cardiovascular and hematopoietic systems in mammals. During development, this process is tightly coordinated, involving the differentiation and interaction of several cell lineages to form the heart, blood vessels and circulating blood cells, and thus is dependent on signals exchanged by these cell types. Our research concentrates on two families of signalling molecules, TIE1 and TIE2 receptor tyrosine kinases, and the TGFβ/BMP receptor, Endoglin. These cell surface receptors are expressed in both endothelial and hematopoietic cells in the embryo and the adult. Consistent with this restricted expression patterns, these molecules have been implicated in a number of human disease states involving the unchecked growth or differentiation of vascular and hematopoietic cells. Using the mouse as a model system, we take a genetic approach both in vivo and in embryonic stem cells to define the cellular processes mediated by these pathways in establishment of the hematopoietic stem cell microenvironment, as well as the patterning of both blood and lymphatic circulatory system during embryogenesis and early postnatal life.

Selected Publications:


Developing blood vessels of midgestation mouse embryo visualized by Tie1-LacZ reporter gene.
Gene Regulatory Programmes that Control Animal Immunity

Research in our laboratory is concerned with characterizing the gene expression programs that underlie animal immunity. These gene regulatory networks encompass transcription regulatory proteins, the DNA control sequences to which they bind and the signaling systems which convey nuclear states among cells. We are specifically interested in understanding regulatory networks that direct immunocyte specification and thereby set up programs for innate immune function. We employ the embryo and larva of the purple sea urchin (Strongylocentrotus purpuratus) as an uncomplicated experimental system in which to carry out these investigations. These animals offer advantages for this work including the availability of enormous quantities of staged embryos, highly efficient transgenesis and the ability to specifically perturb gene function using antisense and transgenic technologies. Importantly, sea urchins are echinoderms, a sister-group of the chordates, and they thus share a genetic heritage with the vertebrates critical for our comparative immune investigations.

The purple sea urchin genome sequence reveals an innate immune system of unprecedented diversity that is rich with experimental opportunities to better understand our own immune system. We approach these issues from two directions:

1.) We are characterizing expression of sea urchin homologs of transcription factors that are well known as regulators of hematopoiesis and immunity in vertebrates. Genes currently being characterized include homologs of vertebrate GATA-1/2/3, PU.1/Spi-B/Spi-C, E2A/HEB/ITF-2, SCL/Tal-2/Lyl-, and Id-1/2/3/4. All of these genes are important in the function of adult immune cells and in the development of larval immune cells.

2.) In a second approach, we are characterizing genes that are responsible for immune recognition, regulation and effector functions. Genes in this category include those encoding immense superfamilies of recognition proteins such as toll-like receptors (TLRs), Nod/Nalp-like proteins and SLCR domain proteins, genes that encode immune signaling molecules such as TNF family members and IL17 homologs, gene families with characteristics of antimicrobial peptides, and perforins. We are also looking at a variety of genes with homologs in the vertebrate adaptive immune system.

Work in this simple model reveals conserved gene regulatory circuitry that can then be characterized in more complicated vertebrate systems, but the sea urchin also provides an invaluable set of “natural experiments” that illuminate the workings of our own immune system and present us with entirely novel solutions to the immune challenges all animals face.

Selected Publications:

Looking for trUbl: Proteomics of ubiquitin and the ubiquitin-like proteins

We are using a combination of biochemical, genetic, and proteomics techniques to study a very interesting, but poorly understood, class of small regulatory proteins - the ubiquitin-like modifiers (Ubls). The Ubls are post-translationally conjugated to other biomolecules to regulate their functions: interestingly, the various Ubls (a family of 12 different isoforms in mammalian cells) appear to be conjugated to several different types of molecules, and possess very different functions. The Ubls have been implicated in a variety of critical cellular functions, such as cell cycle control, the DNA damage response, and intracellular localization. Most importantly, several of the Ubls (e.g. NEDD8 and SUMO) have also been implicated in neurological disorders such as Parkinson’s and Alzheimer’s disease, as well as cancer.

Recently, we developed a novel mass spectrometry-based pattern recognition tool that can identify previously “invisible” Ubl conjugation sites. Using this tool, we were able to demonstrate that, like ubiquitin, the Ubl SUMO can form several different types of SUMO-SUMO multimeric chains. We are in the process of determining the physiological function of these chains using genetic and biochemical assays in mammalian cells and the model organism S. cerevisae. More information can be found at http://www.raughtlab.ca.

Selected Publications:


Glycosidases in human health and disease

Many of the most important macromolecules in physiological processes are neither proteins nor nucleic acids, but carbohydrates (sugars and polysaccharides). Our focus is to investigate the enzymes that process (assemble or break down) complicated polysaccharide structures, and the role of these enzymes in health and disease processes. We emphasize glycosidases because they are particularly suited for inhibitor development. Glycosidase inhibitors have already shown promise clinically as anti-cancer, anti-diabetic and anti-viral compounds.

1. GH38 enzymes and cancer

Several years ago, we first derived the atomic structure of the Golgi enzyme -mannosidase II (GMII). A participant in the N-glycosylation pathway of glycoprotein synthesis, GMII is classified in a family of enzymes, glycosyl hydrolase (GH) 38. Other members of this family are related to HMII structurally and mechanistically, but have very different substrate specificities and localizations in the cell. Inhibition of GMII has been shown to have clinical effectiveness against the progression of late-stage cancer, but with side-effects that are attributed to broad inhibition of other GH38 enzymes. Our approach is to examine the structural characteristics of this family that give rise to cross-inhibition, and to design modified inhibitors with more specificity to GMII.

In a recent, exciting result, we have derived the structure of GMII complexed with its intact polysaccharide substrate (see Figure 1). This has allowed us to identify the precise characteristics of several separate sugar binding sites. By linking together these sites with branched inhibitors, we hypothesize that higher specificity for GMII will result.

2. GH31 enzymes and Diabetes

Starch is the major source of energy from the diet. A series of intestinal enzymes degrade starch, ultimately into glucose. Inhibiting some of these enzymes has been shown to be effective in controlling blood sugar levels. In addition, understanding the specificity of these enzymes for different forms of starch will allow
us to understand what nutritional sources are most effective in generating glucose. This could have applicability in the development of less expensive but more effective nutritional sources for underdeveloped regions.

It turns out that the two main enzymes in the later stages of starch processing, maltase glucoamylase, MGAM, and sucrase isomaltase, SIM, are both made up of dual glycosidase domains, which are all members of the same GH31 family. We are developing a program to study all four of these domains in order to understand how they work in concert to degrade different forms of starch. Recently, we have determined the first MGAM domain structure (Figure 2) and we are studying how it interacts with novel glycosidase inhibitors.

Selected Publications:


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Chemical Biology and Drug discovery

Our lab is interested in chemical biology and drug discovery with a focus on the apoptosis pathway. Using automated and robotic equipment we screen chemical and siRNA libraries to identify chemical and genetic probes and use them as tools to better understand biological pathways with a focus on apoptosis and the pathogenesis of leukemia.

For example, to identify small molecule inhibitors of the anti-apoptotic protein XIAP, a million compound small molecule library was screened. From this screen, small molecule XIAP inhibitors were identified and subsequently used as tools to validate XIAP as a therapeutic target in acute leukemia. Based partly on this work, a clinical trial of XIAP antisense oligonucleotides in combination with reinduction chemotherapy was launched in patients with refractory acute myeloid leukemia. Similar chemical biology approaches have been used to identify small molecules that sensitize resistant cells to death receptor ligands by reducing expression of the caspase-8 inhibitor FLIP. Our work on small molecules that decrease FLIP and activate the death receptor pathway of caspase activation also led to a clinical trial of the synthetic triterpenoid CDDO in patients with refractory leukemia.

Finally, efforts are underway to advance novel molecules from the lab to the bedside. Here, off-patent drugs are screened to identify compounds that impact targets important in the pathogenesis of malignancy and thus have previously unrecognized anti-cancer activity. Through this approach, new insights into molecular pathways are gained. In addition, these old drugs can be “repurposed” and moved rapidly into clinical trial for the treatment of malignancy.

Selected Publications:

T cell-mediated Cancer immunotherapy

The goal of our lab is to develop methods to allow more cancer patients to benefit from the therapeutic potential of tumor-reactive T cells. The two major projects are to learn how best to activate tumor-reactive T cells and how to make tumor cells immunogenic, or able to interact with, and be killed by, T cells. Malignant Melanoma and Chronic Lymphocytic Leukemia (CLL) are used as clinical models.

Tumor-reactive T cells are often in a suppressed state.

We have shown that infusing anergic T cells into lymphopenic hosts “reawakens” them, and are exploring this phenomenon as a strategy to amplify T cell responses after treatment with cytotoxic chemotherapy. We are also investigating the use of antibodies against CTLA-4 (an important negative regulator of T cell activation) as another strategy to recover suppressed tumor-reactive T cell function.

Cancer vaccines can also activate tumor-reactive T cells.

We have developed methods to make leukemia cells differentiate into dendritic cells (the most potent T cell stimulators), and are currently designing clinical trials of these vaccines. We have extended these methods to generate large numbers of “pseudo-dendritic cells” from normal B cells, which can be infected with antigen-expressing viruses to become effective vaccine platforms in immunologically susceptible cancers. The biology and manipulation of these activated B cells in mice and humans is a major project.

To improve the immunogenicity of tumor cells, we are exploring the use of immunomodulators such as Toll-like receptor agonists and type I interferons (IFNs). We have shown that infusions of IFN following melanoma vaccines significantly improved clinical efficacy and that TLR-7 agonists sensitized CLL cells to lysis by both T cells, and chemotherapies. However, the oncogenic events that drive cancers can also corrupt immunomodulator signaling pathways. A central “hallmark” of aggressive cancer cells is their strong dependence on glucose (rather than other fuels) to meet their metabolic needs. A current hypothesis in the lab is that this aberrant glucose metabolism results in glycosylation of intracellular signaling molecules and inhibition of signals from immunomodulators. The molecular explanations for these phenomena are being investigated using cell lines, primary tumor cells, and transgenic mice as tools, and the identification and development of small molecules that can restore proper immunomodulator signaling are being actively pursued.

Recently, we identified an extracellular calcium-sensing receptor on B cells that prepares them to engage in immune responses and appears to be important in the pathogenesis of CLL. Cloning this receptor, developing inhibitors, and establishing its role in other B cell cancers are also current projects in the lab.

Selected Publications:


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Signal Transduction and Cancer: Regulation of Cellular Proliferation, Survival and Apoptosis

Cellular transformation, an early step in tumorigenesis, is almost exclusively caused by malfunction of signal transduction pathways. Immediate cellular environment, including metabolic, mitogenic and positional clues, activity of various modifier genes, as well as selective pressure for tumor growth, profoundly affects cellular signaling throughput and plays key roles in cancer initiation, progression and metastasis. Our laboratory is interested in studying cancer-related signal transduction and its effects on cellular growth, survival and apoptosis. Our primary interest is the PI3K signaling pathway, which has been implicated in the etiology of multiple human malignancies. For instance, PI3K (PIK3A) is a potent oncogene whose mutations are often found in colon, breast, brain and lung cancers, whereas PTEN, a gene whose protein product directly counteracts PI3K activity, is one of the most frequently mutated tumor suppressor genes in a variety of human cancers.

Our previous research in this area included characterization of the physiological function of PTEN, as well as development of several animal model systems that were highly informative in characterizing the role of 3’PI signaling in tumorigenesis and regulation of cell growth and size. More recently, we have become interested in the relevance of subcellular localization of
pathway components as an additional regulatory input into 3'PI signaling and have described the importance of compartmentalization of certain pathway components for proper signal propagation. Our current interests include examination of modes of PTEN regulation, prioritization of PKB/Akt substrates relevant to tumorigenesis and characterization of Rheb, a small GTPase and a downstream target of this pathway. Our work aims to provide a thorough understanding of molecular mechanisms of signal transduction and may lead to the development of effective therapeutic strategies for treatment of cancer.

**Selected Publications:**

- Stambolic V. and Woodgett, JW Functional Distinctions of Protein Kinase B/Akt Isoforms Defined by Their Influence on Cell Migration. Trends in Cell Biology 2006, 16(9):461-6
- Buerger C, Devries B, Stambolic V. Localization of Rheb to the endomembrane is critical for its signaling function. Biochem Biophys Res Commun. 2006 J344(3):869-80

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**Microenvironmental causes of resistance to anticancer drugs**

Mechanistic studies of drug resistance in tumours have concentrated on genetic changes in cancer cells. We are studying equally important, but neglected mechanisms that depend on tumour microenvironment. Anti-cancer drugs access solid tumours via the blood, and must penetrate multiple cell layers to reach all cancer cells. We study drug penetration using multilayered cell cultures ex vivo, and quantitative immunohistochemistry to assess drug distribution in tumours of animals. We have shown limited perivascular distribution of the clinically-used anticancer drugs doxorubicin, mitoxantrone, and topotecan in solid tumours, but uniform distribution in normal tissues other than brain. We are investigating strategies to overcome clinical resistance that include:

i.) Use of the inactive pro-drug AQ4N, which penetrates tissue to reach hypoxic regions where it is activated to AQ4, an analogue of mitoxantrone. We have shown complementary special distributions and synergistic activity of mitoxantrone and AQ4N in human tumour xenografts and are initiating a phase 1 trial, to be followed by a phase 2 trial in patients with hormone-refractory prostate cancer.

ii.) Use of the anti-ulcer agent pantoprazole to inhibit sequestration of the weak bases doxorubicin and mitoxantrone in acidic endosomes of tumour cells (by inhibiting the endosomal proton pump), thereby allowing improved tissue penetration. We expect to initiate a second clinical trial of pantoprazole and doxorubicin for treatment of solid tumours. For drugs that are not themselves fluorescent, we are studying drug distribution in vivo by using antibodies to the drugs, or that recognize downstream targets of drug activity.

**Repopulation of bone marrow allows recovery after chemotherapy**

Repopulation of surviving tumour cells also occurs, and increases in the rate of repopulation between successive cycles of chemotherapy may lead to shrinkage and regrowth of tumours without change in intrinsic drug sensitivity. Another goal of our program is selective inhibition of repopulation of tumour cells using molecular targeted cytostatic agents: giving such agents between courses of chemotherapy (and stopping them prior to the next cycle) is probably the optimal strategy to inhibit repopulation and improve outcome of chemotherapy, whereas concurrent administration, as often used clinically, is likely to be less effective.

**Selected Publications:**

- Kim JJ, Tannock IF: Repopulation of cancer cells during therapy:


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Molecular Evolution, Computational Biology, Bioinformatics, and Genomics

Recent advances in technology have made possible the rapid accumulation of massive amounts of molecular biological data. Namely, rapid sequencing has made possible the complete sequencing of entire genomes, and DNA microchip technology is advancing such that the study of the expression patterns of all genes in a genome can be analyzed rapidly. Our interest is in developing and improving analysis tools that are needed to consider such large amounts of varied data in light of studying biology and evolution on a genomic scale.

Projects in the lab include:
- Developing methods of protein sequence analysis for prediction of aspects of their structure and protein interactions.
- Developing methods of DNA and RNA sequence analysis to predict aspects of their function and regulation.
- Developing new bioinformatics tools for metagenomic applications.

Selected Publications:

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Preclinical Validation of Novel Agents for Treatment of Multiple Myeloma

Our research focus is on novel drug development for the treatment of mature B cell malignancies based on molecular targets. This encompasses drug discovery and pharmacological profiling of candidate compounds, pre-clinical studies to validate molecular and cellular targets, the establishment of appropriate animal models for drug testing, and the development of Phase I/II clinical trials.

Specifically, we have recently validated the tyrosine kinase, Fibroblast Growth Factor Receptor 3 (FGFR3) as a therapeutic target for a subgroup of multiple myeloma patients that express this receptor on tumor surface. We in pre-clinical studies have further identified the small molecule inhibitor, CHIR-258 and a neutralizing anti-FGFR3 antibody, PRO-001 as active agents against FGFR3 expressing myeloma cells. As a direct result, the first ever, clinical trial of FGFR3 inhibition in myeloma has been initiated.

Our efforts are now focused on the development and implementation of relevant biological endpoints to study this novel class of anti-tumor drugs in the context of clinical trials. In addition experiments to validate additional molecular targets in myeloma such as MMSET and the maf and cyclin family of genes are ongoing or currently development.

Selected Publications:
- Trudel, S., Li, Z., Wek, E., Wiesmann, M., Chang, H., Chen, C., Reece, D., Heise, C. and Stewart, A.K. CHIR-258, a novel, multi-targeted tyrosine kinase inhibitor for the treatment of
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Molecular Basis of Lung & Pancreatic Cancer

The laboratory is focused on translational research projects in lung and pancreatic cancer, two of the most deadly cancers with overall five-year survival rates of 15% and 2-5%, respectively. Greater understanding of the molecular basis of their malignancy will provide insights for improving the early diagnosis and treatment against these diseases.

The primary goals of our lung cancer projects are to identify novel genes or proteins that are better than clinical predictors alone in predicting clinical outcome or response to therapies. We profile the gene expression and genomic aberrations of a large number of human lung cancer samples using microarray techniques, apply computational bioinformatic algorithms to identify the predictive genes or gene signatures, and validate them by real-time quantitative PCR technique. The biological functions of the predictive genes are then studied using in vitro lung cancer cell line model, primary lung cancer xenograft models and orthotopic rodent models of human lung cancers.

The primary goal of our pancreatic cancer project is to dissect the molecular basis of human pancreatic cancer, which mostly arises from the duct epithelium. Our laboratory was the first to establish primary cultures of normal human pancreatic duct cells. Immortalized cell lines derived from these primary cultures are used to study the activities of oncogenes and tumor suppressors that are commonly aberrant in pancreatic cancer. The approach provides insights into key molecular events that may be targeted to prevent and treat pancreatic cancer.

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Current Research Interests

The research in the lab is focused on Neurobiology, but is separated into three distinct areas: Neural Development & Stem Cell Biology, Neurobiology of Motivation, and Learning and Memory Genes.

Our Neural Development and Stem Cell Biology project involves the development of the mammalian brain, eye, and pancreas. Neural stem cells also are present throughout the lifetime of the animal, and are being localized characterized. We have also discovered a surprising capacity of the adult mammalian eye to regrow. Our experimental approach involves culturing mouse retinal stem cells from normal and genetically modified mice, in order to understand the factors that control retinal stem cell activity. Finally, we have isolated a rare cell from the adult mouse pancreas that can show extensive proliferation under defined conditions in vitro (Seaberg et al, 2004). These cells may comprise a population of adult mammalian pancreatic stem cells, which might in the future be employed in treating type 1 diabetics.

The primary objective of the Neurobiology of Motivation project is to characterize how the brain processes and distinguishes different types of rewards, e.g. nicotine, food, opiates (morphine). Our overall hypothesis is that two discrete neural mechanisms underlie the rewarding effects of opiates in drug naive animals (processed by TPP) versus drug-dependent and deprived animals (utilizing dopamine). Proposed experiments will further test and reveal the structure of motivational systems in the mammalian brain.
Our Learning and Memory Genes project has resulted in the development of associative and non-associative learning paradigms using olfactory and taste stimuli in the worm C. elegans. Mutational screens in progress have identified new genes that code for critical components of associative and non-associative learning; revealing the separable neuronal and molecular substrates underlying associative learning and habituation. Our goal is to use the power and specificity of modern molecular genetics to reveal the component processes of learning and memory by using the C. elegans' genes.

Selected Publications


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The Nature of the Haematopoietic Stem Cell in Myelodysplastic Syndrome

The myelodysplastic syndromes (MDS) are a group of clonal bone marrow stem cell disorders characterized by low blood cell counts, an increased rate of apoptosis of haematopoietic progenitor cells, and a high risk of transformation to acute leukaemia. The goals of our research program are to gain insights into the biology of MDS, and to use such insights to design rational and effective MDS therapies.

The MDS stem cell advantage – EAR-2, MDS, and AML

A central paradox in MDS biology is how the myelodysplastic stem cell out-competes normal stem cells and comes to dominate the bone marrow. We hypothesized that the competitive advantage enjoyed by the MDS stem cell consists in an enhanced capacity for self-renewal, and identified gene that mediate this property in leukaemia cells. One of the genes we identified, EAR-2, is also more highly expressed in MDS and leukaemia than in normal bone marrow. We have found that EAR-2 expression blocks differentiation of leukaemia cells in culture and leads to the development of leukaemia when overexpressed in mouse bone marrow. We are now studying the mechanism by which EAR-2 alters stem cell behaviour, and its role in the multistep pathogenesis of MDS and AML.

When less is more – Candidate genes in del(5q) and del(7q) MDS

Clonal cytogenetic abnormalities are present in more than 50% of cases of MDS. Two of the most frequently seen abnormalities involve involve deletions of large tracts of the long arms of chromosomes 5 or 7. These deletions are thought to contribute to the development of MDS by resulting in the loss of tumour suppressor genes located on these chromosomes; however, the identity of such tumour suppressor genes remains a mystery. We are investigating two candidate MDS tumour suppressor genes: SPARC (chromosome 5q), a mediator of the interactions between the cell surface and the extracellular matrix, and HIPK2 (chromosome 7q), a serine/threonine kinase that has roles in the regulation of proliferation and apoptosis.

Iron, oxidative stress, and the stem cell

Owing to the failure of normal haematopoiesis, patients with myelodysplastic syndrome commonly require regular blood transfusions in order to survive. This results in accumulation of iron, which results in cellular damage via the generation of reactive oxygen species (ROS). Since accumulation of ROS leads to HSC senescence in mice, we have hypothesized that iron overload in MDS patients creates a vicious cycle, in which iron deposition results in ROS generation, leading to further impairment in haematopoiesis, leading to even greater requirement for blood transfusion. Furthermore, we believe the DNA-damaging effects of ROS may contribute to the progression of MDS to AML. We are conducting experiments to measure this phenomenon, and to explore how it may be reversed by iron chelation and anti-oxidant agents.

Selected Publications:

- Gu C, Teng T, and Wells RA. Synergistic effects of troglitazone in combination with cytotoxic agents in acute myelogenous leukaemia cells. Leukemia Research, 2006; 30; 1447-1451.
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**Effects of ionizing radiation on the central nervous system**

Radiation therapy is a major cancer treatment modality. Radiation injury of the brain and spinal cord has devastating and sometimes fatal consequences. The overall goal of my laboratory research program is to understand the mechanisms of radiation injury in the central nervous system (CNS), and to develop neuroprotective strategies against this injury.

The underlying mechanisms of this injury remain unclear. There is an increasing body of data including those from our laboratory that suggests that the radiation response in the CNS is a continuous, dynamic, and interacting process. It is recognized that clonogenic cell death is not the only mode of radiation-induced cell death. Certain glial, neuronal and endothelial cells including neural progenitor cells in the CNS undergo apoptosis within a few hours after irradiation. There is also component of secondary injury and cell death that is mediated by neuro-inflammation, oxidative stress and microenvironmental alterations. Disruption of the vasculature and microenvironment after irradiation may influence cell fate and lead to inhibition of neurogenesis and neurocognitive deficits.

We are currently studying these effects at the tissue, cell and molecular level. Our work is focused on potentially reversible components of cell death and damage since targeting these damage pathways provide the best opportunities for neuroprotection. Current work includes characterizing apoptosis, neuro-inflammation and perturbations of the vascular/neural progenitor cell niche and cell fate determination after irradiation. Our studies are performed in vivo using transgenic mouse models of radiation-induced neurobehavioral damage and myelopathy, and in vitro using glial and neuronal progenitors cultured from the CNS of these animals.

**Selected Publications:**


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**Tissue-specific Molecular Determinants of Survival, Apoptosis and Metabolism**

Our laboratory is focusing in elucidating tissue-and context-specific roles of molecules that determine cellular survival and function. Interestingly, many of the molecules involved in cancer formation are also involved in obesity and metabolism. Our goal is therefore to understand tissue-specific roles of these complex molecules. Our focus is in caspases, as well as tumour suppressors and oncogenes such as PTEN and myc. Several projects in our lab involve using genetically engineered mice and taking biological, biochemical and molecular approaches to define pathogenic mechanisms of disease. These approaches to clarify tissue-specific molecular mechanisms have wide implications for treatment of type 1 and type 2 diabetes, as well as islet transplantation.

One main area of research in my laboratory is to elucidate genetic determinants of insulin producing pancreatic beta cell growth and apoptosis in healthy states and in diabetes. In type 1 diabetes, beta cells undergo apoptosis from autoimmune mediated islet destruction. In type 2 diabetes, obesity and inflammation are the underlying cause that ultimately lead to glucotoxicity and lipotoxicity of the beta cells. While these two types of diabetes have distinct pathogenesis, beta cell defect and deficiency are features that may utilize common cellular machinery. We use tissue-specific genetic approaches such as the cre-loxP system to analyze the in vivo functions of the critical genes.

PI3K signaling pathway induces a complex pleiotropic biological outcome that is highly context dependent. PTEN is a phosphatase that is a major negative regulator of this important signaling pathway. PTEN, although first discovered as a tumour suppressor, also appears to have critical effects on metabolism and therefore likely plays a key role in the pathogenesis of insulin resistance and type 2 diabetes. Our goal is to understand the molecular mechanism of PTEN type 2 diabetes models.
Functional Characterization of the Wnt & PI3 Kinase Pathways

Our laboratory is focused on the role of specific signal transduction pathways in development and disease. Specifically, we are interested in the Wnt and phosphatidylinositol 3’ kinase (PI3K) pathways as these are frequently activated in human cancers as well as other disorders. We employ molecular and cellular biological approaches to probe the components of these pathways and we are particularly interested in breast and colon cancer as well as diabetes and neurological processes. These signaling pathways share several common elements including regulation of a protein-serine kinase termed GSK-3, which acts as a global suppressor of the functions of a variety of regulatory proteins. Using knockout mice that harbour conditional alleles for the two mammalian isoforms of this kinase, we are investigating the consequences of inactivating this regulatory protein in specific tissues. In embryonic stem cells, GSK-3 inhibition contributes to a block to differentiation and we find a similar effect in progenitor cells in the developing mouse as well as the adult.

To understand how the PI3K and Wnt pathways exert their effects in cancer, we are examining the state of activation of specific downstream components of these systems in transgenic mice as well as cell lines – guided by surveys of human cancer specimens. To fill in the missing connections in the signaling pathways, we are also using mass spectrometry to identify key targets of the protein kinases in these pathways in a systematic approach.

More information can be found at http://www.mshri.on.ca/woodgett/.

Selected Publications:

There is growing but incomplete evidence that cancer may be organized in a hierarchy in which only a fraction of cells, termed Tumor Initiating Cells (TICs; also referred to as Cancer Stem Cells, CSC), is capable of instigating cancer and metastatic disease. In contrast, the majority of tumor cells represents progenitor and partially differentiated cells that have lost their tumorigenic potential. Thus, targeted killing of TICs may be curative.

Our laboratory is using several mouse models of breast cancer to analyze TICs, their biology, the pathways by which they divide and their unique properties relative to mammary stem cells (MSCs). The use of mouse models provides ample supply of primary tumors with defined genetic background to study these rare tumorigenic cells. In addition, powerful genetic manipulations in the mouse allow us to test the basic paradigms of the CSC model in vivo in isogenic mice.

We recently reported on the first identification of TICs in a mouse model of Her2/Neu, one of the most aggressive forms of breast cancer in human. We showed that TICs are functionally indistinguishable from tumorsphere initiating cells, which give rise to spheres in non-adherent conditions. These tumorspheres provide a means by which to screen small molecule libraries for TIC specific therapeutic targets. We are also attempting to purify Her2/Neu TICs to near homogeneity so we can test whether a single TIC can induce Her2/Neu tumors following transplantation into the mammary gland of a recipient mouse, as well as establish a gene expression signature for the Her2/Neu TIC. Our long-term goals are to develop TIC specific inhibitors for the major breast cancer subtypes.

One novel model that we have recently developed involves the targeted inactivation of the tumor suppressor pRb in the mammary gland. Rb is mutated in nearly 30% of human breast tumors and the mice we created produce similar spectrum of breast tumors as in human. We will next attempt to identify TICs and therapeutic targets in these Rb mutant tumors as well as define the effect of Rb status on the response of tumor cells to chemo- and hormonal therapies in vivo.

In addition to mutations that disrupt the Rb gene, pRb is often inactivated in cancer by phosphorylation induced by cyclin dependent kinases. To study the effect of pRb phosphorylation in vivo, we created mutant mice in which key Ser/Thr phospho-acceptor sites are substituted to Ala residues by homologous re-combination. Analysis of these phospho-mutant Rb knock-in mice reveals unanticipated roles for this tumor suppressor in genomic stability and aging.
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