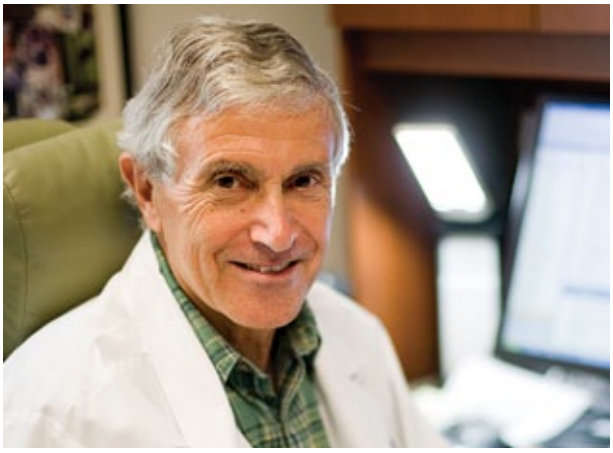
2011 – 2012

Irell & Manella Graduate School of Biological Sciences

Areas of Study

Molecular Biology	Genetics	Diabetes
Cell Biology	Gene Therapy	Chemical Biology
Cancer Biology	DNA Damage/Repair	Structural Biology
Stem Cell Biology	Proteomics	Immunology
Neurosciences	RNA	Biomedical Informatics

City of Hope™



John Rossi

Thank you for your interest in the Irell & Manella Graduate School of Biological Sciences at City of Hope. City of Hope National Medical Center and Beckman Research Institute of City of Hope offer a unique and challenging research and learning environment for graduate education.

Both basic science and translational biomedical research flourish here, in a collegial atmosphere where cross-communication thrives and basic science findings are often applied to the cure of life-threatening diseases.

Our faculty members have made major contributions in the basic biological sciences and biomedicine. They are widely recognized as leaders in their fields. Our diverse students, from the United States, Canada, Argentina, India, Iran, Korea, China, France, Russia, Malaysia and Taiwan, bring to our campus a wealth of interests, experience, enthusiasm, potential, and creativity.

City of Hope's interdisciplinary research programs provide students with many opportunities to enrich their graduate education by interacting with other graduate students, postdoctoral fellows, and faculty members outside of their own areas of specialization. City of Hope has a strong track record in training both predoctoral students and postdoctoral fellows. Each of our Ph.D. graduates has gone on to a prestigious postdoctoral fellowship or a biotechnology or academic position after receiving many excellent offers.

This catalog contains all of the necessary information about applying to the Graduate School. Please contact us if you have any questions. We encourage you to visit our beautiful campus to learn more about this exciting opportunity for doctoral training at City of Hope.

John J. Rossi, Ph.D.
Lidow Family Research Chair
Morgan and Helen Chu Dean's Chair
Dean, Irell & Manella Graduate School of Biological Sciences
City of Hope

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INTRODUCTION

City of Hope was founded in 1913 in Duarte, California, by a small group of working-class men and women who believed in helping those less fortunate than themselves. From humble beginnings — two tents erected on 10 acres of desert — City of Hope has expanded to a 114-acre, 115-building campus landscaped with plants, trees and more than 2,000 rose bushes. In this healing setting, nearly 500 physicians and scientists collaborate to discover, develop and implement innovative strategies for the prevention and treatment of cancer and other catastrophic diseases.

City of Hope is recognized worldwide for its patient care, innovative science and translational research, which rapidly turns laboratory breakthroughs into promising new therapies. We helped launch the biotech industry with the development of technologies that led to the first synthetic human insulin and human growth hormone. Our goal is to shorten the time from scientific breakthroughs to effective new treatments. We strive to find a seamless and fruitful collaboration of basic science, clinical research and patient care that powers important advances in medical progress. Our collaborative pursuits extend beyond City of Hope. We frequently conduct clinical trials in partnership with government agencies and other academic centers, ensuring that patients here and elsewhere receive the potential benefit of promising investigative therapies as quickly as possible.

The campus is located in the San Gabriel Valley, surrounded by one of the greatest concentration of scientific and cultural centers in the United States. Just 10 miles east of Pasadena and 22 miles northeast of Los Angeles in the San Gabriel Mountains, the campus is located in close proximity to a variety of recreational activities, from sunning at the beach to hiking in the desert to skiing in the mountains.

CAMPUS VISITS AND TOURS

The beauty of the City of Hope's campus must be experienced in person. Visitor Services offers tours to prospective students and their families. The tours last about one hour. Reservations are required and may be arranged by calling 626-473-HOPE, ext. 65964.

PH.D. PROGRAM

The Irell & Manella Graduate School of Biological Sciences was founded in 1994 with an incoming class of four students, and the first doctoral degree was conferred in 1997. Since then, the graduate school has grown steadily to almost eighty students and seventy faculty members. The mission of the graduate school is to train students to be outstanding research scientists in chemical, molecular, and cellular biology. Graduates of this program are equipped to address fundamental questions in the life sciences and biomedicine for careers in academia, industry and government.

The program provides students with a solid educational foundation and a research environment that encourages independent thought and challenges current dogma. The program offers coursework to establish a framework for understanding the complexities of scientific problems, research opportunities with investigators who have integrated the latest technologies into their research projects, and professional forums where students can share ideas with colleagues.

Students receive a stipend of $30,000 and health insurance (medical, dental, and vision). Students must pay a registration fee of $50 per semester ($150 per year).

RESIDENCY AND GRADUATE TRAINING PROGRAM IN LABORATORY ANIMAL MEDICINE

The City of Hope/Beckman Research Institute and University of Southern California Residency and Graduate Training Program in Laboratory Animal Medicine is a five-year program whose overall objective is to provide postdoctoral (D.V.M.) trainees (laboratory animal medicine fellows) with intellectual depth and breadth, and appropriate clinical and research training in laboratory animal medicine, laboratory animal/comparative pathology, and comparative medicine. The training program is designed and managed to support preparation toward American College of Laboratory Animal Medicine (ACLAM) board certification and to prepare individuals for academic careers in the biomedical sciences and comparative medicine through completion of the Doctor of Philosophy (Ph.D.) degree in the City of Hope Irell & Manella Graduate School of Biological Sciences.

Residency training includes clinical laboratory animal medicine, laboratory animal resources and facilities management, comparative and diagnostic laboratory animal pathology, and methods and practice of biomedical research. Graduate training consists of a combination of graduate course work, laboratory rotations, seminars, journal club, and scholarly research leading to the Ph.D. in biological sciences. Graduate work provides ample opportunities for specific training in the development of biomedical models and research methodology as well as in other areas important for ACLAM board certification. The training program also includes a full spectrum of clinical rounds, seminars, and special projects pertaining to laboratory animal medicine.

Apply to:
Richard W. Ermel, D.V.M., M.P.V.M., Ph.D.
Director/Professor – Division of Comparative Medicine
Beckman Research Institute of City of Hope
rermel@coh.org

TRAINING PROGRAM IN BIOSCIENCE MANAGEMENT

The Irell & Manella Graduate School in collaboration with the Keck Graduate Institute (KGI) of the Claremont Colleges is proud to offer a Management Training Program for graduate students and postdoctoral fellows. Advanced graduate students and postdocs, with the permission of their research advisor, receive full scholarships to take business-related courses at KGI and to earn a certificate in bioscience management. The program is designed to prepare students for intellectually challenging careers in the biomedical sciences sector.

Required courses include marketing, finance, strategy, accounting and organizational behavior. This program provides excellent preparation for careers in the bioscience industry as science and disease concepts are integrated with management and industry.

VETERANS

The Irell and Manella Graduate School is approved for the training of veterans and eligible persons under the provisions of Title 38, United States Code. Contact Lee Ann Cornell for additional information at 626-471-7396.

ACCREDITATION

City of Hope's Irell & Manella Graduate School of Biological Sciences is accredited by the Accrediting Commission for Senior Colleges and Universities of the Western Association of Schools and Colleges. Contact the Commission at 985 Atlantic Avenue, #100, Alameda, CA 94501 or call 510-748-9001 for questions about the accreditation of City of Hope's Irell & Manella Graduate School of Biological Sciences.

CANCER BIOLOGY

The department, which includes the divisions of Biology, Radiation Biology and Tumor Cell Biology, focuses on understanding the basic mechanisms of genetics, gene expression and function, signaling pathways, mutagenesis, DNA repair and epigenetics as they relate to the development and progression of cancer. Researchers collaborate with clinical and basic research programs within City of Hope and with other centers nationally and internationally. The Division of Biology focuses on understanding the basic biological processes of genetics, gene expression and function, epigenetic mechanisms and DNA repair systems. With a strong foundation in basic research methodology, division scientists are working to bring to light new insights that one day could produce novel therapies for a wide range of genetic diseases. The Division of Radiation Biology focuses on the fundamental mechanisms of radiation resistance in cancer cells and on finding solutions for modulating radiation resistance. Discoveries in radiation biology have the promise of improving the efficacy of radiation therapy in the future. The Division of Tumor Cell Biology works to develop novel translational cancer research in the areas of tumor biology, biomedical informatics, and cancer prevention and diagnosis. Investigators in the division interact closely with basic science researchers focusing in other areas, as well as clinicians at City of Hope.

CANCER IMMUNOTHERAPEUTICS AND TUMOR IMMUNOLOGY

The Department of Cancer Immunotherapeutics and Tumor Immunology (CITI) seeks to advance immunotherapeutic methods to cure cancer, eventually reducing or eliminating the need for chemotherapy, radiation therapy and surgery. Immunotherapy is a powerful weapon against cancer because of its potential to exploit the body's natural defenses against infection. It has been called the "fourth modality" of cancer treatment by the American Cancer Society. The department's goal is to make it a highly effective primary treatment option. Currently, T-cell therapy and radioimmunotherapy offer renewed hope to people who have exhausted other treatment options. They are also effective for eliminating microscopic residual disease which can lead to cancer recurrence, even after chemotherapy, radiation therapy and surgery have been successful. Laboratories in this department are focusing on role of STAT3 in tumor angiogenesis and tumor immune evasion, augmenting the antitumor response of T cells against infection and cancerous cells, and genetically engineering antibodies to withstand chemotherapy.

DIABETES AND METABOLIC DISEASES RESEARCH

City of Hope's newly-created Department of Diabetes and Metabolic Diseases Research encompasses the laboratory efforts of the Division of Diabetes, Endocrinology & Metabolism and the Division of Gene Regulation and Drug Discovery. Combining these resources with an efficient administrative infrastructure allows for a uniquely focused approach to diabetes research. City of Hope has a long and impressive history of groundbreaking discoveries in the field of diabetes, spanning over four decades of intense investigation. The department is focused on advancing islet cell transplantation and related treatments for type 1 diabetes, understanding the genetic and molecular signaling mechanisms that lead to diabetes and its complications, and understanding the structure and function of nuclear receptors in order to design drugs that can prevent activation of undesirable genes. Arthur Riggs, Ph.D., chair of the new Department of Diabetes and Metabolic Diseases Research and director emeritus of Beckman Research Institute of City of Hope, is a pioneer in diabetes research. He is recognized for his work in synthesizing human insulin, which largely replaced animal-derived (porcine or bovine) insulin and became the standard of care for diabetes worldwide.

City of Hope's Division of Diabetes, Endocrinology & Metabolism is also renowned for its islet cell transplantation program, which offers an increasingly viable treatment option for type 1 diabetes.

IMMUNOLOGY

The Department of Immunology continues its original vision, with a dual focus on both immunology and structural biology. The unique combination of biological and structural studies and the intensive exploration of structure-function relationships have created a thriving, productive environment that has encouraged fruitful collaboration among investigators. The research in the department is focused on the role of T cells in immunity, structure analysis with studies on SUMO, computational approaches to study the structure, function and dynamics of G-protein coupled receptors, and determining the role of CEACAM1 in T-cell activation and mammary epithelial cell polarization.

MOLECULAR AND CELLULAR BIOLOGY

Research interests in the department include an array of biological systems and problems, but the unifying theme is mechanisms regulating expression of genetic information at both the transcriptional level (where DNA directs the synthesis of RNA) and the post-transcriptional level (meaning how genes control protein synthesis from newly transcribed RNAs).

MOLECULAR MEDICINE

The department investigates the mechanisms underlying cancer and other diseases to develop novel molecular therapeutics. The mission of the Department of Molecular Medicine includes identifying new molecular targets for cancer therapy, developing small synthetic molecules and natural product derivatives to address these therapeutic targets, and evaluating genomic markers for predicting cancer risk and response to therapy. To accomplish these objectives, molecular medicine researchers employ leading-edge approaches and technologies. These include high throughput screening of medicinal plant extracts and chemical compound libraries, organic and bioorganic synthesis, and analyzing the genetic basis for disease using functional genomics, proteomics, microarray gene expression profiling, and protein X-ray crystallography. The research in the department focuses on the developing and synthesis of molecular-targeted anticancer agents, identifying STAT proteins as new molecular targets for cancer therapy, and using X-ray crystallography to study protein-protein and drug-protein interactions for the design of novel therapeutic agents for the treatment of cancer.

NEUROSCIENCES

Study of the nervous system has a long history at City of Hope, which was one of the first institutions in North America to establish a neurobiology research department. The Department of Neurosciences offers a multidisciplinary research and training environment in neurobiology, with a particular focus on developmental aspects of the nervous system. Research in the department encompasses molecular and cellular neurobiology, genetics and neurophysiology, with ongoing studies in neurogenesis and synaptogenesis, migration and specification, degeneration and embryonic stem cell differentiation. Researchers in the department collaborate with colleagues in other basic science departments and divisions as well as clinical researchers in neuro-oncology programs, focusing on cancer immunotherapy and neurosurgery. The Department of Neurosciences spans a broad range of research interests which include early electrical activity in the developing hippocampus and cortex, regulation of transmitter phenotype, studying the nuclear receptors that control generation and differentiation of neural lineage stem cells in the adult nervous system.

VIROLOGY

The Department of Virology strives to better understand the origin and development of herpes simplex virus and other herpes viruses, the biology of cytomegalovirus (a prime concern for HIV-infected and other immunocompromised patients such as transplant recipients), vaccine development and experimental therapies using gene transfer vectors such as adeno-associated virus (AAV) and lentivirus. Viral vectors have shown great promise in treating both cancers and HIV. The research in this department is focused on the role of cytokines in herpes simplex virus (HSV) infections in vivo, developing vaccines to combat hematologic malignancies, solid tumors and infectious pathogens, and developing lentiviral vectors such as HIV and FIV to deliver transgenes into cells for potential treatment of human diseases.

To facilitate scientific progress, City of Hope provides investigators and their laboratories access to sophisticated support services and state-of-the-art equipment through many of our core facilities.

ANALYTICAL CYTOMETRY CORE

This core provides researchers with high-quality flow cytometry instrumentation as well as expertise in analyzing and/or sorting sample populations of interest via interpretation of their physical fluorescent and/or light-scattering properties.

ANALYTICAL PHARMACOLOGY CORE

This core encourages and facilitates collaborative research between basic scientists and clinicians by conducting pharmacokinetic studies for both chemotherapy clinical trials and peer-reviewed preclinical studies.

ANIMAL RESOURCES CENTER

City of Hope maintains a centralized animal care and use program, which is registered by the United States Department of Agriculture and accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC). The mission of the Animal Resources Center (ARC) is to enhance excellence in research and teaching through the provision of high quality animal care consistent with the Public Health Service Policy on Humane Care and Use of Laboratory Animals, the Guide for the Care and Use of Laboratory Animals, the Animal Welfare Act, and other applicable state and local regulations.

BIOMEDICAL INFORMATICS

This core consists of hardware, software, and an interdisciplinary team of experts with a few objectives. They support the information collection, classification, storage, dissemination, integration and visualization needs of our investigators. It identifies and forges opportunities for synthesizing the results of clinical and basic science research.

CENTER FOR APPLIED TECHNOLOGY DEVELOPMENT

To help bring new discoveries and developments to market, the Sylvia R. and Isador A. Deutch Center for Applied Technology Development provides both internal and external entities licensing services and patent documentation.

CENTER FOR BIOMEDICINE & GENETICS

Our Center for Biomedicine & Genetics manufactures promising new genetic and cellular agents created by researchers for use in clinical trials. We are uniquely equipped to evaluate therapies swiftly and move lifesaving drugs into the marketplace with great speed.

CLINICAL PROTOCOL MANAGEMENT

This core provides expertise in the areas of protocol administration and data management to the investigators of the comprehensive cancer center. The staff assists with screening patients for eligibility, managing studies, collecting study data, monitoring regulatory compliances, IRB submissions, and participating in training.

DNA SEQUENCING

The objective of the DNA Sequencing Lab is to provide convenient, rapid, and cost effective DNA analysis for all City of Hope investigators.

ELECTRON MICROSCOPY

The core is available to define ultrastructural details in their experimental systems. The EM Core assists with all aspects of studies brought to the lab including training, experimental design and interpretation of results.

FUNCTIONAL GENOMICS

This core comprises the following four services: microarray, genetic markers, RNAi, and data analysis services.

GRAFF MEDICAL AND SCIENTIFIC LIBRARY

The Graff Medical and Scientific Library serves the information needs of the City of Hope community. It furnishes materials and services that support the institution's clinical, scientific and educational mission. The Graff Library offers an expensive collection of scientific and biomedical journals and online databases, as well as a wide array of other services. There are study spaces and a computer lab with Internet/intranet-accessible workstations and printers. A copy room allows both black-and-white and color printing and the creation of .pdf files.

HIGH THROUGHPUT SCREENING (HTS)

This core's mission is assist in identifying novel lead compounds for anticancer drug development and chemical probes useful for biochemical mechanistic studies via sophisticated high throughput screening assays of synthetic compound and natural product libraries.

MASS SPECTROMETRY AND PROTEOMICS

The core provides high-quality analysis of biomolecules. Notable capabilities include: Purified proteins can be rapidly identified; relative protein expression levels can be determined for lysates of whole cells or subcellular organelles; and absolute levels of specific peptide components in complex biological samples can be determined via multidimensional separation and MS analysis.

NUCLEAR MAGNETIC RESONANCE (NMR) SPECTROSCOPY

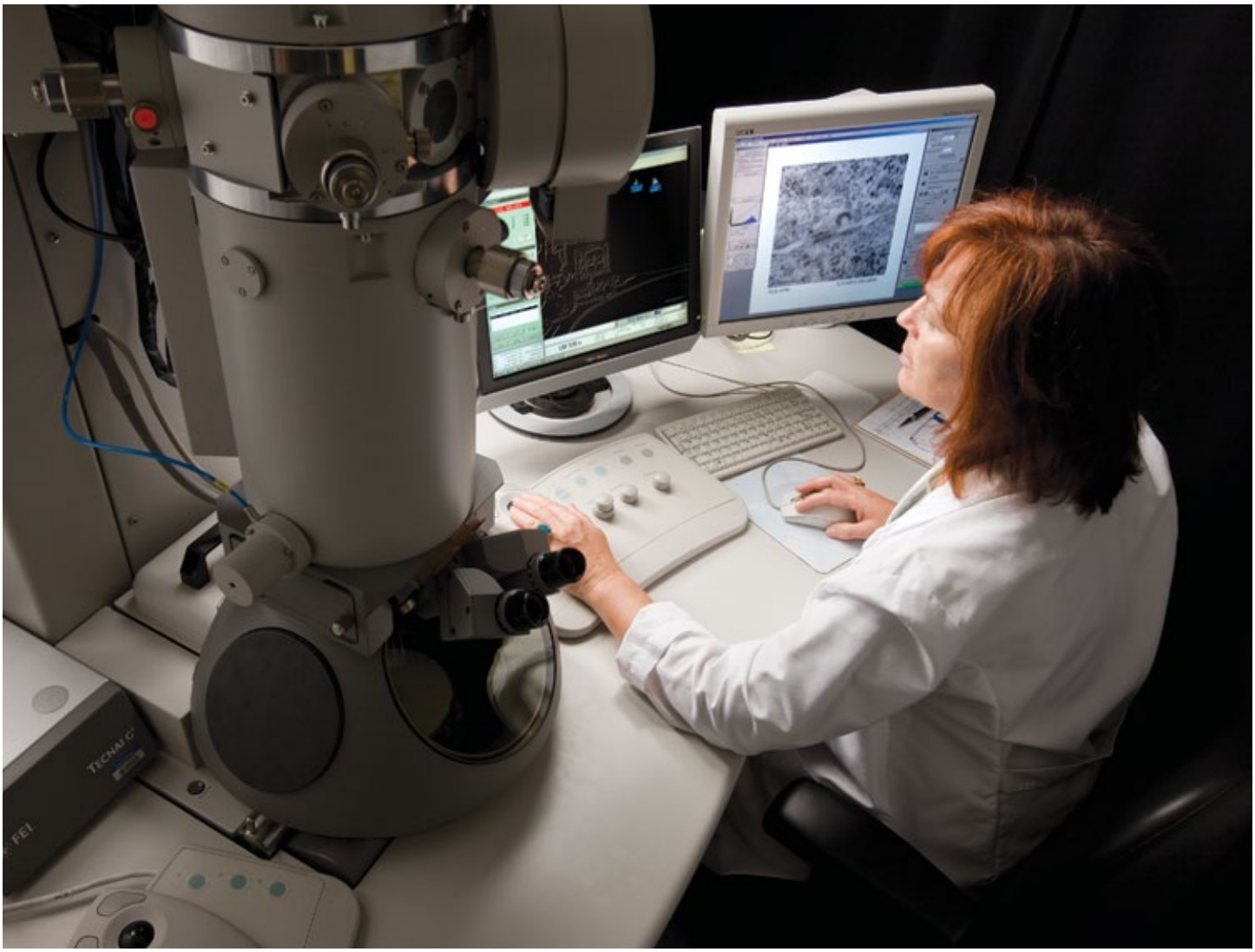
The main focus of the NMR is to apply high resolution NMR spectroscopy to the structural characterization of biomolecules, small organic compounds, natural products, and biomolecular complexes including protein-protein, protein-nucleic acid and protein-ligand complexes.

PATHOLOGY

This facility provides histologic processing of tissues including paraffin embedding, sectioning, and hematoxylin and eosin staining of human tissue, animal tissue, and preparations from cell lines. The facility also performs and interprets immunohistochemistry for the detection of cell products in normal and disease states.

SYNTHETIC AND BIOPOLYMER CHEMISTRY

The general capabilities of the core include the design and synthesis of highly specialized biopolymers, including siRNA-aptamers, DNA-peptide hybrid derivatives, and peptides. The core is capable of synthesizing very complex molecules, as well as the synthesis of small-molecule agonists and antagonists, imaging agents, affinity ligands, nanoparticles and focused combinatorial libraries.



ADMISSIONS

Admission to study at the Irell & Manella Graduate School of Biological Sciences is offered to students who are curious about science and demonstrate sound undergraduate training in biochemistry, chemistry, biology or a related biomedical field. Students lacking preparation in a particular subject, but otherwise qualified, may be admitted and given the opportunity to strengthen their background by taking the appropriate courses.

The graduate school strongly encourages applications from students regardless of national or ethnic origin, sex, sexual orientation, marital status, race, age, color, citizenship or disability.

Applicants should provide official transcripts documenting grades in the following courses:

- Mathematics
- General physics including laboratory
- Chemistry (general and organic) including laboratory
- Biology
- Biochemistry

Courses in genetics, cell biology, physiology, developmental biology, molecular genetics, and neurobiology help to strengthen an applicant's background and are recommended.

In addition to academic achievement, the Admissions Committee also evaluates an applicant's potential for scientific achievement through letters of recommendation, a personal statement of his/her experience, goals, prior research experience, GRE scores, and TOEFL scores, if applicable.

A minimum of three letters of recommendation are required. Recommenders should provide evidence of the applicant's potential to conduct significant research. Letters should be submitted through the online system by registering your recommenders' names and email addresses. The recommender will then receive instructions on how to upload and submit a recommendation electronically. Recommendations will also be accepted by email or mail.

Official scores must be sent by ETS to the Graduate School Office. The general GRE test is required and a subject test is strongly recommended. The school code for Irell & Manella Graduate School is 4076. The TOEFL test is required for all students whose native language is not English. The school code for the TOEFL is 2268.

Colleges and universities must mail official transcripts directly to the Graduate School Office.

Supplemental materials should be sent directly from the institutions and testing agencies, but we will also process your application if all of the supplemental materials are sent initially to your address in officially sealed envelopes and then you place all the supplemental documents (still in their officially sealed envelopes) in a larger envelope and send them directly to our office at one time. Your application will be evaluated only if all the materials have been received.

Applications must be submitted online at https://apply.gradschool.cityofhope.org. There is no application fee. The application deadline is January 1. Orientation begins in late August and classes begin the first week of September.

For information, please contact:
City of Hope
Irell & Manella Graduate School of Biological Sciences
Graduate School Office
1500 East Duarte Road
Duarte, CA 91010

Tel: 877-715-GRAD
Email: gradschool@coh.org
www.cityofhope.org/gradschool

PH.D. REQUIREMENTS

The Graduate Program in Biological Sciences grants a Ph.D. degree upon completion of all of the requirements, which include coursework, lab rotations, qualifying examinations, dissertation research, and dissertation defense. Each requirement is described in more detail below. Students devote full time to study and research. The number of years dedicated to this pursuit depends on the student's prior training and the dissertation project chosen.

During the first year, students must complete the core curriculum, which is comprised of lecture courses and lab rotations. There are six courses taken during the first year: Biochemistry, Molecular Biology, Genetics, Cell Biology, Biostatistics/ Bioinformatics, and Current Topics in Biology. Core courses are described below. Students must achieve a grade of A or B in order to receive credit in a course.

Students are expected to spend half of their time on coursework and half of their time in the rotation labs. Students are required to take two rotations to increase their exposure to different laboratories and select a thesis laboratory that matches their research interest. By the end of the first year, all students should have achieved a broad-based understanding of biology through the didactic coursework, lab rotations, and scientific seminars.

Elective courses are offered on a two-year cycle in the following areas: RNA Biology, Immunology, Cancer Biology, DNA Damage and Repair, Chemical Biology and Stem Cell Biology. In consultation with the associate deans and their advisers, students may choose additional courses and/or tutorials.

Students must have passed all relevant requirements prior to taking the qualifying examinations. There are two qualifying examinations, one focused on research distinct from the student's thesis research and one focused on the student's thesis research. The goals of these exams are to rigorously test the ability of the student to design a research plan, present the proposal in a formal seminar and discuss the material presented. The written proposal should be in the style of a NIH grant application. After the proposal is approved by the Graduate Oversight Committee and/or the qualifying exam committee, the student must defend the proposal orally. Students are required to pass both qualifying examinations for advancement to doctoral candidacy.

Beginning in year three, an annual thesis committee meeting is required. This will help the student remain on track and bring his/her thesis project to a successful and timely completion. The final requirements for the doctoral degree are the thesis manuscript and the oral defense of the thesis. The oral defense will be in front of a panel of faculty members, an external expert in the field and the entire City of Hope community.

The diagram on the next page outlines the progression through coursework, qualifying exams, thesis work and graduation.

Occasionally, students will require more than five years to complete their degree. All sixth year students and beyond are monitored very closely by the graduate school administration and thesis committee to ensure timely completion of their degree.

PATH TO GRADUATION

	Fall Semester	Winter Semester	Spring Semester	Summer Semester
Year 1	Biochem/MolBiol/Genetics	Biostatistics/Bioinformatics	Current Topics in Biology	*Qual. Exam part 1 — Research Proposal due end of September*
		Cell Biology		
	Rotation 1	Rotation 2		
	Leading-edge Lecture (two per year in the first year)			
Year 2	Advanced Topics Class		Scientific Writing	
	Journal Club			
	Leading-edge Lecture (six per year)			
			Qual. Exam part 2 — Thesis Proposal due end of June	
Year 3	Advanced Topics Class			
	Journal Club			
	Leading-edge Lecture (six per year)			*Yearly Dissertation Committee meeting*
Year 4	Journal Club			
	Leading-edge Lecture (six per year)			*Yearly Dissertation Committee meeting*
Year 5	Journal Club			
	Leading-edge Lecture (six per year)			*Dissertation Defense*

CITY OF HOPE GRADUATE COURSES

BIOSCI501.	Advanced Biochemistry [R]
BIOSCI502.	Advanced Molecular Biology [R]
BIOSCI503.	Advanced Genetics [R]
BIOSCI504.	Advanced Cell Biology [R]
BIOSCI505.	Advanced Biostatistics / Bioinformatics [R]
BIOSCI506.	Current Topics in Biology [R]
BIOSCI507.	Laboratory Rotation [R] Graded pass/fail.
BIOSCI536	Responsible Conduct of Research [R] Graded pass / fail.
BIOSCI537.	Scientific Writing [R]
BIOSCI538.	General Seminar in Biological Sciences (Journal Clubs) [R] Seminar, 1 hour; discussion, 1 hour. Oral reports by graduate students on current research topics in the biological sciences. Graded pass/fail. Course is require every year in the second year and beyond.
BIOSCI539.	Leading-edge Lecture Seminar [R] Graded pass/fail.
BIOSCI550.	Advanced Topics in RNA [E]
BIOSCI551.	Advanced Topics in Cancer Biology [E]
BIOSCI552.	Advanced Topics in Stem Cell Biology [E]
BIOSCI553.	Advanced Topics in Immunology [E]
BIOSCI554.	Advanced Topics in Neurosciences [E]
BIOSCI555.	Advanced Topics in Stem Cell Biology [E]
BIOSCI556.	Advanced Topics in DNA Repair [E]
BIOSCI557A, B557B.	Advanced Topics in Comparative Medicine [E]
BIOSCI591.	Independent Study [E]
BIOSCI 599.	Research for Dissertation [R] Graded pass/fail. Prerequisite: Advancement to Candidacy. Graded Satisfactory (S) or No Credit (NC). Course is repeatable.

R = required; E = elective.

Students are required to take two electives after the first year of study. Students in the Residency/Graduate Training Program in Laboratory Animal Medicine track are required to take BIOSCI557A and BIOSCI557B and one elective.

BIOCHEMISTRY — B501 — 3 CREDITS

This course covers several areas of biochemistry congruent with teaching faculty members' areas of interest and expertise. The course is divided into two halves, with the first half focusing on structural and functional aspects of nucleic acids and proteins, and the second half on the structural and functional aspects of carbohydrates, lipids, enzyme kinetics, biomolecules, and metabolic diseases.

Course Coordinator: David Horne, Ph.D.

MOLECULAR BIOLOGY — B502 — 3 CREDITS

This course is meant to bridge the gap between study of molecular biology at the undergraduate and the advanced course levels. Students will become familiar with the primary literature covering fundamental topics of molecular biology including DNA replication and repair, transcription and processing, RNA interference, protein biosynthesis and degradation, chromatin remodeling and cell cycle. It is expected that this course will form the intellectual foundation for further investigation into the student's field of interest, as well as to foster awareness of the connections between different fields.

Course Coordinator: Adam Balis, Ph.D.

ADVANCED GENETICS — B503 — 3 CREDITS

Lectures are designed to introduce students to basic concepts of genetics, as currently applied to model systems and mammals, to genomics and epigenetics, and to cancer susceptibility. The field of genetics is rapidly advancing, and so it is important to refine skills for evaluation of published literature, and for framing meaningful questions. Lectures and exams provide opportunities to exercise analytic skills through speaking and writing. Major themes include model systems, mammalian genetics, genomics, epigenetics, and genetic epidemiology.

Course Coordinator: Adam Balis, Ph.D.

CELL BIOLOGY — B504 — 3 CREDITS

This course is divided into three sections: (i) cell structure and interactions; (ii) signal transduction and cell physiology; and (iii) development, stem cell biology and virus-cell interactions. Topology of the cell, including cytoskeleton structures, is discussed as a prelude to understanding protein trafficking. The basics of innate immunity also will be covered, from the cells involved to the pattern recognition receptors and the pathogen they recognize.

Course Coordinators: David Ann, Ph.D. and Nagarajan Vaidehi, Ph.D.

BIOSTATISTICS/BIOINFORMATICS — B505 — 3 CREDITS

This course is divided into two sections. In the first section, biostatistics, students are trained in theory and applications of statistical methods. In the second section, bioinformatics, students apply information technology to the study of biological problems. Students learn to use and search various types of databases.

Course Coordinators: Jeffrey Longmate, Ph.D. and Yate-Ching Yuan, Ph.D.

CURRENT TOPICS IN BIOLOGY — B506 — 4 CREDITS

The main goals of this course are the development of the ability to read scientific journals proficiently, to think and write logically about experimental approaches to biological problems, and to successfully debate scientific issues with colleagues. The primary literature is the route by which professional scientists obtain information regarding their field of endeavor, and contribute to it. Therefore, graduate students must develop some mastery of the literature in their field before they can commence their studies. This represents one of the greatest barriers to transitioning from an undergraduate to a graduate student. The course consists of eight topics. TAs will hold two classes: one to introduce the topics and one for Q & A.

Course Coordinators: Timothy O'Connor, Ph.D. and Markus Kalkum, Ph.D.

Lauren Liddell

Graduating from Michigan State was an exciting time for me. I was finally joining the "real world." As an undergraduate, I worked as a teaching assistant, and in two research labs. I always knew that I wanted to do research; however, I didn't know that I wanted to continue this journey at City of Hope.

After taking the GRE, I received an abundance of letters advertising the benefits of a specific university. I remember opening the letter from City of Hope and being initially intrigued by the "bench-top-to-bedside" research approach. Because I'd never heard of the City of Hope Graduate School of Biological Sciences, I almost threw the letter away, but then I reconsidered. What did I have to lose? I began to research the program.

A few months and six long hours later, I stepped off a cramped plane into beautiful Southern California. I liked the small, close-knit atmosphere, which was very different from Michigan State's 50,000-student campus. City of Hope students and faculty were very friendly and approachable. I was also intrigued by the accessibility of the core facilities to the graduate students. On top of everything else, the campus was beautiful.

Three years later, I'm glad that I decided to join City of Hope for my graduate studies because my research lab is an ideal environment for scientific training. My thesis advisor, Adam Bailis, Ph.D., is the perfect amalgamation of teacher and motivator. He provides intellectual stimulation and scientific discussion on a regular basis: formally through lab meetings and journal club, and informally at the bench.

Our lab is concerned with understanding the basic mechanisms that drive genomic instability. We use the budding yeast, Saccharomyces cerevisiae, to study a subset of DNA repair called homologous recombination. My thesis project focuses on further defining the role of a key, but not well understood, protein in this process: Rad59. By making mutants in residues homologous to the mammalian protein, and then studying their genetic and molecular effects, I plan to break Rad59 apart to better understand its role in DNA repair.

After graduate school, my overall goal is to combine my passions for teaching and research by managing my own research laboratory at a university so that I can spread my love for science and contribute to our growing knowledge of the biological world.

Sergey Nechaev

Before coming to City of Hope, I graduated from the Novosibirsk State University, located in the heart of Russian Siberia. My first acquaintance with international science took place in Germany during a cancer immunology internship, and I firmly decided to seek for a Ph.D. position abroad. I was asked many times: "How did you find City of Hope?" And yes, I should admit that my destiny was changed by Google. The first thing to catch my eye was this unusual name, second, the broadness of the program, and after reading the research profiles of professors, I told myself, "this is a scientific paradise."

My interests changed while I was going through multiple research rotations, and finally I have chosen to do my thesis research in the laboratory of Marcin Kortylewski, Ph.D. His lab turned out to be an unbelievable blend of RNA, which I mastered before, and my new interest, cancer immunology. My current research focuses on understanding the molecular mode of action of CpG-STAT3-siRNA conjugate, a novel therapeutic agent developed in our lab, which is able to penetrate certain types of immune cells without the use of potentially toxic transfection agents. Use of this construct enables to overcome immunosuppressive effect of the tumor microenvironment, and effectively suppresses tumor growth and metastasis. I have always enjoyed working with Dr. Kortylewski. He is an open-minded and intelligent mentor, respecting new ideas and always open to discussions.

I should also mark out an incredible amount of emotional and financial support from the graduate school. Apart from prestigious Berger and Parson's fellowships that I was awarded, the school provides a unique chance to get a certificate degree in bioscience management in collaboration with the Keck Graduate Institute. As a goal-oriented person, I cannot underestimate the opportunity to look at the life sciences from both scientific and market points of view. After graduation, I am planning to develop my further career in the bioscience industry, and I am sure that a Ph.D. from City of Hope will serve as a solid platform for those goals.

Swati Kadam

Swati Kadam is a third-year graduate student at City of Hope. She moved to the United States from Mumbai, India, when she was thirteen. She attended high school in California and earned her B.S. degree from the University of California, Davis, majoring in genetics and taking a minor in psychology.

To explore the field of epigenetics, she chose to do her thesis research in the laboratory of Gerd Pfeifer, Ph.D. Her thesis focuses on epigenetic regulation and how it can be manipulated. In addition to completing her courses at City of Hope, she is taking MBA courses at the nearby Keck Graduate Institute, working toward a certificate in bioscience management. "I have learned so much in such a small amount of time. I knew I'd be exposed to a myriad of ideas, but coming here and being part of the system sculpted me into an all-rounded researcher. You know when you are in the right place," she said, "when something feels like home. I would say that about Dr. Pfeifer's lab and City of Hope. It feels like home."

Kadam has been involved in student activities. "Living in student housing during my first year helped me grow a lot. We would have late night study sessions before exams, where we could all meet and help clarify concepts the other would have. Not only did we study hard, but because we all lived nearby, we would also have a lot of social events, which helped us mold into a large family. It was nice knowing that someone was around in a place where we didn't know anyone."

Marisa Bowers

Marisa Bowers, a third year Ph.D. candidate in the laboratory of Ravi Bhatia, M.D., is studying the role of the normal bone marrow in both supporting leukemic stem cell survival and in protecting leukemia stem cells from treatment, those of which can lead to relapse.

Bowers joined the City of Hope community in 2005 as part of the Eugene and Ruth Roberts Summer Student Academy, during which time she worked in Dr. Karen Aboody's laboratory studying glioblastoma. Marisa returned to the Summer Student Academy, and Dr. Aboody's laboratory, the following summer, and continued working with Dr. Aboody during her senior year at Occidental College.

In 2008, Bowers joined the Irell and Manella Graduate School of Biological Sciences, and as a first year student was awarded a Berger Foundation Fellowship. In 2009, she received a three year CRIM grant to fund her thesis research. Bowers is excited at the path her project has taken over the last two years.

Marisa is an active member of the Graduate Student Organization, having served as First Year Representative, Treasurer, President, and Student Recruitment Committee co-chair, and she organizes the Grads for Hope team for the annual Los Angeles Walk for Hope. In the future Bowers hopes to obtain a faculty position in order to teach and run a laboratory.

STUDENT HOUSING

Living in student housing connects you to our vibrant campus and all of its resources. Each house or apartment has unique qualities based on its architecture, size and location, and your living experience is ultimately shaped by the residents who live there. Each residence's personality changes every year based on the residents' contributions to their community.

First-year students may choose to live in on campus housing, which ranges from $600 – $750 a month. Housing is also available within the immediate area at a cost of approximately $800 – $1200.

Students may express their interest in student housing by filling out an 'Application for Occupancy' form which is available on the website or in the Office of Graduate Education. The housing lease is an eleven-month term starting the day you move in through July 31.

Each room is supplied with a bed, desk, desk drawers, chair, and dresser. Additional room furnishings may be supplied depending on the type of room being rented. In addition to bedrooms, each apartment or house contains a living room, dining area and kitchen. The living area is furnished with a couch, chair, coffee table and television. The dining area contains a table and chairs. The kitchen is supplied with dishes, utensils, pots and other kitchen supplies. Each of the houses contains a washer/dryer, and the apartments share a washer/dryer. Additional appliances and furnishings may be supplied depending on the unit rented.

RECREATIONAL ACTIVITIES

Living in Southern California places you at the center of local tourism and the entertainment industry, where each year millions of tourists visit Disneyland, Magic Mountain, Universal Studios, and television studios. The area is a thriving cultural center, with wonderful museums including the Getty Center, Norton Simon Museum, Huntington Library, Museum of Contemporary Art, and the California Science Center. Stand-up comedy, jazz, rock and comedy clubs are sprinkled throughout the area. Nearby Pasadena hosts the Rose Parade on New Year's Day and the exciting street life in Old Town Pasadena. Sports fans can enjoy professional baseball (Dodgers and Angels), basketball (Lakers and Clippers), hockey (Kings and Ducks) and soccer (Galaxy), as well as outstanding collegiate sports teams.

In this ethnically diverse region, one can explore the food and culture of peoples from different countries. Likewise, the City of Hope campus has an international, cosmopolitan atmosphere, attracting talented scientists from all over the world who feel at home here. Additionally, Southern California is home to many great universities, and our faculty members collaborate with their colleagues at nearby institutions including Caltech, USC, and UCLA.

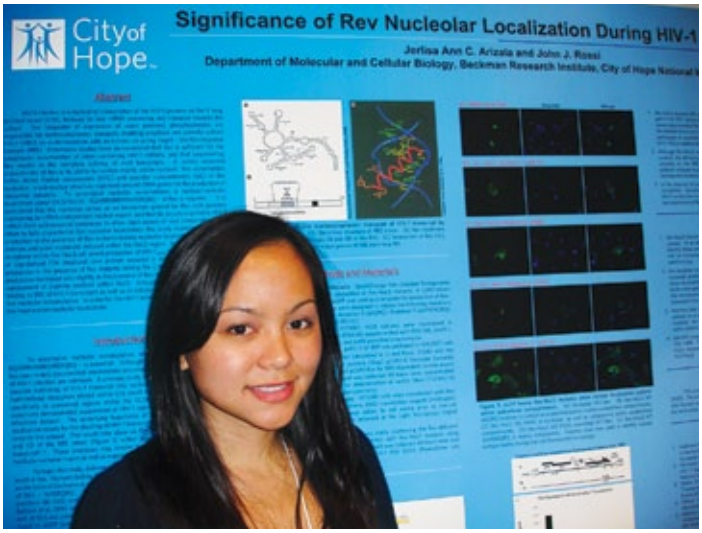
Nestled in the foothills of the San Gabriel Mountains, Duarte is a serene community in which to live and work. The subtropical and semi-arid climate offers year-round outdoor activity. The San Gabriel Mountains are lined with hundreds of miles of hiking and mountain biking trails. An hour's drive in different directions can take you to ocean beaches, snow skiing, or desert camping. Many of our students and faculty are avid outdoorsmen, and several ride their bikes to work.

STUDENT SUCCESS

Graduation Rate: 81 percent
Time-to-Degree: 5.1 years

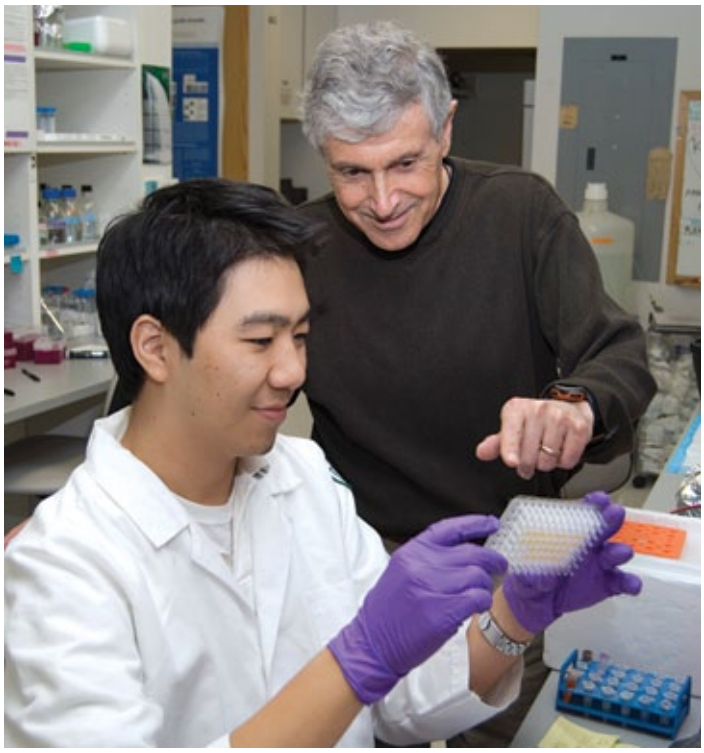
By the time he or she graduates, the typical student has published three scientific papers and attended two scientific conferences.

Students are encouraged to present their research at national and international meetings, including Germany, Italy, England, Hong Kong, Shanghai, and British Columbia. One of the students wrote, "*I recently presented a poster at a national conference and was pleasantly surprised at the response generated from my work. Presenting work at a specialized conference where many scientists understand and appreciate your work is a very rewarding experience.*"



Jerlisa Arizala presents her research at the 2010 Lake Arrowhead Conference.

City of Hope students have won a number of fellowships, awards and prizes. While working with John Rossi, Ph.D., **Daniel Kim** won the National Academy of Sciences' Cozzarrelli Prize for his 2008 first-author, graduate student paper selected as the most outstanding contribution in the biological sciences published last year in the *Proceedings of the National Academy of Sciences USA*. Six PNAS papers received the Cozzarrelli Prize out of 3,500 published.



Kim is the second City of Hope graduate student whose research has been honored by PNAS. The first was **Jing Song**, whose thesis advisor was Yuan Chen, Ph.D.

In 2008, The American Chemical Society's Division of Toxicology presented graduate student **Daniel Tamae** with a young investigator award at its national meeting in Philadelphia, one of only six students to receive such an award at the conference.

A 2008 *PLoS Genetics* paper by students **Seung-Gi Jin** and **Cai Guo**, with Professor Gerd Pfeifer, Ph.D., was selected for the prestigious Faculty of 1000 database of important publications.

A 2010 *Nucleic Acids Research* paper by **Seung-Gi Jin** (alumnus), **Swati Kadam** (current student), and Professor Gerd Pfeifer, Ph.D., also was selected for the prestigious Faculty of 1000 database of important publications.

Mike Lewis, Ph.D.

Mike Lewis was City of Hope's first graduate and the first to earn tenure at a research university. He arrived with a B.S. in chemistry from the University of North Carolina, Chapel Hill, and an M.S. in chemistry from California Institute of Technology. His thesis research with John E. Shively, Ph.D., developed means of attaching radioactive metals to monoclonal antibodies for tumor imaging and therapy. His work contributed to a radioimmunotherapy clinical trial.

After graduating, Lewis took a postdoctoral fellowship at Washington University, St. Louis, and then received a faculty appointment at the University of Missouri, Columbia, where he is now an associate professor. Looking back, he recalls, "The unique strength of the graduate school is having access to all components of a research project under one roof. During my studies there, I was able to work in a collaborative manner with chemists, biochemists, radiation oncologists, medical physicists, nuclear pharmacists, molecular biologists, and engineers of protein design. That is exactly like the collaborative nature of the work I do now. The unique research experiences I had at City of Hope have been a tremendous advantage throughout my career."

Yuqing "Kate" Tu, Ph.D.

Kate was the second graduate. She came to City of Hope with a bachelor's degree in molecular science from NanKai University and a master's degree in biology from East Tennessee State University.

At City of Hope, she conducted her thesis research with Gerd Pfeifer, Ph.D., studying genes involved in deficient repair of DNA damage caused by ultraviolet light, leading to mutations and the development of skin cancer.

After a postdoc at UCLA, she went back to school and earned an MBA from the University of Southern California. Since then she has moved up the corporate ladder and is currently a vice president for Citbank, stationed in Shanghai.

"Gerd was a great mentor for me," she says. "With his guidance, I completed my Ph.D. in four years, with three publications in major scientific journals. The school provided an excellent curriculum and learning environment."

Rob Ring, Ph.D.

Rob Ring, Ph.D., earned a bachelor's degree in fine arts and biology at Westmont College in Santa Barbara and a master's degree in molecular biology at California State Polytechnic University, Pomona.

While at Cal Poly, he conducted his master's research at City of Hope and then applied to the graduate school.

Ring conducted his thesis research jointly with Judith Singer-Sam, Ph.D., and Michael Barish, Ph.D. His dissertation was a microarray analysis of the genes involved in the central nervous system's response to immunogenic challenges.

After graduating, Ring became a senior scientist and team leader at Wyeth Pharmaceuticals in Princeton, New Jersey, where he is now the head of Neurobiology. He is also an adjunct associate professor at Mount Sinai School of Medicine.

"The breadth of my training and quality of the mentorship I received at the City of Hope have had a significant impact on my success in the pharmaceutical industry. Training in an institution like the City of Hope, where basic research is being translated into medical practice in front of your eyes, has provided me with an excellent understanding of the challenges that all fields face as we aim to discover and develop treatments for human disease. Exposure to this translational environment distinguishes the City of Hope graduate from, and perhaps provides them an edge over, their peers as they advance into the varied professional paths of research science."

Reena Vishwanath Thomas, Ph.D.

Reena Vishwanath Thomas, Ph.D., did her undergraduate studies at Cornell University, specializing in neurobiology and Latino studies. Before applying to the graduate school, she spent two summers as an intern in City of Hope's Eugene and Ruth Roberts Summer Student Academy. In her application to the graduate school, she wrote, "I am proud to be a first-generation American, having a father from India and a mother from El Salvador. Although it saddens me to see so few Latina women holding positions of power in the scientific community, this struggle gives me the inspiration to work hard in life. I hope to not only make a difference in the scientific community, but contribute to the diversity that should exist in this environment."

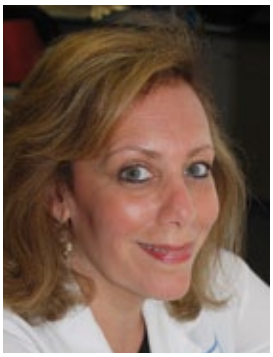
She conducted her thesis research with Michael Jensen, M.D., studying the ability of genetically modified tumor-specific cytotoxic T-lymphocytes to home to glioma tumors. After graduating, Thomas earned an M.D. at Georgetown University School of Medicine and she is now a resident in internal medicine at Stanford University Hospital. She plans to be a physician-scientist in the field of neurology.

She writes, "A unique aspect of the City of Hope graduate school is the intimate community of scientists who are dedicated to advancing the academic pursuits of their graduate students. This environment provides the opportunity for mentorship across disciplines, fosters collaboration, and promotes critical thinking in thesis project development. I feel fortunate to have been a part of this rich academic environment and gained a diverse portfolio of both technical experience but, more importantly, the tools necessary to become an independent investigator."

City of Hope™

Graduate School of Biological Sciences

Faculty



Karen S. Aboody, M.D.

Associate Professor, Department of Neurosciences; Division of Neurosurgery

NEURAL STEM CELLS — THERAPEUTIC APPLICATIONS

My translational research laboratory focuses on neural stem cells (NSCs) and their therapeutic applications for primary and metastatic tumors. Our novel findings have demonstrated the inherent tumor-tropic property of NSCs, and their use as cellular delivery vehicles to effectively target and disseminate therapeutic agents to invasive tumors, including glioma, neuroblastoma, breast carcinoma and melanoma. Their capacity for tracking infiltrating tumor cells and localizing to distant micro-tumor foci make NSCs a novel and attractive gene therapy vehicle with tremendous clinical potential.

We use brain tumor and systemic solid tumor animal models to test intracranial and intravenous administration of NSCs to target various therapeutic agents directly to tumor sites, resulting in localized chemotherapy production. Our lab has many cutting-edge, collaborative projects in progress. Our translational research focuses on genetically modifying human NSCs to produce different therapeutic agents, including pro-drug activating enzymes and antibodies, in pre-clinical tumor models, using real-time imaging techniques to monitor tumor growth and NSC migration (xenogen and MRI). We currently have an investigational new drug (IND) application pending with the FDA, to conduct a first-in-human NSC-mediated therapeutic study for recurrent glioma patients. We have also been granted a California Institute of Regenerative Medicine (CIRM) Disease Team Award to move a more advanced therapeutic toward clinical trials, which involves a large interdisciplinary collaborative team.

We are also trying to identify the biological mechanisms and signaling pathways involved in the directed migration of NSCs to tumor cells. We are collaborating with the Glackin laboratory to investigate NSC applications for metastatic breast cancer; the Barish laboratory to develop novel 3D reconstruction imaging techniques of NSC-tumor distribution and study tumor development and progression; and the Synold laboratory to study pharmacokinetics in an intracranial microdialysis animal model.

The field of stem cell research is promising, but still relatively new. There are many exciting directions of investigation to pursue in order to better understand stem cell function and development, with a wide array of potential clinical applications to explore. Our lab offers a unique experience for interested students with exposure to many laboratory techniques, including stereotactic animal surgery, tissue processing and immunocytochemistry, fluorescent microscopy, xenogen imaging, molecular biology, RT-PCR, cell culture, and flow cytometry studies.

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David K. Ann, Ph.D.

Professor, Department of Molecular Pharmacology

MOLECULAR MECHANISMS OF MAINTAINING GENOMIC INTEGRITY

The goals of my research program are to understand both the molecular mechanisms and signal transductions for maintenance of genomic integrity following DNA damage and to develop novel molecular therapies for human cancers by targeting dysfunctional or deregulated DNA damage responses. The DNA damage-induced signaling pathway consists of a kinase-dependent signaling cascade that regulates cell cycle progression, DNA repair, and cell cycle arrest/apoptosis. It is the coordination of these events that ensures genomic stability. Our current interests are (1) deciphering the crosstalk of SUMOylation, phosphorylation and ubiquitylation utilized by ATM activation to regulate KAP1-mediated chromatin remodeling, rendering genomic stability, and (2) elucidating the role of other post-translational modifications and signaling transduction pathways in cellular adaptive responses to hypoxia. Understanding these molecular pathways will assist us to develop better therapeutic approaches against solid tumors. In addition, we also focus on investigating the role of autophagy and autophagy-associated events in cellular adaptive responses to genotoxicity or extracellular stress.

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Adam M. Bailis, Ph.D.

Associate Professor, Department of Molecular and Cellular Biology

HOMOLOGOUS RECOMBINATION IN THE MAINTENANCE OF GENOME STABILITY AND TUMOR SUPPRESSION

Homologous recombination (HR) is the process by which similar or identical sequences in the genome exchange information. This process can be elicited by DNA damage or disruptions in DNA replication. The exchange of information may be conservative, preserving the normal sequence of genes on chromosomes, or non-conservative, leading to rearrangements of chromosomal material.

Translocations are rearrangements that transfer chromosomal material within and between chromosomes, and can result from HR between the dispersed, repetitive sequences that populate eukaryotic genomes. Such rearrangements are common in tumor cells, and are thought play an important role in tumorigenesis. Recently, we found that the loss of Rad51, a protein required for conservative mechanisms of HR leads to increased non-conservative HR, including translocations in the model eukaryote, *S. cerevisiae*. Additionally, loss of the function of Rad52 that facilitates Rad51 activity, which is analogous to the function of the human breast cancer susceptibility protein, BRCA2, also stimulates translocation formation. Genetic analysis shows that the HR that propagates these events is distinct from the normal mechanism of non-conservative HR, suggesting that disruptions of conservative HR stimulate a novel pathway toward genome instability. This is consistent with the observation of genetic linkage of mutations in the human Rad51 and BRCA2 genes with an increased risk of cancer.

The Rad51 protein forms filaments on single-stranded DNA in order to execute its function in HR. While this function is essential in human cells to rescue disruptions in DNA replication, the orderly and timely disassembly of these filaments is required for DNA replication to resume. Our recent genetic and molecular data suggest that removal of Rad51 in *S. cerevisiae* requires its replacement by Rad52 and its paralog Rad59, and that this is essential for life under conditions of DNA replication failure. Given the similar function of the analogous human factors, BRCA1 and Rad52 in HR and cell viability, we suggest that the primary function of HR is to restart DNA replication subsequent to failure. Partial loss of this activity in patients with mutations in the genes that encode these functions may induce tumor formation by leading to an accumulation of partially replicated DNA that contributes to widespread genome instability.

BRCA1 is a tumor suppressor protein whose function has been linked to HR, but whose mechanism of action is poorly understood. One of the barriers to understanding this mechanism is that BRCA1 is essential in mammalian cells, such that mutations that abrogate its function cannot be easily studied. Recently, we developed an assay where the function of BRCA1 in HR can be studied in *S. cerevisiae*. Genetic and molecular screens have been developed to investigate thousands of possible mutations in an effort to build a map of which amino acids in the BRCA1 sequence are important for HR. A detailed structure-function map of BRCA1 could be instrumental in predicting the potential pathogenicity of mutations in the BRCA1 gene found in patients, and could additionally be useful in designing more effective chemotherapeutic strategies.

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Michael E. Barish, Ph.D.

Professor and Chair, Department of Neurosciences
Associate Dean for Academic Affairs

IMAGING STUDIES OF NEURAL PROGENITOR AND BRAIN TUMOR CELLS

Malignant primary brain tumors, the majority of which are gliomas, are essentially uncurable despite aggressive treatments. Therapeutic failure is largely attributable to the presence of engrafted microfoci that are seeded by infiltrating glioma cells and that are exceptionally difficult to treat by surgical resection, irradiation, chemotherapy or gene therapy. In rodent models, immortalized neural progenitor cells (NPCs) introduced into the brain parenchyma or circulating blood will home to sites of engrafted human glioma. Further, close inspection of published micrographs reveals that individual NPCs can have extensive physical contact with tumor cells. Our major research interests are to understand mechanisms of NPC homing and binding to tumor cells in the context of on-going glioma cell infiltration of normal brain. In these investigations we are interested in determining the extent to which NPCs and brain tumor cells may be recapitulating processes active in developing brain.

a) Physiological mechanisms by which NPCs natively home to and recognize tumor cells.

As part of a California Institute of Regenerative Medicine-supported Disease Team program targeting glioma, and in collaboration with Karen Aboody, M.D. (Neurosciences), we are examining mechanisms of NPC migration and tumor cell recognition, and quantitatively assessing the efficiency and intratumoral distributions of NPCs that ultimately will be used to delivery therapeutic payloads. In one investigation, we are studying NPC recognition of brain tumor cells, the specificity of these contacts, and the molecular components of NPC-tumor contact zones. We observe that in a highly simplified culture system containing only two cell types, NPCs and tumor cell targets encapsulated in a peptide hydrogel, the NPCs will contact co-cultured glioma cells, and in time-lapse sequences will either proceed to encircle them, less often, move off them onto other neighboring cells. Our observations suggest that NPC–tumor cell interactions can occur in the absence of additional signals including those originating in surrounding brain. Another major effort, complementary to the highly restricted system described above, is approaching NPC-tumor interaction in vivo, clearly a much more complex system. Using an in vivo orthotopic xenograft model, and serial sectioning and 3-dimensional reconstruction of tumor and neighboring brain, we are determining the spatial relationships between NPCs and different regions of engrafted tumors: hypoxic core, proliferating periphery, disseminating edges, etc. Under these conditions the tumor microenvironment may be as important as the tumor itself, and we are examining the signals responsible for shaping the distributions of NPCs in and around tumors.

b) Processes of engraftment and niche creation by disseminating brain tumor cells.

In collaboration with Christine Brown, Ph.D. (Cancer Immunotherapeutics and Tumor Immunology), we are using a variety of characterized patient-derived brain tumor cell lines to understand the balance between cell intrinsic, environmentally-determined, mechanisms driving tumor cell dissemination to establish new tumor foci in normal brain tissue. Of particular interest is defining the meaning of "engraftment": when, where and why does a tumor cell stop migrating and pause to form a tumor focus? We are approaching this question using confocal microscopy and immunochemistry to visualize the interface between orthotopic human glioma xenografts and surrounding normal brain. We observe that in cortex growing tumors form tendrils penetrating the normal brain parenchyma, and believe that these structures are tumor cells migrating along blood vessels. At the same time, in striatum we see a different pattern in which cells emerging from the same tumors migrate along axon tracts. Immunochemical comparisons indicate that cell surface receptors and underlying cytoskeletal organization differ between cells in these two tumor microenvironments. Our goal is to understand the signaling processes in these brain microenvironments and their consequences for the tumor cell disseminating and disease progression.

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Jacob Berlin, Ph.D.

Associate Professor, Department of Molecular Medicine

NANOPARTICLES FOR THE DIAGNOSIS AND TREATMENT OF CANCER

Jacob Berlin's research group is focused on the application of nanomaterials for the diagnosis and treatment of cancer. As part of City of Hope's "bench-to-bedside" continuum, the Berlin lab is committed to developing novel therapies that will improve patient outcomes. City of Hope is a world leader in clinical trials, and with on-campus facilities capable of producing materials suitable for clinical trial use, the Berlin lab is focused on getting nanoparticle treatments into the clinic in a rapid manner.

Selective drug action on cancerous cells is the future of cancer therapy. Current targeting methodologies for nanoparticles usually result in an increase in tumor accumulation relative to non-targeted particles, but the majority of both classes accumulate in the liver and spleen. We are developing novel responsive nanoparticle coatings that result in more specific tumor accumulation. We are also interested in new antibody selection techniques to improve their efficacy when conjugated to nanoparticles.

In a parallel effort, we are investigating new classes of particles that allow effective delivery of biopolymer therapeutics. We are interested in preparing nanoparticles that hide the biopolymer payload from detection and destruction while in the blood stream and then release this payload once a cancerous cell is detected. As part of this work, we are also developing methodologies to control the assembly of very small nanoparticles into well defined larger nanoparticles.

We are also interested in evaluating side by side nanoparticles prepared from different materials. Most current studies focus on the efficacy of nanoparticles of just one type (e.g. gold, carbon, silica, etc). We believe that it is important to evaluate a variety of materials in one model to better understand what role the composition of the nanoparticles plays in their therapeutic utility.

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Ravi Bhatia, M.D.

Professor, Department of Hematology and Hematopoietic Cell Transplantation
Chief, Division of Hematopoietic Stem Cell and Leukemia Research

REGULATION OF NORMAL AND MALIGNANT HEMATOPOIETIC STEM CELL GROWTH

Hematopoietic stem cells in the bone marrow cavity provide a continuous source of blood cells during the lifespan of the individual. As with normal hematopoietic cells, leukemia cells may also arise from small subpopulations of leukemia stem cells. Our goal is to investigate mechanisms of regulation of normal hematopoietic stem cell growth; mechanisms underlying stem cell transformation in leukemia; and preclinical development of mechanism-based therapeutic interventions directed against malignant stem cells.

We are investigating molecular mechanisms crucial for stem cell transformation in CML, AML and MDS, which are lethal malignancies of stem cell origin. In addition to studying primary stem and progenitor cells from patients we are using novel human leukemia models based on viral delivery of oncogenes into human stem cells and transgenic mouse models of leukemia to investigate the specific molecular and cellular mechanisms responsible stem cell transformation. We are currently studying the role of stem cell-microenvironment interactions and mechanisms regulating quiescence, self-renewal and survival in growth, maintenance and drug resistance of leukemia stem cells.

An ultimate goal of these studies is to develop strategies for selective targeting of leukemia stem cells. We have shown that malignant stem cells persist in CML patients in complete remission following treatment with the BCR/ABL kinase inhibitor imatinib (Gleevec) and investigated key mechanisms underlying resistance of malignant stem cells to this agent. We have shown in preclinical studies that epigenetic regulation of gene expression in malignant stem cells with HDAC inhibitors can effectively target malignant stem cells. These observations have resulted in a clinical trial aimed at targeting residual leukemia stem cells in imatinib-treated CML patients using a combination of imatinib and HDAC inhibitor treatment. Additional therapeutic strategies involving inhibition of other key microenvironmental and intrinsic factors contributing to leukemia stem cell maintenance are currently being pursued.

We are also investigating the process of HSC transformation in response to mutagenic insults. Therapy-related myelodysplasia/acute myelogenous leukemia (t-MDS) is a lethal complication of cancer therapy, resulting from exposure to DNA-damaging chemotherapy and radiation therapy. We have initiated a prospective, longitudinal evaluation of patients are at high risk for development of t-MDS. Blood and marrow samples are collected and stored at various time points to investigate early events sequential changes associated with development of t-MDS. This project is aimed at understanding mechanisms of susceptibility and evolution of cellular and molecular abnormalities in stem cells during myeloid leukemogenesis, identification of patients at risk for leukemia, and development of novel preventive and therapeutic strategies. We are investigating the role of altered DNA repair, DNA damage response and hematopoietic regulation in development of t-MDS. The results of this study will have broader implications for the pathogenesis of de novo AML and MDS in general.

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Mark Boldin, Ph.D.

Associate Professor, Department of Molecular and Cellular Biology

THE ROLE OF NONCODING RNAS IN INNATE IMMUNITY AND CANCER

The long-term research goal of our laboratory is to decipher the molecular mechanisms that govern the development and function of the immune system and to understand how dysregulation of immune cell signaling can lead to autoimmunity and cancer. The immune response to nonself is a highly complex reaction that requires an orchestrated action of multiple cell types mediated by a continuous flow of extracellular cues in the form of cytokines and secondary messengers. Understanding how immune cells receive and translate these signals into gene changes is our fundamental aim.

miRNAs represent a newly discovered class of small noncoding RNAs that have recently emerged as key post-transcriptional regulators, controlling diverse biological processes, including response to nonself. A few years ago, we postulated a hypothesis that miRNAs might comprise a novel layer of regulation of the innate immune response and we have carried out a systematic effort to identify miRNAs that might be involved in the mammalian response to microbial infection. We identified three miRNA genes (miR-146a, miR-132 and miR-155) whose expression is sharply upregulated in response to bacterial lipopolysaccharides (LPS), and this list was later expanded to include miR-9, miR-21 and miR-147 through the work of others.

Current work in the lab is focused on understanding the biological roles of miRNAs of the miR-146 family—miR-146a and miR-146b—in immune cell development and function. Our results suggest that miR-146a functions as an important negative regulator of inflammation. Mice with targeted deletion of miR-146a gene mount an exaggerated inflammatory response to endotoxin challenge and develop a spontaneous autoimmune disorder, characterized by splenomegaly, lymphoadenopathy, and multiorgan inflammation later in life. Mechanistically, autoimmunity in the miR-146a null mice correlated with hyperresponsiveness of primary macrophages to bacteria, loss of peripheral T cell tolerance and a defect in regulatory T cell function. Based on our initial molecular studies, we proposed a model suggesting that miR-146a acts as a negative feedback regulator of the innate immune response by silencing expression of two adapter proteins, TRAF6 and IRAK1, that are crucial for proinflammatory and antigen receptor signaling. Presently, we are continuing to characterize physiological functions of miR-146a and to investigate molecular mechanisms of its action using loss- and gain-of-function mouse models.

While very close in sequence to miR-146a and perhaps targeting the same pool of target genes, miR-146b and its biological roles still remain a mystery. Thus, we are creating miR-146b null and miR-146a/b double knockout mice to study the nonredundant and shared physiological functions within this miRNA family.

In addition, we previously found that miR-146a plays a role in the control of immune cell proliferation: aging miR-146a null mice display an excessive production of myeloid cells and develop frank tumors in their secondary lymphoid organs suggesting that miR-146a can function as a tumor suppressor in the context of the immune system. A growing body of evidence suggests that inflammation and cancer are intimately linked; however, the molecular nature of this relationship is poorly understood. We propose that miR-146a can serve as a molecular bridge between these phenomena and are actively investigating its role in the suppression of tumorigenesis.

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Edouard M. Cantin, Ph.D.

Professor, Department of Virology

HERPES SIMPLEX VIRUS INFECTIONS IN THE NERVOUS SYSTEM

Herpes simplex virus (HSV) is a ubiquitous human pathogen associated with mild disease as in cold sores. Less frequent are more serious diseases like herpes keratitis, a leading cause of blindness, and herpes encephalitis (HSE), a debilitating neurological disease with relatively high mortality and morbidity. Susceptibility to HSV disease is genetically controlled, and our studies indicate that a complex genetic system determines resistance or susceptibility to fatal HSE in C57BL/6 and 129S6 mice respectively. Resistance is also strongly influenced by sex such that females are more resistant than males. Using a variety of approaches including bone marrow transplantation, in vivo siRNA gene silencing, flow cytometry analysis of brain infiltrating cells and MRI imaging, we determined that fatal HSE resulted primarily from unrestrained inflammatory responses in the CNS rather than from virus cytopathology. An important finding was that once CNS inflammation was initiated the antiviral drug Acyclovir was ineffective in preventing mortality despite early and sustained inhibition of virus replication.

Intravenous immunoglobulin (IVIG) is a clinical product comprised of pooled human IgG collected from thousands of donors. Initially, IVIG was used to treat primary and secondary immunodeficiencies but its use has expanded to include treatment for a host of other autoimmune and systemic inflammatory diseases due to its potent anti-inflammatory and immunomodulatory properties. IVIG protects against fatal HSE by preventing hyper-inflammatory CNS responses through a mechanism(s) independent of virus specific antibodies that includes induction of regulatory T cells and monocytes, which confer long-term protection. We confirmed a report that sialylated IgGs (sIgG) present in limiting amount in IVIG can mediate its anti-inflammatory activity, and we have uncovered another sIgG independent mechanism. Further studies of IVIG's mechanism(s) of action, including a novel capacity to block intracellular virus replication, are under way. These ongoing studies are clinically relevant with a strong translational potential.

HSV establishes lifelong latent infections in the sensory ganglia from which it periodically reactivates to cause recurrent disease and spread in the population. Understanding the mechanisms regulating latency is critical for devising strategies to interfere with latency and reactivation. To this end, we have exploited IVIG to develop a novel model of latency establishment in immunodeficient Rag mice that will facilitate deciphering immunological mechanisms regulating latency. Latency is characterized by epigenetic silencing of lytic promoters, and we are investigating the potential role of miRNAs in this process as well as their involvement in other aspects of HSV pathogenesis.

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Saswati Chatterjee, Ph.D.

Professor, Department of Virology

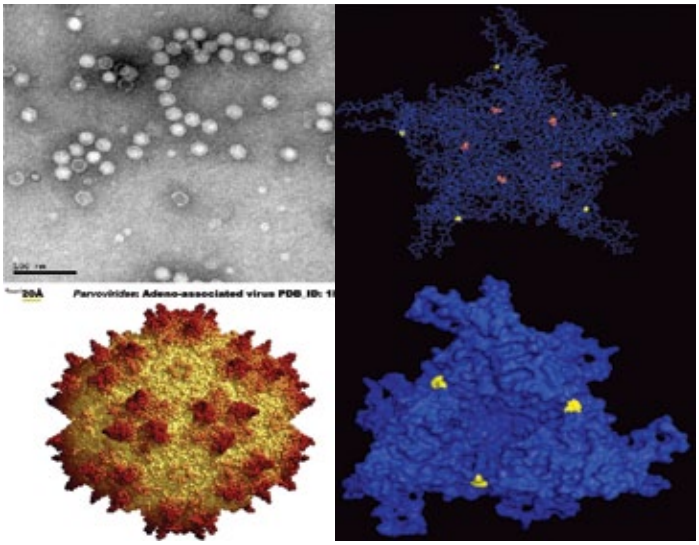
ADENO-ASSOCIATED VIRUS VECTORS FOR STEM CELL GENE THERAPY

Our research group is interested in therapeutic gene transfer to treat inherited and acquired diseases. Therapeutic gene-based strategies targeting stem cells are an efficient approach for the treatment of diseases of genetic origin. However the search for the ideal stem cell gene therapy vector continues as recognized problems persist with currently available gene transfer modalities. Meanwhile, recombinant adeno-associated virus (rAAV) gene transfer vectors are rapidly being developed for a wide variety of in vivo applications, based upon their profile of safety, in vivo efficiency, non-pathogenicity and possible anti-oncogenicity. Our research focuses upon both, elucidating the basic biology of recombinant AAV-mediated gene transfer as well as developing therapeutic applications for eventual clinical use.

To this end, we have developed gene transfer strategies that lead to safe and effective long-term genetic modification of primitive human hematopoietic stem cells, capable of persisting in vivo, differentiating normally into progeny cells and expressing transgenes with no evidence of genotoxicity.

More recently, following reports of abundant natural AAV sequences in primate tissues, including the bone marrow, we have isolated a panel of endogenous AAV sequences from healthy human hematopoietic stem cells. We reasoned that stem cell-derived AAV viruses would have evolved optimally for efficient stem cell transduction. Thus we derived a series of novel rAAV using capsid genes cloned from the stem cell AAV isolates. Our results indicated that rAAV vectors derived from stem cells have uniquely high tropism for these cells, suggesting that these novel rAAV are likely ideally suited for safe and efficient stem cell gene transduction. Furthermore, studies on the biodistribution and specific in vivo tissue tropisms of these novel rAAV reveal highly efficient localization to specific target tissues, including the liver and the heart.

Our studies indicate that vectors derived from our panel of novel, naturally occurring AAV capsids are capable of high level sustained targeted transgene expression following in vivo delivery, even at low doses and, therefore have important implications for gene therapy of numerous human diseases.



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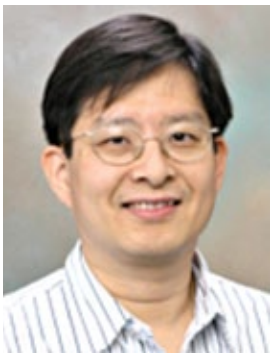

Ching-Cheng Chen, Ph.D.

Assistant Professor, Division of Hematopoietic Stem Cell and Leukemia Research

CELLULAR AND MOLECULAR CHARACTERIZATION OF THE HEMATOPOIETIC NICHE

Hematopoiesis occurs in specialized hematopoietic niches in the adult bone marrow, which provide key regulatory factors and interactions that regulate self-renewal and differentiation of hematopoietic stem cells (HSC). Perturbation within these specialized microenvironments may impose aberrant function on HSC and/or other targets to result in a variety of hematological diseases and dysfunctions including leukemia. Increasing evidence suggests that leukemia cells can take over normal hematopoietic niches to support their self-renewal and to escape from chemotherapy. The overall goal of our laboratory is to develop a complete cellular and molecular understanding of the bone marrow microenvironment and its role on leukemia initiation and progression. Better understanding of the bone marrow microenvironment will allow us to devise specific strategies to improve current therapeutic solutions and provide better treatment and prevention for leukemia.

We have pioneered an in vivo ectopic bone-forming assay to dissect the developing HSC niche and identified a novel osteochondral progenitor as the HSC niche-initiating cell (Nature 457:490-494, 2009). These findings have allowed for the very first time the development of an in vivo hematopoietic niche model to dissect the cellular/molecular components of niche and the role of niche on leukemia initiation and progression. Reconstruction and more complete definition of the hematopoietic niche are necessary to maximize as well as manipulate HSC potential in both normal and disease states. The ability to manipulate the niche will have great therapeutic potential. We will use the combination of in vivo ectopic bone-forming assay, single cell gene assay and flow cytometry to address fundamental and longstanding questions related to the HSC niche.

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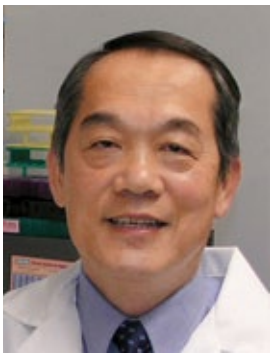

Shiuan Chen, Ph.D.

Director and Professor, Division of Tumor Cell Biology

HORMONES AND CANCER

A major research focus of this laboratory is to investigate the roles of aromatase in breast cancer development. In estrogen-dependent breast tumors, estrogen binds to the estrogen receptor protein and induces the expression of peptide growth factors that are responsible for the proliferative responses of cancer cells. Aromatase is an enzyme that converts androgen to estrogen. Since aromatase is the enzyme responsible for the synthesis of estrogen, and estrogen can have a major effect in the development of breast cancer, an abnormal expression of aromatase in breast cancer cells and/or surrounding adipose stromal cells has a significant influence on breast tumor development and growth in cancer patients.

Studies are being conducted in this laboratory to analyze the tissue specific regulation of the promoter elements in the human aromatase gene. Such experiments have helped identify a histone deacetylase inhibitor LBH589 as a drug against hormone-dependent breast cancer. In addition, structure-function studies are performed to characterize the structural features of the active site of aromatase. Since the suppression of estrogen formation by aromatase inhibitors is considered an important breast cancer treatment strategy, it is vital that the structural nature of the inhibitor-binding site of aromatase be determined. We have succeeded in the expression and purification of functionally active human aromatase from E. coli. Efforts are under way to determine the molecular basis of the interaction between aromatase and its inhibitors. This information will be critical for the design of potent and selective aromatase inhibitors for breast cancer treatment.

During the last five years, aromatase inhibitors have been demonstrated to be superior to tamoxifen for the treatment of hormone-dependent breast cancer. This new generation of aromatase inhibitors has been shown to be useful in the treatment of hormone-responsive breast cancer, although resistance to such endocrine therapy still develops. We are conducting gene expression array experiments, microRNA profiling analyses, and estrogen receptor-ChIP sequencing studies on aromatase inhibitor-responsive as well as inhibitor-resistant cell lines that have been generated in our laboratory. We plan to identify and functionally confirm the roles of genes/miRNAs involved in resistance. It is hypothesized that these studies will produce valuable molecular information regarding the mechanisms of aromatase inhibitor resistance, and the information will help design approaches to reduce resistance and improve the efficacy of aromatase inhibitor treatments of breast cancer.

We also conduct research to determine how environmental chemicals modulate the activity and expression of aromatase in human tissue. Experiments are being conducted to provide a molecular and mechanistic basis as to how phytochemicals and organochlorine compounds affect estrogen biosynthesis (i.e., aromatase function) in women. In addition, our laboratory has found that grapes, mushrooms, and red wine contain chemicals that can suppress aromatase activity. Therefore, a diet that includes grapes, mushrooms, and red wine would be considered preventative against breast cancer. We have purified and characterized the natural anti-aromatase chemicals and evaluated their in vivo effects using animal experiments. Dr. Melanie Palomares (Population Sciences), Dr. Tim Synold (Experimental Therapeutics) and our laboratory have collaborated and initiated a clinical trial based on the chemoprevention studies against breast cancer using grape seed extract. Together with Dr. Melanie Palomares and Dr. Przemyslaw Twardowski (Medical Oncology), two clinical trials (breast cancer and prostate cancer) on mushrooms have been also initiated.

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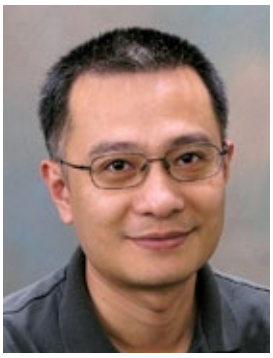

WenYong Chen, Ph.D.

Assistant Professor, Department of Cancer Biology

EPIGENETICS, CANCER AND AGING

DNA methylation and histone modification affect mammalian gene regulations and functions. These epigenetic modifications play important roles in many normal biological processes, such as development, stem cell differentiation and aging, as well as in several mental retardation diseases and cancer. Cancer evolves with the genetic loss of function of tumor suppressor genes and overactivity of oncogenes. Meanwhile, cancer progression also involves profound epigenetic deregulation of tumor suppressor genes, and chromatin alterations that affect genomic stability and gene expression. We are interested in understanding the biology of cancer with a focus on epigenetic regulation of tumor suppressor genes and oncogenes in development, aging and tumorigenesis, and developing mouse models for studying cancer epigenetics and testing strategies for cancer prevention and therapy.

Using mouse genetics, we demonstrate the important roles of an epigenetically regulated gene, hypermethylated in cancer 1 (HIC1), in tumor suppression. We show that a key role for loss of HIC1 function in tumorigenesis is to activate the mammalian stress-response gene SIRT1 (an NAD-dependent protein deacetylase) and thereby deacetylate and inactivate p53. SIRT1 is a homologue of yeast Sir2 protein that is involved in epigenetic gene silencing and suppression of recombination and maintenance of chromatin functions. Increasing Sir2 or SIRT1 gene dosage or enzymatic activity promotes longevity in multiple lower organisms and protects mammalian cells from apoptosis under stress and DNA damage. Since aging increases promoter hypermethylation and epigenetic silencing of HIC1, these studies suggest that the resultant upregulation of SIRT1 may be a double-edged sword that both promotes survival of aging cells and increases cancer risk in mammals.

SIRT1 over-expression is prevalent in human and mouse cancers; however, the precise roles of SIRT1 in tumorigenesis are not well defined. Other members of the SIRT1 gene family termed sirtuins may also regulate cell metabolism and aging, but little is known of their functions in cancer. The current research themes in the lab include investigating the roles of SIRT1 in BCR-ABL transformation of primitive hematopoietic progenitor cells and chemoresistance of chronic myelogenous leukemia to tyrosine kinase inhibitors, and studying the functions of sirtuins and NAD metabolism in prostate epithelial cells and carcinogenesis. Our goals are to discern how sirtuins are involved in regulating cellular longevity and tumorigenesis and to develop strategies for healthy aging and reduced cancer risk.

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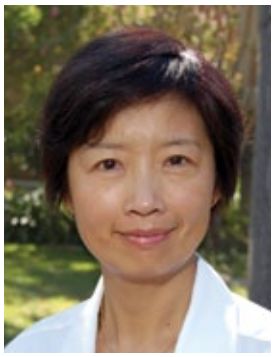

Yuan Chen, Ph.D.

Professor, Department of Molecular Medicine

PROTEIN MODIFICATIONS

Our current research interest is in protein modifications by a family of small proteins known as ubiquitin and its homologues. These modifications control life-spans, trafficking, assembly, and enzymatic activities of cellular proteins, and are important in nearly every aspect of biological functions. We employ nuclear magnetic resonance (NMR) and other structural approaches in combination with biochemical, molecular and cellular biological methods to understand these processes at an atomic level. Such knowledge is necessary for developing research and therapeutic approaches to target these processes, where aberrations are responsible for the development of numerous life-threatening human diseases. Specific areas of interest are outlined below.

Enzyme Mechanism of Protein Modifications by Ubiquitin-like Proteins

Covalent attachments of ubiquitin or its homologues to other proteins are macromolecular chemical reactions that, similar to other macromolecular reactions (e.g., transcription, translation and DNA repair), require several steps catalyzed by multiple enzymes. These processes involve dynamic protein-protein interactions for which NMR methods are particularly suited and can provide atomic resolution of structural information. Knowledge of how macromolecular reactions are catalyzed will lead to improved design of pharmacological intervention strategies, targeting these modifications for the treatment of a wide variety of human diseases.

The Downstream Effect of SUMO Modifications

Our recent studies have shown that biological consequences of modifications by the ubiquitin-like SUMO protein occur mostly through the interaction between SUMO and a SUMO-binding motif (SBM, also known as SIM). We have found that SUMO-mediated protein-protein interactions play key roles in the repair of DNA double-strand breaks through the non-homologous-end-joining pathway. We are developing reagents that will enhance cancer cell sensitivity to DNA damaging chemotherapeutic drugs and radiation.

NMR-based Metabolomics

Metabolic activity is a reflection of cellular functions. Our laboratory utilizes NMR spectroscopy in metabolomic studies, that is, global analysis of metabolic activities. Our goal is to provide information on cellular pathways and to discover biomarkers for diagnosis, as well as prognosis assessment in cancer therapy.

Application of NMR Methods in Drug Discovery

We are collaborating with several other laboratories with expertise in synthetic chemistry, molecular biology and medical oncology to develop new therapeutics for cancer. State-of-the-art NMR methods are employed to screen fragment compound libraries, and provide information on protein-ligand interactions at an atomic resolution, information that is critical for the rational design of new therapeutics.

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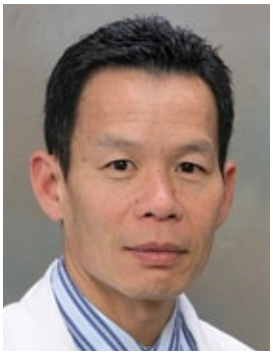

Warren Chow, M.D., FACP

Associate Professor, Department of Clinical and Molecular Pharmacology;
Department of Medical Oncology & Therapeutics Research

CELL SIGNALING AND CANCER

Regulated intramembrane proteolysis

Regulated intramembrane proteolysis (RIP) is a conserved, cellular signaling process whereby transmembrane proteins are cleaved within the membrane to generate cytosolic fragments that enter the nucleus to control gene transcription. This mechanism of cellular signaling regulation governs processes such as lipid metabolism, cellular differentiation, and response to unfolded proteins. Originally described for sterol regulatory element binding protein-1 (SREBP-1), the master transcription factor for adipogenesis, it has been found to also regulate notch and activating transcription factor-6 (ATF6), which regulate differentiation and the unfolded protein response (UPR). Interruption of the normal function of these processes can initiate two forms of programmed cell death, apoptosis and autophagy. Our laboratory has been studying approaches to inhibit this process through small molecules and siRNA, to understand the biological consequences in cancer.

Lipogenic Phenotype of Cancer

Sarcomas are a rare type of malignancy, accounting for less than 1% of all cancers. The most common type of soft-tissue sarcoma is liposarcoma, which develops from adipocytic tissue. This type of cancer is inherently resistant to chemotherapy. Importantly, development of a lipogenic phenotype in other more common types of cancer, such as prostate cancer, is now a recognized process whereby these tumors develop fat-like properties that are associated with progressive malignant characteristics, as well as resistance to chemotherapy. The fatty acids and lipids generated by the lipogenic phenotype provide energy and molecules for membrane biosynthesis in these cancers. Interruption of RIP inhibits RIP and reverses this malignant phenotype. Our laboratory has been studying approaches to inhibit this process through small molecules and siRNA for therapeutic purposes.

High-Throughput Screening

We have begun collaborating with investigators at City of Hope to perform high-throughput screening for novel inhibitors of RIP based upon structure-based and ligand-based inhibitor design. These novel inhibitors will further our basic understanding of RIP, and may eventually serve a therapeutic purpose.

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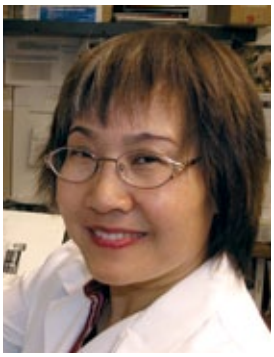

Fong-Fong Chu, Ph.D.

Associate Professor, Department of Radiation Biology; Department of Cancer Biology

THE IMPACT OF GENETICS AND DIET ON INFLAMMATORY BOWEL DISEASE AND INTESTINAL CANCER

There is a strong association between chronic inflammation and cancer in the gastrointestinal (GI) tract. Patients suffering from ulcerative colitis and Crohn's disease, which are the major inflammatory bowel diseases (IBD), have higher risk for cancers in small and large intestine. We have generated a mouse IBD model by virtue of disruption of GPx1 and GPx2 genes, which encode two intracellular glutathione peroxidases (GPx). GPx is a family of selenium-dependent enzymes, which reduce hydroperoxides including H2O2 by oxidizing glutathione. The etiology of ileocolitis in these GPx1/2-double knockout (DKO) mice shares similar characteristics with human diseases. First, luminal microflora played an essential role in disease onset and perpetuation. Germ-free DKO mice are virtually disease-free. Second, diet impacts disease severity, neoplastic progression, and tumor location. Diet exerts its effect either through changing microflora content or via its components/metabolites inducing inflammatory responses. High-cholesterol diet exacerbates colitis. Third, genetic factors also influence disease severity and tumor incidence. The DKO mice on C57BL/6J (B6) genetic background have mild disease, when mice on 129S1Sv/J (129) background have severe disease. The major strength of this model over other mouse IBD models is that the immune system is not altered and does not require any artificial stimulus.

A major goal of my lab is to find a cure for IBD. We have been testing different diets and dietary supplements, as well as drugs, on 129 DKO mice which have early-onset aggressive IBD. Studying the effective drugs may elucidate the essential molecules triggering IBD.

Another focus of my lab is to identify the gene(s) affecting disease severity. Using genetic approach, we found a colitis locus, Gdac1 (GPx-deficiency-associated colitis-1), which partially differentiates disease severity between B6 and 129 strains of DKO mice. We are testing dual oxidases (Duox) as a candidate gene at this locus, because Duox is stimulated by inflammatory cytokines and generates H2O2.

Given the best circumstances, only 25% DKO mice have tumor; thus it is not a robust tumor model. The most used colon tumor model is combining a carcinogen, azoxymethane, and an irritant, dextran sulfate sodium, to induce multiple tumors. We plan to develop a protocol to increase tumor incidence and multiplicity in the DKO mice to make it a robust tumor model in the future.

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Don J. Diamond, Ph.D.

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TRANSLATIONAL RESEARCH IN CANCER VACCINES

The Division of Translational Vaccine Research (TVR) is developing strategies to combat cancer (hematopoietic malignancy and solid tumors) and infectious disease using a variety of vaccine delivery strategies. The use of small hairpin RNA as a means to block immunosuppressive molecules in the tumor microenvironment has become a major interest in the laboratory highlighted by our recent publication in *Cancer Research* and ongoing studies evaluating novel approaches to inhibit molecules that prevent successful immune clearance of solid tumors. Mouse models of leukemia/lymphoma and solid tumors, such as melanoma and pancreatic cancer, are being used to evaluate the potential of our strategies for clinical application.

Salmonella as a Platform for Antigen and shRNA Delivery

In an exciting new chapter for the TVR, we are using attenuated salmonella as a vaccine vehicle to deliver tumor antigens in mouse models for the purpose of future clinical development. More provocatively, we are also using salmonella as a DNA delivery vector that encodes shRNA targeting the tumor microenvironment. The combination of delivery of cancer antigens to stimulate the T cell response and shRNA to tame the immunosuppressive tumor microenvironment is an exciting new strategy that shows great promise. Opportunities for training and participation in the breakthrough discoveries in the system are currently available.

MVA as a Platform for Vaccine Delivery

p53 — We are using a vaccine delivery platform that is based on a highly attenuated virus referred to as modified vaccinia Ankara or MVA. This crippled virus was developed in Europe in the 1970s as a safer alternative to the licensed vaccine for smallpox infection. We have recently submitted an application to FDA for approval to initiate therapeutic immunization of oncology patients at City of Hope using our vaccine. Opportunities exist in monitoring the results of the clinical trial and planning for more advanced phase II trials combining our vaccine with other FDA-approved immunomodulators (CTLA-4, YervoyTM).

Wilms' Tumor Antigen and Survivin — Similarly, we are also developing MVA vaccines for both of these antigens in the context of both hematologic malignancy and solid tumor. These antigens have also been inserted into salmonella and listeria vectors and prime-boost strategies are being investigated in mouse models.

CMV — We received approval in April 2007 from the National Cancer Institute to produce clinical-grade MVA vaccine targeting CMV. The CMV vaccine will have expanded breadth compared to our 1st generation peptide vaccine. The goal is to vaccinate donors of transplant recipients in 2011. Opportunities to work on all of these projects are available, including immune monitoring of vaccine recipients and further molecular vaccine construction.

Primate Model of CMV Infection

We are utilizing Rhesus macaque (RM) housed at the California National Primate Research Center (CNPRC) as a primate model for CMV infection. A unique resource at the CNPRC is RM that have been selected to be CMV-negative at birth. Using the bacterial artificial chromosome cloning system, we have constructed an MVA expressing five antigens representing a key molecular complex involved in an entry route for CMV. We are using RM to test the efficacy of our BAC-derived MVA to successfully prevent infection after challenge with a pathogenic Rhesus CMV strain. Many opportunities exist for collaboration on this exciting project.

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Richard W. Ermel, D.V.M., M.P.V.M., Ph.D.

Director/Professor, Division of Comparative Medicine
Director, Animal Resources Center
Director, Residency and Graduate Training Program in Laboratory Animal Medicine

APPLIED ANIMAL RESEARCH

The Division of Comparative Medicine (DCM) is an academic and clinical division within the Beckman Research Institute of City of Hope. The DCM contributes to City of Hope and Beckman Research Institute research missions by providing excellent quality and humane animal care; by providing comparative medicine, laboratory animal science, and veterinary medicine expertise through teaching and consultation; and by promoting animal welfare and improving the quality and integrity of animal-based biomedical and translational research. The DCM consists of academic programs (graduate and professional level teaching programs; residency and graduate training program in laboratory animal medicine; and independent and collaborative research programs) and animal model related cores/shared resources which provide technical expertise and services. The DCM assures that all care and use of animals by City of Hope and Beckman Research Institute faculty and staff is humane and complies with all relevant policies, procedures, legal requirements, and accreditation standards. The laboratory animal care and use program at City of Hope and Beckman Research Institute is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC) and registered by the United States Department of Agriculture (USDA).

The DCM research mission is to engage in collaborative and independent service-directed applied research in laboratory animal science and comparative medicine. Areas of research emphasis include: development of new biomodels (animal models of human disease); refinement of laboratory animal research environments and techniques; study of spontaneous and induced animal diseases and/or zoonotic diseases (diseases transmitted from animals to humans); development of new diagnostic techniques for infectious/immune mediated diseases; and, development of therapeutic protocols/intervention and prevention strategies for human and animal diseases.

Recent research projects have included the following:

Collaborative research to determine the anti-cancer potential of readily available and affordable natural compounds alone and in combination using cell culture, xenograft rodent models, and comparative oncology methods and approach. The primary research objective is to determine the anti-cancer effects of cardiac glycosides and related compounds on human and canine cancers in cell culture assays and rodent xenograft models and to evaluate their therapeutic potential through clinical trials using a comparative oncology approach.

Collaborative research on the effect of novel inhibitors of advanced glycation endpoint (AGE) to prevent arterial stiffening and improve arterial elasticity and compliance in a streptozotocin (STZ)-induced diabetic Sprague Dawley rat model. The primary research objective is to determine the effect of AGE inhibitors on the mechanical properties of the arterial system in STZ-induced diabetic Sprague Dawley rats based on the aortic input impedance analysis. Novel AGE inhibitors may mitigate the development of diabetic vascular disease, which remains one of the most severe complications of diabetes mellitus.

Collaborative research on the evaluation and assessment of cage microenvironment and inflammatory cytokine responses of mice housed on a mixture of absorbent bedding/ammonia neutralizer additives and standard bedding. The primary research objective is to assess microenvironment temperature, relative humidity, and ammonia concentrations within cages housing mice on standard bedding material with urease inhibitor or absorbent additive or combinations thereof. The secondary objective is to assess microscopic evidence of ammonia damage to respiratory and ocular tissue and to assess inflammatory responses in lungs by detecting cytokine concentrations (IL-6, -10, -12, IFNγ and TNFα) in lung homogenates.

Collaborative research to develop new diagnostic techniques for infectious diseases of laboratory animals. The primary research objective is to develop enhanced polymerase chain reaction (PCR) tests for murine norovirus, Helicobacter spp., Pasteurella pnuemotropica, and other infectious agents of rodents. A secondary objective is to develop service-directed applied diagnostic and clinical pathology research in conjunction with the initiation of the DCM rodent diagnostic/clinical pathology laboratory.

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Barry Marc Forman, M.D., Ph.D.

Ruth B. and Robert K. Lanman Chair in Gene Regulation and Drug Discovery
Professor and Director, Division of Gene Regulation and Drug Discovery

ORPHAN NUCLEAR RECEPTORS: A NEW ENDOCRINOLOGY

Nuclear hormone receptors comprise a superfamily of ligand-activated transcription factors that mediate the biological effects of steroid, retinoid, and thyroid hormones. Members of this superfamily are characterized by their conserved DNA-binding, ligand-binding, dimerization, and C-terminal transactivation domains. After nuclear receptors had been isolated for all known nuclear-acting hormones, a large number of additional receptor-like proteins were identified. These proteins are known as orphan receptors, as they appear to represent receptors for yet-to-be-discovered ligands. Our lab uses a variety of approaches to identify orphan receptor ligands including automated robotic screening and affinity isolation/mass spectrometry. The physiological aspects of receptor and ligand function can then be studied via gene expression profiling and in vivo studies using knockout mice.

We believe that orphan receptors provide unique tools for the identification and study of novel signaling systems. Indeed, over the past few years the orphan nuclear receptor PPAR has been shown to play a central role in maintaining lipid homeostasis. The PPARα subtype binds polyunsaturated fatty acids. When activated by these ligands, PPARα stimulates transcription of a battery of genes required for fatty acid catabolism. In addition, PPARα is activated by the fibrate class of hypolipidemic agents, indicating that activation of this receptor serves as a clinical treatment for hyperlipidemia. PPARα is also expressed in the endothelial cell where it can regulate atherogenic gene expression. As endothelial cells are normally exposed to flowing blood, we are currently exploring the effects of this mechanical stress on PPARα-activity in these cells. Our data suggest that mechanical forces can regulate gene transcription by altering the levels of endogenous lipid ligands for PPARα. This work has fundamental significance in understanding and treating atherosclerosis.

We have also identified bile acids as ligands for FXR. We now find that these bile acid ligands can act via non-genomic mechanisms to lower glucose levels in diabetic mice. This offers a potentially new approach toward treating type 2 diabetics. We have also found that FXR expression is linked to overall survival in patients with colon cancer and in mouse models of the disease. We are now working to identify synthetic ligands that can act as pharmacologic agents to treat colorectal cancer and to lower glucose via FXR. One of these drugs is now in clinical trials at City of Hope.

A major interest in the lab is to identify "true" endogenous ligands for orphan nuclear receptors. We have developed a combined affinity purification and mass spectroscopy approach to identify these ligands. Our ongoing studies have demonstrated the utility of this approach for identifying natural ligands for PPAR and HNF4. For example, our work with HNF4 has shown that hepatic derived HNF4 is normally associated in the nucleus with a single essential fatty acid. Fasting animals results in loss of binding of this endogenous fatty acid suggesting that HNF4 ligand-occupancy plays a role in the fasted state. This technology offers a novel approach toward ligand identification for other orphan nuclear receptors.

We have applied this technology toward the problem of illicit use of performance-enhancing steroids. Current testing protocols utilized by the Olympics and major league sports are inadequate as athletes can evade detection by using novel steroids that are unknown to authorities. Our testing strategy overcomes this limitation by virtue of its ability to detect "designer steroids" as novel ligands for the androgen receptor. Thus, rather than searching for the chemical signatures of known anabolic steroids, our approach identifies the illicit drug by virtue of its ability to bind to the androgen receptor. We could successfully detect pharmacologically relevant concentrations of anabolic steroids in normal human serum; detection limits are well within the range specified by World Anti-Doping Association.

Finally, our lab is interested in synthetic approaches toward ligand identification. We have recently identified agonists for the nuclear receptor ERR and are in the process of identifying synthetic ligands for other receptors. These chemical tools will provide unique reagents for biological discovery.

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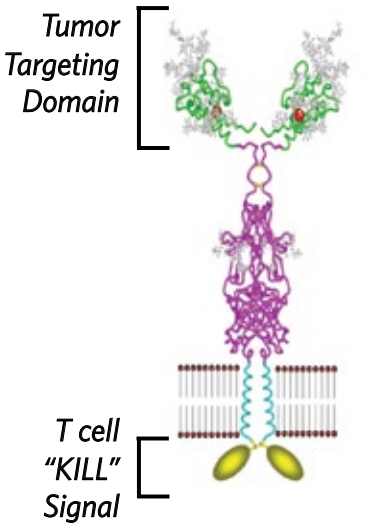
Stephen J. Forman, M.D.

Francis and Kathleen McNamara Distinguished Chair in Hematology and Hematopoietic Cell Transplantation
Director, T-Cell Therapeutics Research Laboratory

T-CELL IMMUNOTHERAPY FOR TREATMENT OF CANCER

Harnessing the power and specificity of the immune system to directly target and eliminate malignant disease is emerging as a promising clinical approach. Our laboratory is focused on the development of antigen-specific adoptive T-cell immunotherapy for the treatment of cancer by the use of T cells that have been genetically modified to convey specificity to tumor targets. Our experimental findings suggest that the engineering of T cells to express a chimeric antigen receptor (CAR, see figure) allows for the targeting of antigens on tumor cells in an HLA independent manner. The laboratory is also studying ways to enhance the ability of T cells to recognize tumor cells in patients, mediate an antitumor response, and become part of the adaptive immune system, resulting in an ongoing antitumor response. Over the years, our focus has been on genetically engineering T cells that can recognize such tumors as lymphoma (CD19), acute lymphoblastic leukemia (CD19), AML (IL-3 receptor), glioblastoma multiforme (IL-13Rα2), neuroblastoma and ovarian cancer (L1-CAM). We have conducted phase I clinical trials in neuroblastoma, glioblastoma and lymphoma.

Chimeric Antigen Receptor



In addition to developing T-cell-based therapies for treatment of human disease, our laboratory conducts experiments focused on understanding the impact of the tumor microenvironment on T-cell function and persistence. This has been accomplished by further genetic modification of the T cells or combining them with other molecules that reduce the immunosuppressive environment and facilitate the effectiveness of adoptively transferred antigen-specific cells.

Our lab has conducted studies to determine the optimal cell population for use in adoptive therapy. There are multiple types of T cells, some of which are designed to mediate a rapid response to antigen, predominantly on microbial agents, while others, in addition to their effector mechanisms, are able to become part of a long lived memory compartment. We have identified a T-cell subset, the central memory T cell that is intrinsically programmed for persistence following adoptive transfer and is capable of repopulating functional immunologic memory. We have furthermore developed a lentiviral vector that directs the expression of CD19-specific CAR with methodologies to isolate, transduce and expand central memory T cells for the purposes of conducting clinical trials in patients with CD19+ disease, initially in relapsed B cell lymphoma.

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Carlotta A. Glackin, Ph.D.

Associate Professor, Department of Neurosciences

UNDERSTANDING GENE REGULATION FROM STEM CELLS

The Glackin laboratory plans to focus on two major areas of research. The first is to understand the molecular mechanisms that maintain pluripotent human stem cells and tumor-initiating or cancer stem cells. The second is to elucidate the mechanisms by which elevated expression of TWIST1 (and related HLH proteins) promote epithelial to mesenchymal transition (EMT), tumor invasion, malignancy and metastases. To achieve our first goal we are focusing on the roles of TWIST (and other helix-loop-helix (HLH) proteins) in maintaining uncommitted progenitor and cancer stem cell populations and their interactions with targets which promote EMT and their differentiation into mesenchymal lineages. By investigating these transcriptional mechanisms, a more comprehensive understanding should enable us to develop therapies to regulate human stem cell growth and development for the efficient repair of a variety of human tissues including skeletal and neuronal tissue regeneration.

Our pioneering studies have focused on the role of TWIST-1 signaling in regulating and maintaining mesenchymal stem cells (MSCs) during osteogenesis. Normally, TWIST-1 is an abundant protein in MSCs and acts to maintain adult pluripotent MSCs by regulating periostin, and type 1 collagen enabling the cells to be poised to differentiate into cartilage, bone, muscles and fat. The primary focus of our research is to extend the knowledge of these inter-relationships to the mesenchymal stem cell (hMSCs) lineage, derived from human induced pluripotent (iPSCs) and embryonic stem cells (hESCs). Overall, the use of human stem cells is of primary importance to my research; whether they are derived from a variety of human adult tissues, iPSCs or heSCs. An environment where the hESC, iPSC, and adult stem cell technologies and expertise is available will be a valuable resource for the continued success of these stem cell studies.

While we initially demonstrated that TWIST-1 is an inhibitor of differentiation and necessary for the maintenance of uncommitted mesenchymal progenitors, we also found TWIST to be expressed at higher levels in human tissues samples with advanced stage glioblastoma multiforme (GBM), neuroblastoma, and breast, ovarian and prostate cancer when compared to normal tissues. Thus, the second area of research addresses our recent observations that TWIST-1 activates multiple cellular pathways that regulate the normal migration of adult stem cells, a process called EMT which is exacerbated in metastatic and invasive cancers. Furthermore, we have investigated the molecular mechanisms of TWIST-1 regulation in breast cancer cells. Results from theses studies demonstrate convincing evidence that TWIST-1 directly interacts with NFκB to regulate IL-8 transcription and EGF receptor localization affecting multiple signaling pathways to promote tumor cell invasion and metastasis. Since Twist1 such a powerful master regulator of multiple signaling pathways, it should be an ideal therapeutic target for invasive cancer. Thus, we are currently developing specific soluble twist inhibitors (STIs) as novel therapeutics to selectively reduce cell differentiation and invasion in breast cancer pre-clinical models. The impact of intravascular injections of STIs as therapeutics on our preclinical studies will serve as proof-of-principle for the use of STIs as therapeutic candidates for reducing aggressive tumor metastases as well as tissue regeneration applications. We believe that localized STI based therapeutic approaches will significantly decrease the toxic side effects that are associated with currently available therapies.

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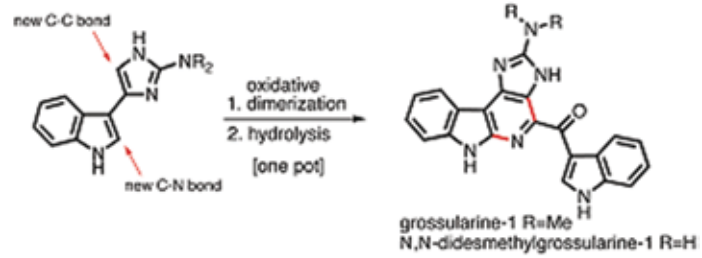
David A. Horne, Ph.D.

Professor and Chair, Department of Molecular Medicine

DEVELOPING NATURAL PRODUCTS AS NOVEL ANTICANCER AGENTS

Natural products possess a long and storied history in the discovery of lead compounds and important medicines for the treatment of human disease. This is particularly notable in the area of cancer research, where, according to data published by the National Cancer Institute, 74 percent of the small-molecule chemical entities introduced as new drugs worldwide from 1981-2001 can be traced to or were inspired by natural products. Moreover, natural products synthesis is an integral part of the drug development process. Pharmacophore identification, optimization of lead compounds, and creating novel structural scaffolds inspired by nature are achieved by synthetic means.

An integral aspect of our research program at City of Hope involves the application of complex molecule synthesis to the generation and development of novel therapeutic agents for the molecular-targeted treatment of cancer and related conditions. Our research interests in general lie in developing new synthetic methods and strategies for the total synthesis of architecturally complex biologically active natural products, particularly from marine sources. One such natural product that is currently under development is grossularine. This metabolite shows potent anticancer properties against solid tumors and is derived from the tunicate (sea squirt) *Dendrodoa grossularia*. That this metabolite is only produced in very minute quantities has hampered further development. Our lab has completed a total synthesis of grossularine-1 and prepared a focused library of α-carboline analogs upon which grossularine is structurally based. The α-carboline hetercyclic framework is unique among natural products and represents a new pharmacophore for anticancer activity. The synthesis is highly efficient, allowing access to gram quantities of synthetic material for biological evaluation. Our group, in collaboration with Dr. Richard Jove's laboratory at City of Hope, has recently demonstrated that grossularines have potent anticancer activity against DU145 prostate cells. In vivo studies are currently in progress and as well as molecular target identification.



From Nature to the Laboratory

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Wendong Huang, Ph.D.

Assistant Professor, Division of Gene Regulation and Drug Discovery

METABOLIC REGULATION, CANCER AND AGING

We are interested in the molecular link between metabolism, cancer and aging. One particular direction is the functional studies of a group of nuclear receptors. Nuclear receptors are a special group of transcriptional factors that are activated by their cognate ligands. A new branch of nuclear receptors was recently identified to play pivotal roles in regulating different metabolic pathways. These nuclear receptors work as sensors of metabolic signals and regulate the expression of essential genes in different pathways. Studies in this area have generated significant impact in many human diseases such as diabetes, obesity, cancer and aging. A second approach is focused on identification and characterization of novel signaling pathways in metabolism and cancer through both genetic and epigenetic approaches. We have identified several novel miRNA-mediated pathways in metabolic diseases as well as cancer growth and metastasis. We have generated transgenic mouse models of those miRNAs and their roles in obesity, diabetes and cancer development are being investigated currently. Moreover, we screen and identify novel small chemical compounds to target cancer stem cells. By using the chemicals we have identified, we are also identifying and characterizing novel signaling pathways in cancer stem cells. In summary, taking advantage of both genetically engineered mouse models and molecular pharmacological tools, our long-term goal is to identify novel signaling pathways in metabolism and cancer in order to provide new targets for drug discovery.

The current research projects in my laboratory focus on:

1) Identification and characterization of physiological signal molecules and pathways by which nuclear receptors regulate metabolism and cancer;
2) Cancer stem cell, novel signaling pathways and targeting therapy; and
3) Epigenetic and miRNA mechanisms in organ regeneration and cancer.

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Janice M. Huss, Ph.D.

Assistant Professor, Department of Diabetes and Metabolic Diseases Research

THE ROLE OF ORPHAN NUCLEAR RECEPTORS IN CARDIAC AND SKELETAL MUSCLE BIOLOGY

Our laboratory investigates how the estrogen-related receptor (ERR) family of orphan receptors regulates mitochondrial energy metabolism and growth in cardiac and skeletal muscle. Genome-wide target gene identification has revealed ERR's involvement in regulating genes encoding contractile proteins and enzymes involved in oxidative metabolism in the mitochondria. We employ primary myocyte models to investigate the upstream signaling pathways that regulate ERR activity and the mechanisms by which ERRs coordinate expression of its target gene programs during myocyte differentiation. We use mouse models, genetically modified to lack ERRα or ERRγ expression, to study how muscle-specific deletion of each isoform affects metabolic and growth/repair adaptations in response to physiologic (e.g., exercise) or pathologic (type 2 diabetes) stimuli. Our studies have revealed the ERR pathway as a potential therapeutic target for diseases associated with metabolic dysregulation, including type 2 diabetes, heart failure and muscle atrophy/growth in aging.

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Keiichi Itakura, Ph.D.

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FUNCTIONS OF MRF-1 AND -2

Molecular Events in Energy Balance

During the study of mechanisms underlying the activation of human cytomegalovirus (HCMV), we have cloned modulator recognition factors (Mrf)-1 and -2 that interact with the modulator region of the HCMV major immediate early gene. These proteins belong to the family of AT-Rich Interaction Domain (ARID) proteins, which regulate differentiation and gene expression in fungi, plants and animals. To study functions of the proteins, we have generated transgenic mice with a deletion of Mrf-2 (Mrf-2KO) by homologous recombination. The two most significant phenotypes associated with the deletion are their leanness and craniofacial abnormalities in comparison to wild type mice. Weight measurements of several organs revealed that accumulation of fat in white adipocytes was impaired. To investigate causes for less fat accumulation, we exploited an in vitro system in which embryonic fibroblasts are induced to differentiate to fat cells. The results showed that fat accumulation was severely impaired in cells derived from Mrf-2KO embryos compared to cells derived from wild type. The examination of gene expression at several stages of differentiation revealed that in cells derived from Mrf-2KO, mRNA levels of various adipogenic genes were significantly reduced in later stages. This result suggests that differentiation of adipocytes was suppressed by the deletion of Mrf-2 that could affect gene expression involved in adipogenesis. To study how Mrf-2 is involved in differentiation of adipocytes at molecular levels, we used RNA interference technology to knock down Mrf-2 (Mrf-2KD) in differentiation of 3T3-L1 pre-adipocytes that is the best model system to study adipocyte differentiation. We observed that compared to controls, knock down of Mrf-2 prolonged the expression of CHOP-10 that is an inhibitor of a key transcriptional factor family for adipocyte differentiation, C/EBPs. The examination of gene expression at later stages of differentiation showed reduced expression of the two key transcriptional factors controlling adipogenesis, C/EBP alpha and PPAR gamma, resulting in lower expression of adipogenic genes such as fatty acid synthase, perilipin, aP2, PEPCK, etcetera. It seems therefore that Mrf-2 controls indirectly the expression of C/EBP alpha through CHOP-10 that in turn affects the expression of PPAR gamma.

In the future, we will investigate more details of how Mrf-2 regulates gene expression and whether Mrf-2 plays a role in maintaining adipogenicity of fat cells. The craniofacial abnormalities of Mrf-2KO mice suggest that Mrf-2 could affect differentiation of bone. Since fat and bone cells are derived from mesenchymal stem cells and have in general a reciprocal relationship in quantity, it might be possible that Mrf-2 is involved in determination of the cell lineage between the two cells.

Metastasis

To study functions of Mrf-1, we investigated conditions in cancer cells such as hypoxia, growth factors or cytokine treatment and anti-cancer reagent treatment, enhance or suppress expression. We found that hypoxia and growth factor (EGF, IGF) treatment enhanced expression two to four times leading to our speculation that Mrf-1 transcription factor may regulate transcription of genes associated with cell proliferation, apoptosis, or migration. Knockdown of Mrf-1 (Mrf-1KD) in human prostate cancer cells (DU145) did not affect either cell proliferation or apoptosis, but blocked cell migration in vitro suggesting that Mrf-1 controls gene expression involved in cell metastasis.

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Linda Iverson, Ph.D.

Professor, Division of Stem Cell and Leukemia Research

STEM CELLS AND CANCER

Our research is focused on stem cells and cancer. Both basic and translational research projects are ongoing in the laboratory and include 1) identification of novel biomarkers that can be used to identify and isolate normal stem cells (hESCs and iPSCs) from potentially tumorigenic variants and 2) identification of novel biomarkers of astrocytic cancer stem cells that may be developed for diagnostic and therapeutic purposes.

Human embryonic stem cells (hESCs) and induced pluripotent stem cells (iPSCs) can be differentiated in vitro into cells of numerous lineages. The use of these pluripotent stem cells in regenerative medicine requires caution since transformed variants spontaneously arise in culture. The transformed variants exhibit many properties indistinguishable from their normal counterparts; they self-renew, express markers of 'stemness', retain pluripotency, and can be differentiated in vitro. However, these stem cell variants are also strikingly similar to cancer stem cells; they exhibit a reduced doubling time and other features of neoplastic transformation. Global gene expression profiling indicates that stem cell variants are similar to the normal lines from which they were derived, suggesting that changes in expression of relatively few genes are sufficient to drive transformation of a normal stem cell into a cancer stem cell. Given the propensity of stem cells to undergo premalignant transformation in culture, it is imperative that all transformed, and potentially tumorigenic, stem cells are eliminated prior to their use in the clinical setting. With this goal in mind, our laboratory has recently completed global gene expression profiling of a number of normal and transformed human stem cell lines by hybridization of RNA to exon-specific microarrays. Unlike cDNA arrays, the use of exon-specific microarrays allows identification of changes in expression of the full-length transcript as well as identification of alternative splice variants. A number of differences in gene expression have been validated by qRT-PCR analysis and used to identify unique protein epitopes that are specifically expressed in normal pluripotent stem cells, but absent from the transformed variants. One of these epitopes has been used to generate an antibody that specifically recognizes normal stem cells. Both the novel nucleic acid markers and antibodies constitute a valuable 'tool kit' that can be used to facilitate the isolation and purification of normal pluripotent stem cells from potentially tumorigenic stem cell variants.

Neural stem cells arising in the subventricular zone are thought to be a source of origin of malignant brain tumors (gliomas). These slowly cycling cancer stem cells are refractory to radiation and chemotherapy and create a major obstacle for treatment of malignant gliomas. Specific targeting of astrocytic cancer stem cells that are the source of inevitable tumor recurrence following conventional therapy may be a more effective therapeutic strategy. Identification of markers characteristic of brain tumor stem cells is a major challenge complicated by their low numbers, elusive nature, as well as the highly invasive properties and heterogeneity of brain tumors. Our laboratory has devised a method for obtaining abundant quantities of premalignant astrocytic stem cells via in vitro directed astrocytic differentiation of transformed hESCs (described above) that are suitable for sophisticated molecular analyses. Comparative gene expression profiling indicates that hESC-derived aberrant astrocytes exhibit gene expression patterns similar to astrocytoma patient samples and can be used to identify novel markers of astrocytic cancer stem cells. We recently generated monoclonal antibodies to nine of these biomarkers, which are being used to validate expression in patient samples. Any validated antibody can be used for improved diagnostic purposes and, conceivably, may be adapted to provide new treatment options for patients with malignant gliomas. This combination of in vitro directed differentiation of aberrant hESC lines, global gene expression profiling and robust bioinformatic analyses provides a powerful model system that can be used to identify unique biomarkers of cancer stem cells in heterogeneous tumors.

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Jeremy Jones, Ph.D.

Assistant Professor, Department of Molecular Pharmacology

THE ANDROGEN RECEPTOR IN HUMAN DISEASE

We study the androgen receptor (AR) and its involvement in human disease. Therapies targeting the AR are used to treat a wide array of human diseases, including prostate cancer (PCa). AR activation is often considered to be a simple consequence of hormone ligand binding; in truth, AR activation is much more complicated, with tissue-specific cellular pathways regulating the process at multiple points. Each of these points represents a potential therapeutic target, one that is distinct from ligand binding. However, all current approaches to control AR activity function through the ligand binding pocket of AR, either activating AR activity or inhibiting it by limiting endogenous hormone binding. In the setting of PCa, these 'hormone-blocking' therapies are initially effective at controlling disseminated PCa, but they eventually fail, leading to terminal castration resistant prostate cancer (CRPC). Furthermore, these therapies act systemically, inhibiting AR activity in other tissues, leading to debilitating side-effects that cause many patients to discontinue the treatment.

Regulating AR activity by means other than manipulating ligand binding could lead to new AR inhibitors that retain efficacy against PCa when current anti-androgen treatments fail. It may also lead to the development of tissue-selective AR modulators (SARMs), which could be used to circumvent the side-effects associated with systemic anti-androgen treatment or to treat other conditions associated with AR activity.

The goals of our lab are to understand how the cell 'indirectly' controls AR activity through cross-talk and leverage that information to create treatments for a variety of AR-dependent diseases. Two of the main clinical objectives are to identify prostate-selective AR inhibitors that are effective against CRPC and muscle/brain/bone-selective AR agonists for 'prostate-safe' androgen replacement therapies for aging men. During the process of developing treatments for these diseases, we hope to learn more about the mechanisms of this 'indirect' regulation and how such regulation is involved in the tissue-selective activities of AR.

In order to identify drugs and cellular pathways that 'indirectly' control AR activity, we created a cell-based assay to monitor AR conformation change and nuclear translocation, two points of regulation that are distinct from ligand binding.

Using this system, we discovered several anti-androgens with unique, non-competitive mechanisms of action. One of these compounds, pyrvinium, was found to inhibit AR activity in vivo, and appears to have some selectivity for the prostate. A biotech company was formed to foster the clinical development of this and related compounds, but we are still working to understand how pyrvinium inhibits AR in a tissue-selective fashion (Project 1). By performing chemical and genetic screens, we have identified several cellular pathways that potentially control AR activity by cross-talk mechanisms. We are working to understand how these pathways control AR activity, and more importantly, use that information to find new ways to treat PCa (Project 2). We are also using our cell-based AR conformation change assay to identify tissue-selective AR modulators to develop novel PCa drugs that have fewer side-effects, as well as new classes of SARMs to treat other diseases associated with AR activity (Project 3). We are developing a rodent model to assess the tissue-selective activity of these potential SARMs (Project 3). In the process, we hope to learn more about the distinct mechanisms by which AR is controlled in different tissues. We are also investigating the relationship between age-related decline in serum testosterone levels and prostate disease, and exploring the possibility that testosterone replacement therapy could reduce the incidence of benign prostatic hyperplasia and PCa (Project 4). Finally, we have begun a new line of research investigating the relationship between circulating tumor cells (CTCs) and prostate cancer metastases and the use of CTCs for predicting disease progression and response to therapy. Please see the research projects tab on my website for more details.

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Richard Jove, Ph.D.

Morgan and Helen Chu Director's Chair
Director, Beckman Research Institute of City of Hope
Professor, Department of Molecular Medicine

DEVELOPMENT OF MOLECULAR TARGETED THERAPEUTICS

Several decades of research on oncogenes have established the causal roles of signal transduction proteins in cancer development. Many of these signal transduction proteins are tyrosine kinases or transcription factors that convey signals from cell surface growth factor or cytokine receptors to the nucleus. The first oncogene to be identified encodes the SRC tyrosine kinase, which is involved in transmitting signals from many receptors for growth factors such as EGF. In cancer cells, persistent activation of the SRC kinase results in phosphorylation and activation of downstream substrate proteins that participate in tumor progression. Recent development of small-molecule drugs that inhibit SRC kinase has allowed us to investigate their cellular and molecular mechanisms of action against cancer cells. Our studies reveal that inhibition of SRC kinase in a wide diversity of human solid tumor cells suppresses their migration and invasion, suggesting that SRC inhibitors may prevent metastasis of cancer cells. Similar findings were observed in breast and prostate cancers, melanomas and sarcomas. Moreover, we have shown that inhibition of SRC kinases in endothelial and myeloid cells in the tumor microenvironment contributes to the antitumor activity of SRC inhibitors. Collectively, these studies provide the rationale for clinical trials to evaluate the effects of small-molecule SRC inhibitors on metastasis of solid tumors in patients.

More recently, we have validated persistent activation of the JAK/STAT signaling pathway as a promising new molecular target for cancer therapy. In response to stimulation with cytokines such as IL-6, the JAK tyrosine kinase is activated and phosphorylates cytosolic STAT proteins. Activated STAT proteins then translocate to the nucleus and function as transcription factors. Our studies show that one STAT family member in particular, STAT3, induces the expression of genes that enhance tumor cell survival and proliferation, as well as tumor angiogenesis and immune evasion. One strategy to inhibit STAT3 activation involves small-molecule inhibitors of JAK kinase. We have shown that novel JAK inhibitors block the growth of breast, prostate and ovarian tumors through inhibition of downstream STAT3 signaling. A second strategy involves the targeted delivery of siRNA directed against STAT3 to immune cells in the tumor microenvironment, resulting in enhanced antitumor immunity. A third strategy is based on design and selection of small-molecule drugs that directly inhibit the function of STAT3 protein, thereby inducing growth arrest and apoptosis of tumor cells. Finally, we have identified the JAK/STAT3 signaling pathway as a molecular target for many natural products derived from traditional herbal medicines and marine life that possess antitumor activity.

Of particular importance are recent studies linking JAK/STAT3 signaling to inflammation associated with both cancer and diabetes. Ongoing research is directed at further development of more potent and specific inhibitors of this signaling pathway. Thus, molecular targeting of JAK/STAT3 signaling holds promise for treatment of two major diseases, cancer and diabetes, previously thought to be unrelated.

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Markus Kalkum, Dr. Rer. Nat. (Ph.D.)

Associate Professor, Department of Immunology

BIODEFENSE AND EMERGING INFECTIOUS DISEASES

The bacterially-produced botulinum neurotoxins (BoNTs) are the most toxic biological substances known. Their abuse as a biological threat agent is feared, but at the same time BoNTs represent important medical drugs for the treatment of various human diseases. Reliable methods for the sensitive detection of BoNTs are of great public and pharmaceutical interest. We have recently developed a novel assay for the attomolar detection of BoNTs in serum and liquid foods. Our new method, BoNT "assay with a large immuno-sorbent surface area" (ALISSA), captures active BoNT molecules on antibody-coated beads such that the intrinsic metalloprotease activity of BoNT is preserved. The presence of immobilized BoNT molecules is then revealed by conversion of a specific fluorogenic peptide substrate (Figure 1). The BoNT ALISSA is at least 4 orders of magnitude more sensitive than the current "gold standard" mouse bioassay. Remarkably, the enzymatic activity of immobilized BoNT is dramatically increased over that of non-immobilized BoNT. Our laboratory is exploring the scientific basis of this acceleration effect and has been successful in expanding the ALISSA technology to other diagnostic targets.

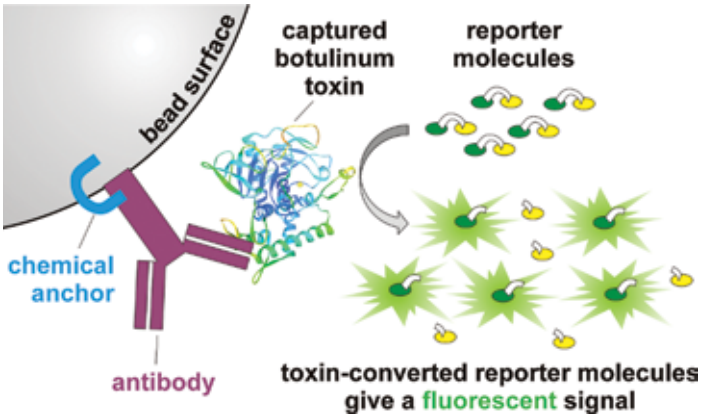

Figure 1 – The ALISSA principle.

Diagnostics for Invasive Aspergillosis

We are pursuing the sensitive detection of fungal proteases to create novel diagnostic techniques to identify early stages of invasive aspergillosis (IA), an often fatal disease that is common in patients with suppressed immune systems. Organ transplant recipients, AIDS patients, burn victims and cancer patients are at risk of contracting IA. Preliminary data suggests that the ALISSA principle can be extended to proteases secreted by the pathogenic fungus *Aspergillus fumigatus* upon invasion of host tissues. The fungal proteases targeted by this approach are not known to recognize specific amino acid sequences; however, they reproducibly cleave protein and peptide substrates with specific tertiary structures. Mass spectrometric and biochemical methods help us to study how specific host proteins are degraded by fungal proteases and how structure-dependent cleavage sites can form the basis of novel substrates for diagnostic assays.

Discovery and Testing of Anti-Fungal Vaccine Candidates

Another research goal is to reconstitute antifungal immunity in cancer patients. Recently, we discovered the *A. fumigatus* protein Asp f 3 is a promising vaccine candidate. Asp f 3 is a known allergen with a described bi-partite IgE-binding epitope. The epitope can be disrupted without diminishing the protective effect of the resulting, modified recombinant Asp f 3, making it an interesting vaccine candidate for potential human use.

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Susan E. Kane, Ph.D.

Professor, Division of Tumor Cell Biology

DRUG RESISTANCE AND CANCER

Drug Resistance

The development of multidrug resistance remains one of the most serious impediments to effective, curative chemotherapy in cancer patients. Resistance develops from a cancer cell's natural response to anticancer drugs. We believe that by understanding these cellular responses, we will learn more about the mechanism of action of specific drugs and about why treatments fail. Ultimately, we hope to contribute to the design of more effective therapeutics and/or treatment protocols and to the advancement of customized therapies based on an individual patient's likelihood of response. Therefore, the overriding theme in my laboratory is to understand, on a molecular level, the various cellular responses and resulting resistance mechanisms that arise in cancer cells treated with anticancer agents.

Multidrug Resistance-1

Perhaps the best characterized mechanism of drug resistance is that mediated by the Multidrug Resistance-1 (*MDR*1) gene in humans. *MDR*1 codes for P-glycoprotein (Pgp), an ATP-dependent plasma membrane protein that acts as a drug pump to prevent intracellular drug accumulation and render cells drug resistant. Pgp can transport and make cancers resistant to a variety of anticancer agents and other xenobiotics, whereas Pgp expression in normal tissues can affect drug pharmacodynamics, pharmacokinetics, and blood-brain distribution in patients. Tissue culture systems have been used extensively to study the functional properties of Pgp and the control of *MDR*1 expression, but adequate animal models for studying the in vivo regulation and activity of *MDR*1 have been lacking.

Our current studies are focused on using a novel mouse model to perform bioimaging of mouse *mdr*1 expression in vivo, in real time, and under the influence of various developmental, environmental, and genetic influences. This model has the potential to provide new, heretofore unattainable information about the role of *mdr*1 in drug resistance and normal organ function. We are also adapting the bioimaging capability to measure real-time in vivo expression of other genes involved in tumorigenesis, tumor progression, and drug resistance.

Breast Cancer Resistance

In a second project, we are investigating a novel resistance mechanism conferred by regulatory components of the protein kinase A (PKA) signaling network. Originally identified in an in vitro model of resistance to trastuzumab (Herceptin), a monoclonal antibody used to treat patients with the Her2+ type of breast cancer, the PKA network may be more broadly involved in response to chemotherapy in multiple types of breast cancer. One component of the PKA pathway, the *PPP1R1B* gene, appears to be dysregulated in breast cancer and it is highly up-regulated in our drug-resistant cell lines. *PPP1R1B* codes for the Darpp-32 protein and its truncated variant, t-Darpp; it is t-Darpp that confers the resistance phenotype when it is overexpressed. Current studies are focused on clarifying the molecular mechanisms by which t-Darpp and PKA affect drug resistance and on dissecting the underlying regulation of *PPP1R1B* gene expression in breast cancer. We are also investigating the role of t-Darpp, Darpp-32, and PKA proteins in breast cancer patients and their response to chemotherapy.

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Mei Kong, Ph.D.

Assistant Professor, Division of Tumor Cell Biology

SIGNAL TRANSDUCTION AND CANCER METABOLISM

Tumor cells often display fundamental changes in metabolism and increase their uptake of nutrients to meet the increased bioenergetic demands of proliferation. Glucose and glutamine are two main nutrients whose uptake is directly controlled by signal transduction and are essential for tumor cell survival and proliferation. Altered glucose metabolism in cancer cells is termed the Warburg effect, which describes the propensity of most cancer cells to take up glucose avidly and convert it primarily to lactate, despite available oxygen. In addition to glucose, glutamine is another essential nutrient whose uptake is directly controlled by oncogenes, and it is critical for cancer cell survival and proliferation. During tumor growth, increased uptake of nutrients and rapid accumulation of cells can outstrip the supply of essential nutrients, including glucose and glutamine. How tumor cells survive these temporary periods of nutrient deprivation is unclear, but is necessary for tumorigenesis to persist.

Exciting progress has been made over the past twenty years in elucidating how cancer cells survive glucose deprivation via mTOR, AMPK, and p53 pathways. In contrast, less is known about the signal transduction pathways that regulate tumor cells' survival during glutamine deprivation in spite of the evidence that has been noticed for many years that glutamine fell from a high level in normal tissue to a level not detectable in different solid tumors. Thus, identifying the critical regulators that control tumor cell survival during glutamine deprivation may lead to the development of novel and safer cancer therapies.

We recently discovered that protein phosphatase 2A (PP2A) plays a key role in regulating the glutamine sensing pathway. In future work, we will focus on understanding how PP2A complexes regulate tumor cell survival under glutamine deprivation, and identify PP2A binding proteins and substrates that play critical roles in cancer cell survival under glutamine limitation. Our long-term goal is to identify the signals that allow communication between oncogenic pathways and tumor cell metabolism and develop novel therapeutics targeting metabolic differences between rapidly-proliferating cancer cells and normal cells.

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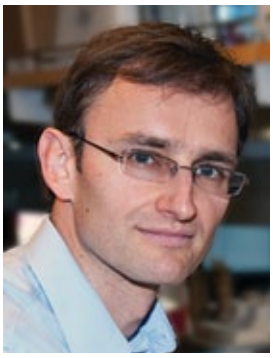

Marcin Kortylewski, Ph.D.

Assistant Professor, Department of Cancer Immunotherapeutics and Tumor Immunology

IMMUNE CELLS AS TARGETS FOR CANCER THERAPY

Fighting cancer by activating immune system to search and destroy tumor cells with high precision could overcome the problem of serious side-effects observed after conventional cancer treatments. Recent advances in the understanding interactions between tumor and immune system demonstrated that immune cells accumulated within tumor tissue are essential therapeutic targets for cancer therapy. Dysfunctional dendritic cells (DCs), macrophages and other myeloid cell populations within the tumor microenvironment secrete growth factors, promote blood vessel formation and dampen antitumor effects of the immune system. Blocking oncogenic and immunosuppressive signaling pathways in tumor-associated immune cells can restore the antitumor immunity inducing complete regression of established tumors. However, targeting immune cells in the tumor microenvironment poses problems due to the lack of specific therapeutics.

We previously developed a novel reagent, CpG-siRNA molecules, that allow for specific delivery of the therapeutic agent (siRNA) into various toll-like receptor 9 (TLR9)-positive target cells, including mouse dendritic cells, macrophages and B cells in mouse. CpG-siRNA conjugates are rapidly internalized by target cells inducing simultaneously immune activation and gene silencing. Our current studies disclose mechanisms of CpG-siRNAs processing, including siRNA uncoupling and translocation from endosomes to processing complexes in ER to induce target gene silencing.

CpG-siRNA molecules directed against oncogenic and immunosuppressive signals demonstrated potent immunostimulatory and antitumor activity in mice. We recently adapted this strategy for siRNA delivery to human immune cells, including monocyte-derived DCs, underscoring the possibility that CpG-siRNAs could revive the antitumor immune responses in cancer patients. Furthermore, we validated the use of CpG-siRNA against several TLR9-positive hematologic malignancies, including acute myeloid leukemia (AML) and multiple myeloma (MM). Our current studies demonstrate that CpG-siRNAs targeting oncogenic and/or pro-survival genes induce tumor cell death and inhibit growth of human AML tumors transplanted into immunodeficient mice. We anticipate that with better understanding of the mechanism(s) underlying CpG-siRNA effect and with reagents optimized for use against disseminated tumors, our studies will produce a cutting-edge technology platform applicable to broad clinical application.

Our current research goals are:

A. Identification of therapeutic targets in the tumor microenvironment that limit immunogenic effects of local tumor radiation therapy.

B. Elucidation of intracellular effects of CpG-siRNA molecules.

C. Optimization of CpG-siRNA strategy for targeting disseminated human tumors.

Lab webpage address: http://www.cityofhope.org/directory/people/kortylewski-marcin/Pages/default.aspx

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Theodore G. Krontiris, M.D., Ph.D.

Professor, Department of Molecular Medicine

GENETIC RISK AND DISEASE

We study a particular type of unstable repetitive element, designated the hypervariable minisatellite or variable number of tandem repeats (VNTR), as a causal factor in commonly occurring human cancer. Minisatellites occur throughout the human genome, often next to genes and gene clusters. We showed several years ago that rare alleles of the highly unstable minisatellite immediately adjacent to the HRAS proto-oncogene were associated with a two-fold increased risk of several common cancers. Because more than 20 percent of cancer patients bear these alleles, one in 11 cancers of the colon, breast, lung, prostate and urinary bladder may be attributed to the influence of the HRAS minisatellite. Now there is evidence that the HRAS minisatellite interacts with other genetic loci to modify their effects on cancer type and age of onset. For example, carriers of disease alleles at the familial breast cancer locus, BRCA1, have a higher risk of getting ovarian cancer at an earlier age if they also bear high-risk VNTR alleles at HRAS.

The mechanistic basis of the VNTR association with breast and other cancers is unknown. However, with the advent of robust databases of human DNA sequences, tissue- and tumor-specific RNA expression patterns, and comparative data from other mammalian species, it has been possible to revisit the VNTR cancer association through new genomic and bioinformatic analyses, and additional molecular biologic studies. As a result of these investigations, we have recently demonstrated that the VNTR is expressed on a capped, spliced, polyadenylated transcript distinct from HRAS; the two genes are transcribed tail-to-tail, with a 10bp overlap of their 3' UTRs. Furthermore, a transcript is produced across the VNTR on the opposite strand, the "antisense" (AS) strand. Expression of both strands occurs widely across normal human tissues and human cancer cell lines. The AS is processed internally, leaving the flanking RNA 5' and 3' to the VNTR spliced together. At the present time, the trigger for, disposition of, and function of short pieces of repetitive RNA removed from the center of the AS transcript are unknown. In addition to our molecular biologic studies, we have learned through bioinformatic analysis of expression databases that expression levels of at least five genes are correlated with the allele state at the HRAS VNTR gene in multiple, independent sample sets, implying that the VNTR locus could participate in transregulation of other genes. Finally, our curation of expressed human RNA sequences has identified dozens of unique transcripts bearing internal, tandemly-repetitive sequences (10 or more copies of 10 bp or larger repeat sizes; 84 bp repeats are most common), implying that processes and potential functions we have uncovered at the HRAS VNTR gene may represent a general mechanism for integrating gene expression.

We have also constructed knock-in mouse strains bearing common and rare HRAS VNTR alleles. Knock-in mice develop massive thymic hyperplasia after a year of age; the rare allele mice develop a higher frequency of cancer of all organs. These mice also delete the VNTR knock-in within a few generations. We are using the mouse model to study VNTR function and to understand the evolutionary implications of using unstable elements to regulate genetic expression.

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Hsun Teresa Ku, Ph.D.

Assistant Professor, Division of Diabetes, Endocrinology & Metabolism

PANCREATIC ENDOCRINE STEM CELLS

1. Embryonic stem cell therapy for type 1 diabetes

Type 1 diabetes (T1D) is marked by a deficiency of the insulin-secreting β cells residing in the Islets of Langerhans within the pancreas due to autoimmune destruction. One of the long-term goals of our laboratory is to advance clinical cell-replacement therapy for patients with severe forms of T1D by developing a safe, reliable and abundant source of cells, derived from human stem cells that function like pancreatic islets. Toward this end, we have established an efficient and potentially cost-effective differentiation protocol, originally adapted from a mouse embryonic stem cell (ESC) differentiation method previously established in our laboratory, and generated a population of glucose-responsive, insulin-producing and secreting cells derived from human ESCs while in vitro. This cell population will be a suitable development candidate for clinical cell replacement therapy for T1D at City of Hope in the future.

2. Embryonic stem cells as a cellular tool for screening of molecules for treatment of diabetes

In the small molecule drug discovery field, the "bottom-up" approach, based on structural considerations of known targets, has not been as fruitful as once promised. ESC technology offers a potential solution to this bottleneck. ESCs can be grown in large numbers and maintained in a pluripotent state in vitro. They can also be induced in culture to differentiate into cells from all three germ layers in a relatively normal fashion that is faithful to development in vivo. Three properties make ESCs cells an ideal platform for drug discovery: first, ESCs cells can provide virtually inexhaustible quantities of target cells, which is necessary for screening of large numbers of compounds; second, ESCs cells can differentiate into mature cells with phenotypes that mimic their counterparts in vivo; and third, compared with immortalized cell lines, ESCs cells and their derivatives will provide a much more accurate platform for the "top-down" drug screening approach. Our laboratory is interested in high throughput screening (HTS) of small molecules that may affect proliferation, differentiation and/or maturation of the pancreatic insulin-producing β cells and their immediate progenitors. As mentioned, we have established a relatively simple and inexpensive differentiation protocol that allows efficient generation of the pancreatic like, insulin-expressing cells from murine ESCs. Thus, this ESC to pancreatic lineage differentiation assay will be valuable to serve as a cellular tool for screening large number of molecules that could be used to treat diabetes.

3. Identification and characterization of pancreatic stem cells

The existence of self-renewing multipotential pancreatic stem cells in the adult pancreas remains elusive. Our laboratory is interested in the identification and characterization of pancreatic stem/progenitor cells, using both mouse models and cadaverous human pancreatic tissues for studies. We have established a quantitative and clonogenic progenitor cell assay in our laboratory, which will be a powerful tool to study the cellular and molecular mechanisms that govern the differentiation and proliferation of the pancreatic stem/progenitor cells at the single cell level.

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Ya-Huei Kuo, Ph.D.

Assistant Professor, Division of Hematopoietic Stem Cell and Leukemia Research

MOLECULAR GENETICS OF HEMATOPOIETIC STEM CELLS AND LEUKEMIA STEM CELLS

Acute myeloid leukemia (AML) is the most common type of leukemia in adults with approximately 13,000 new cases diagnosed in the United States each year. Despite considerable improvement of survival rates for patients with leukemia over the past 50 years, the overall five-year relative survival rate for AML patients is only approximately 20 percent. Using a genetic mouse model of a common chromosome aberration, inv(16), found in AML patients, we have shown that leukemia arises as a result of a multi-step transformation process involving mutations that disrupt hematopoietic differentiation, and those that deregulate proliferation and self-renewal programs. Our research efforts are directed toward understanding the molecular pathways and functional mediators regulating normal hematopoiesis as well as those underlying leukemogenesis. Leukemia stem cells (LSCs) are now recognized as the critical "seeds" of leukemia, and represent a fundamental challenge for leukemia therapy due to the inability to eradicate them through conventional treatments. Our long-term goal is to elucidate the molecular mechanism(s) regulating the maintenance of LSCs, to understand how they differ from normal hematopoietic stem cells, and to develop novel targeted therapeutics against LSCs. We use a combination of genetics, genomics, biochemistry, proteomics, molecular and cellular biology, and in vivo transplantation approaches in our studies.

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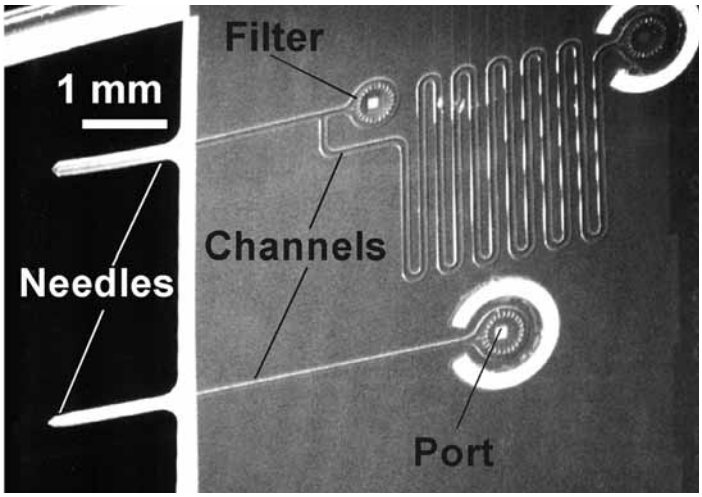
Terry D. Lee, Ph.D.

Professor, Department of Immunology

MASS SPECTROMETRY OF BIOMOLECULES

With the advent of new ionization methods, such as electrospray and matrix assisted laser desorption (MALDI), it is now possible to produce gas phase ions of almost any size and class of molecular compounds. A major part of our work is to develop new technologies to bridge the gap that exists between the inherent sensitivity of mass spectral analysis and its application to "real life" biological problems. More often than not, compounds of interest are present in vanishingly small amounts as part of complex mixtures. Additionally, there are often components in the mixture that interfere with the mass spectral analysis. The technological improvements that we have developed include innovative instrumentation and novel methods of data acquisition and analysis. The sensitivity of our LC/MS technology has been greatly improved by the creation of a micro-electrospray (microspray) interface. The output from a normal electrospray source far exceeds the pumping capacity of a mass spectrometer vacuum system. Consequently, a major portion of the sample does not make it into the instrument. With the microspray interface, all of the sample is analyzed, greatly reducing the sample amount needed to obtain quality MS data. With the feasibility of very low flow electrospray demonstrated, future work will be centered on the construction of silicon chip devices that incorporate the electrospray source and separation device as a single disposable integral unit. These devices will be micromachined using the same technology used to manufacture computer chips. The result will be a universal, low-cost, disposable sample preparation device for mass spectrometers. The efficiency of mass spectral analyses can be vastly improved in both time and sample amount by using expert systems to analyze incoming data and make real time decisions in analysis parameters. In the context of an LC/MS run, all of the required information to solve a given problem can be acquired in a single analysis. This methodology will make it possible to more easily transfer the expertise needed to solve particular analytical problems without the need for extensive training of the individual actually doing the analysis. The net effect will be to make mass spectrometry a more useful tool for solving biomedical problems.



Scanning Electron Micrograph of a Polymer (Parylene) Electrospray Source for Mass Spectrometry Micromachined on a Silicon Chip.

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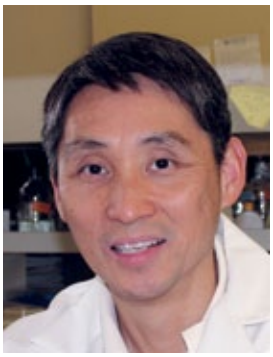

Ren-Jang Lin, Ph.D.

Professor, Department of Molecular and Cellular Biology

RNA SPLICING AND POST-TRANSCRIPTIONAL GENE REGULATION

Spliceosome and Pre-mRNA Splicing

In eukaryotes, the primary transcripts are processed in order to be functional. One of the processing events is the removal of introns and the splicing of the exons by the spliceosome. The spliceosome is assembled by incorporating small nuclear ribonucleoproteins (snRNPs) along with RNA-binding proteins and RNA helicases onto the intron-containing precursor RNA. We have been studying the role of RNA helicases in controlling the dynamics and functionality of the spliceosome. For example, yeast Prp2 helicase works in conjunction with Spp2 RNA binding protein to remodel the spliceosome. We have also shown that U6 snRNA coordinates a magnesium ion that is essential for the first transesterification reaction of splicing.

Recently, we have characterized human homologues of yeast Prp2 and Spp2. Inactivating the human protein (hPRP2 or DHX16) through mutations or antibodies impaired splicing, and the unspliced, intron-containing RNAs were retained in the nucleus. By using genomic tiling microarray, we identified endogenous unspliced RNAs that preferentially accumulated in DHX16 mutant cells. We proposed the stability of unspliced RNAs plays a pivotal role in determining which RNA will be sensitive to splicing perturbation and regulation. We are now testing this hypothesis using splicing drugs, RNA deep sequencing, and molecular and biochemical characterization of Prp2/DHX16 and Spp2/GPKOW. The study may also provide mechanistic clue to human diseases that are linked to dysfunctional spliceosomes.

Post-transcriptional Gene Regulation in Human Disease

MicroRNAs are a major component in post-transcriptional gene regulation, and dysregulation of microRNA biogenesis is of critical importance in human diseases including cancer. Increasing lines of evidence indicated that RNA processing and RNA decay are imperative yet poorly understood regulatory steps in microRNA biogenesis. We are setting up a cell-based screen to identify genes and small molecules that could modulate the level of a specific microRNA in lymphoma cells. The candidates identified from the first screen will be further evaluated for processing/decay regulators using an RNA-based assay. The scheme will be applicable to studying microRNA biogenesis in other systems.

Spinal muscular atrophy, a major genetic disease in causing infant mortality, is linked to SMN protein deficiency. SMN is involved in the biogenesis of snRNPs, which are the major spliceosomal components. SMN is also associated with certain mRNAs in the cytoplasm and may modulate mRNA localization in motor neurons. Recently, the level of certain neuronal specific microRNAs is reported to be affected in SMN-deficient mice. In collaboration with Jiing-Kuan Yee, Ph.D. at the Virology Department, we are beginning to investigate the role of SMN in splicing and microRNA biogenesis in motor neurons derived from patient induced pluripotent stem cells.

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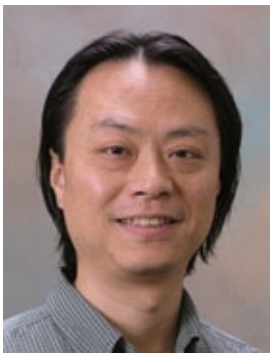

Chih-Pin Liu, Ph.D.

Professor, Department of Diabetes and Metabolic Diseases Research,
Department of Immunology

IMMUNE REGULATION OF AUTOIMMUNE DISEASE AND TUMOR

Regulation of Immunity and Intervention of Type 1 Diabetes

The incidence of various types of autoimmune disease has significantly increased in the U.S. and other parts of the world in recent years. Recent studies have demonstrated that while some T cells can cause autoimmune disease, such as type 1 diabetes or multiple sclerosis, others play a regulatory role in preventing autoimmunity and disease. These unique regulatory T cells (Treg cells) play a central role in causing immune tolerance to occur in both animals and humans. A deficiency in the number and/or function of these Treg cells can lead to the development of autoimmune disease. Therefore, in order to prevent or to treat autoimmune disease, it is necessary to restore the function or to expand the number of functional Treg cells in patients or in disease-sensitive individuals. It is known that antigen-specific Treg cells are much more potent than a heterogeneous population of Treg cells. The major goal of our research is to evaluate the in vivo behavior and function of autoantigen-specific Treg cells, to determine the molecular and cellular mechanisms regulating Treg cell function, and to determine how Treg cells regulate disease-causing T cells, thus leading to the inhibition of autoimmune disease.

Several lines of autoantigen-specific Treg cells have been successfully isolated that can suppress the proliferation and the trafficking of disease-causing T cells. We are evaluating how these novel Treg cells function to inhibit disease-causing T cells during the progression and regulation of diabetes. We are investigating the molecules that control the regulatory function of Treg cells. And we are seeking novel methods that can expand Treg cells while suppressing pathogenic T cells in order to induce effective immune tolerance. It is expected that these studies will form the basis for the design of a novel Treg cell-based therapy which seeks to enhance the function and/or increase the number of functional Treg cells in order to induce immune tolerance and prevent diabetes in patients or patients receiving islet transplantation.

Inhibition of Activation and Cell Death of T Lymphoma Cells

Signals mediated through T cell antigen receptors (TCRs) on T cells play a critical role in regulating not only the function but also the activation and activation induced cell death of T cells. The Syk kinase, a non-receptor protein tyrosine kinase, plays a critical role in signaling events leading to proliferation and differentiation of hematopoietic cells. It has been demonstrated in epithelial breast cancer cells that Syk kinase functions as a tumor suppressor by inhibiting the malignant growth rather than inducing apoptosis of the cells. Moreover, it has been shown that expression of Syk, but not ZAP-70 kinase, in a mouse T lymphoma cell line inhibits their activation and activation-induced cell death. However, the molecular mechanisms underlying the regulation of tumor cell death remain largely elusive. Further studies have shown that expression of Syk in T lymphoma cells induces a unique set of tyrosine-phosphorylated proteins in the cells in response to TCR stimulation. Phosphorylation of these proteins is either absent or not detectable in T lymphoma cells expressing ZAP-70. It is likely that the expression/phosphorylation of these unique downstream proteins plays a critical role in the tumor suppressive function of Syk that controls the activation and cell death of T lymphoma cells. We have used state-of-the-art proteomics approaches to identify a panel of proteins uniquely expressed in Syk or ZAP-70-expressing T lymphoma cells, and will further evaluate the in vitro and in vivo function of the identified molecules in regulating the activation and cell death of both T lymphoma cells and normal T cells.

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Qiang Lu, Ph.D.

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UNDERSTANDING THE MECHANISMS THAT CONTROL SELF-RENEWAL AND DIFFERENTIATION OF NEURAL PROGENITOR/STEM CELLS

Neural progenitor/stem cells can self-renew and are capable of generating neurons and glia, the major cell types that constitute the central nervous system. They are fundamentally important in the development of a functional nervous system. The multipotent potential and the ability to self-renew also make them promising candidates in cell-based replacement therapy for repairing damaged nerves or treating neurological disorders. Under physiological conditions, neural progenitor/stem cells maintain a balance between the two cell states — self-renewal and differentiation — and can shift the balance in response to environmental cues. Loss of this homeostasis can lead to defects in brain development and may be the cause of brain cancers. The long-term goal of our laboratory is to understand the mechanisms that govern self-renewal and differentiation and to apply this knowledge to developing novel treatments for neural regeneration and brain cancers. We study neural progenitor/stem cells using combined molecular, cell biological and genetic approaches.

Our current research was directed towards identifying molecular determinants important for the self-renewal and differentiation of neural progenitor cells in the mammalian cerebral cortex. During development, neural progenitor cells of the cerebral cortex initially expand within the ventricular zone (VZ). As corticogenesis proceeds, they differentiate into neurons, which migrate out of the VZ into their final residence in the cortical plate (CP). This developmental progression of cortical neurogenesis provides a particularly good platform for studying neural progenitor cell self-renewal, differentiation and migration.

Our on-going studies include two parallel approaches. (1) We have recently discovered that ephrin-B and heterotrimeric G protein signaling pathways coordinately regulate the balance between self-renewal and differentiation of neural progenitor cells in the developing mouse cerebral cortex. Thus, we are continuing to investigate the functions and the mechanisms of action of the ephrin/Eph family signaling molecules and G proteins in neural progenitor/stem cells. (2) We also want to more broadly identify molecules and mechanisms critical for neural progenitor/stem cell regulation, in particular the epigenetic control of the neural progenitor cell state. Our strategy is to use genetic modification to differentially label neural progenitor cells and their immediate neuronal progeny in the mouse cerebral cortex. This differential labeling strategy provides us a platform to achieve co-purification of endogenous neural progenitor cells and their progeny. We can then apply advanced global genetic and epigenetic analysis to identify candidate regulators and mechanisms with respect to the decision of neural progenitor cells to either self-renew or differentiate.

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Marcia M. Miller, Ph.D.

Professor, Department of Molecular and Cellular Biology

MOLECULAR IMMUNOGENETICS

We are interested in the genes that exist in populations as the result of pathogen selection over evolutionary time. Such genes/alleles exist because they provided protection from disease and allowed individuals to survive and reproduce. Some of these genes become fixed. Others are polymorphic: that is, different individuals in the population have different alleles, some more effective than others in minimizing disease caused by a particular infection or by transformed cells. We focus primarily on genes within the MHC. The MHC is highly polymorphic in man and in many other organisms. Particular alleles encoded at MHC loci may, in a sense, be "customized" through evolutionary selection. We would like to understand in detail the relationship between MHC alleles and disease so as to be able to predict immune responses and thereby intervene, when needed, to better harness and direct the powerful immune responses that humans and other higher organisms possess.

We are using the chicken as our experimental model. Chickens, just like humans, are challenged by a variety of viral and bacterial pathogens. Resistance to several viral diseases has been genetically mapped to the MHC region. Which genes within the region are responsible and how they protect against disease is mostly unknown. In recent work we were able to identify one locus, BG1, with a major influence in whether tumors form following infection with a highly oncogenic herpesvirus. We are now focused on the mechanism by which BG1 influences the incidence of tumors. In additional projects, we continue to map chicken MHC genes so that all the MHC genes within this region that influences disease are identified. We are expanding the understanding of the role of MHC-encoded molecules in the immediate innate and intrinsic responses to viruses. Some of these genes appear to be pirated by viral pathogens and are apparently deployed to subvert immune recognition in the continuing contest for survival between pathogen and host.

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Rama Natarajan, Ph.D., FAHA, FASN

National Office Products Industry Professor in Diabetes Research,
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DIABETIC VASCULAR COMPLICATIONS

The major focus of our research is to determine the cellular and molecular mechanisms that play key roles in the development of diabetic vascular complications. We use genomics, transcriptomics and epigenomics approaches in cell culture and animal models to examine our hypothesis that accelerated vascular complications result from enhanced vascular and renal cell growth, and also monocyte activation due to altered expression of inflammatory cytokines, chemokines and lipids under diabetic conditions.

Our results suggest that high glucose (HG) and advanced glycation end products (AGEs) can promote vascular complications by increasing inflammatory and growth promoting genes in smooth muscle cells, renal mesangial cells and monocytes. We have uncovered key pathways and molecular signals, including NF-kB transcription factor, that integrate and amplify the effects of HG or AGEs and demonstrated that the diabetic phenotype can be attenuated by blocking these pathways. We are also examining how oxidized lipids such as lipoxygenase products can promote pathological effects under diabetic conditions. The in vivo functional significance is studied with relevant animal models. These data will also be used in the City of Hope Islet Cell Transplantation Program to track patients pre- and post-transplantation.

We are actively evaluating epigenetic mechanisms involved in the expression of pathological genes under diabetic conditions and in promoting metabolic memory. We have demonstrated the role of specific chromatin histone posttranslational modifications in the regulation of inflammatory genes. We use epigenomic profiling approaches to map histone modifications, DNA methylation and binding of chromatin factors at diabetes-regulated genes with techniques such as chromatin immunoprecipitation (ChIP) assays, ChIP-linked to microarrays, and ChIP-Sequencing. We have uncovered key epigenetic alterations under diabetic conditions in vitro, in vivo in diabetic mice, and cells from diabetic patients, and shown relevance to the phenomenon of metabolic memory.

Another active area is the evaluation of specific microRNAs in regulating the expression of inflammatory and fibrotic genes under diabetic conditions. We are evaluating molecular mechanisms of microRNA regulation, and how key microRNAs co-operate with each other in a circuit to amplify the effects of growth factors under diabetic conditions. To test the functional significance in the kidney, we are generating microRNA knockout mice and also evaluating novel modified inhibitors of specific microRNAs (antagomirs) on the progression of diabetic kidney disease in mouse models.

Together, these approaches could lead to new therapeutic modalities for the debilitating complications of diabetes.

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Susan Neuhausen, Ph.D.

Morris & Horowitz Families Professor in Cancer Etiology and Outcomes Research,
Department of Population Sciences, Division of Molecular Epidemiology

GENETIC EPIDEMIOLOGY OF COMPLEX DISEASES

My research is centered on identifying genes and environmental stressors that predispose to disease. We are focused primarily on breast cancer and celiac disease, funded through extramural grants. We are starting a prostate cancer research project, and hope to collaborate with others on pharmacogenetics of drugs in development.

Our breast cancer research, using different populations and study designs, tests the hypothesis that variation in genes in the IGF signaling pathway plays a key role in the etiology and progression of breast cancer. The IGF system is a rare example where the hypothesis is supported from both population studies and laboratory research. Our first study was targeted to African Americans who present with more aggressive breast cancer. We found significant associations of genetic variants at the *IGFBP2* and *IGFBP5* locus and risk of breast cancer, and replicated these associations in Nigerian women. This study was the first to provide evidence that genetic variation in IGF signaling plays a role in risk of breast cancer in women of African descent. Another study investigates variants in 45 genes involved in IGF signaling. We are in the process of replicating our significant findings. We are also investigating disease-free survival after breast cancer in this population. The other NCI-funded study is to identify genetic and lifestyle factors that modify risks of developing cancers in a cohort of women carrying deleterious mutations in *BRCA1* and *BRCA2*. We hope this information can be used to assist in individual risk assessment, and to identify women to target for prevention/treatment strategies. We found highly significant associations of variants, including in *IRS1*, suggesting that IGF signaling modifies the risk of cancer in women who carry *BRCA1/2* mutations. Collaborators and I are investigating the roles of prophylactic surgeries, hormone replacement therapy, and reproductive and lifestyle factors for risk of developing the disease. I am also a co-investigator on the California Teachers Study, looking primarily at breast cancer.

My other primary research is celiac disease, a common auto-immune disease with a frequency of 1 percent. In an NIDDK-funded study, we hope to identify genes that cause the disease in order to provide the foundation for better screening, therapeutics and prevention. In another study, we are investigating the public health management issues of retesting in high-risk individuals who previously tested negative and whether the development of other auto-immune diseases in celiacs can be mitigated with a gluten-free diet. We have two pending grants to do deep sequencing to identify susceptibility variants both within and outside the HLA region, including looking at shared alleles across auto-immune diseases.

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Edward M. Newman, Ph.D.

Associate Professor, Department of Molecular Pharmacology

BIOCHEMICAL PHARMACOLOGY OF ANTIMETABOLITES

The principal area of investigation of the laboratory is the biochemistry of antineoplastic drugs and potential antineoplastic drugs.

Drug Development of 5-Fluorodeoxycytidine (FdCyd)

Initial studies have demonstrated novel metabolic pathways for the activation of FdCyd, as compared with the activation of approved fluoro-pyrimidine anticancer drugs. These studies suggest the potential for enhanced transformation to 5-fluoro-deoxyuridylate, an inhibitor of thymidylate synthase, as well as direct incorporation into DNA, which can cause inhibition of DNA cytidine methyltransferase. We have recently completed a phase I (dose-finding) trial of FdCyd in patients with advanced solid tumors at City of Hope and collaborating institutions. On the basis of data obtained in the solid tumor phase I trial, we have opened a phase I trial at City of Hope in patients with myeloid malignancies and the National Cancer Institute (NCI) is conducting a phase II (initial efficacy) trial of FdCyd in patients with solid tumors of multiple histologies.

Early Drug Development with Phase I Emphasis

City of Hope continues a Cooperative Agreement with the NCI entitled "Phase I Molecular and Clinical Pharmacodynamic Trials," which is now in its 18th year of continuous funding. This grant funds the infrastructure for conducting clinical trials of NCI-sponsored investigational agents and selected other agents, including FdCyd, of interest to the NCI Cancer Therapy Evaluation Program. A consortium of three institutions (City of Hope, University of Southern California, and University of California Davis) participates in these trials, with City of Hope as the coordinating center.

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Vu Ngo, Ph.D.

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MOLECULAR PATHOGENESIS OF LYMPHOID MALIGNANCIES

Leukemias and lymphomas can develop when elaborate control mechanisms governing cell proliferation and survival break down. Germinal centers are the critical environment where antigen-activated B cells undergo clonal expansion, affinity maturation and selection to become long-lived antibody-secreting or memory B cells. Within germinal centers, B cells diversify the antibody repertoire by somatic hypermutation and class switch recombination of the immunoglobulin genes. This process subjects B cells to potential loss of genomic integrity through aberrant mutations and chromosomal breakage and hence increases risk of tumor development. The research in our laboratory aims to determine the molecular mechanisms regulating proliferation and survival in normal germinal center B cells as well as the aberrant signals promoting B cell tumor development. We use functional genomics and biochemical approaches to discover key molecules essential for tumor cell viability and validate findings in human biopsy samples and mouse models. One of these approaches makes use of the RNA interference principle by which small RNA molecules can suppress gene expression. We have developed a bar coded, inducible small-hairpin RNA interference library targeting over 3,000 human genes. Using this library, we uncovered multiple signaling components that are critical for the survival of diffuse large B-cell lymphomas, a form of germinal center-derived B cell tumors. We demonstrated that oncogenic activation of NF-kB in these lymphomas is caused by somatic mutations in various NF-kB upstream regulators including CD79A/B, CARD11 and MYD88, which could potentially be targeted therapeutically. Currently, our research efforts focus on: 1) the role of the activating mutant MYD88 and how to inhibit its activity in human lymphomas; and 2) identification and characterization of novel targets in various B cell tumors including mantle cell lymphomas and marginal zone lymphomas. Better understanding the molecular basis of aberrant signal transduction pathways critical for cancer growth will allow us to identify effective drug targets and devise rational treatment strategies for cancer.

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Timothy R. O'Connor, Ph.D.

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DNA REPAIR

DNA in cells is constantly exposed to damage from both endogenous and exogenous sources. To remove damage and maintain genomic stability, cells have evolved DNA repair systems. The protein levels in these pathways are finely tuned, and DNA damage may induce production of DNA repair proteins. We study DNA damage and repair from several aspects. Our work involves the study of adducts, the repair enzymes involved in adduct removal, how a repair system functions to remove an adduct, how repair systems interact, and finally the response of cells to DNA damage. One system that we focus on is the base excision repair (BER) pathway replacing mismatched or modified bases in DNA. BER is one of the most important systems in the elimination of endogenous DNA damage. The goal of our research is to understand how DNA repair proteins function to eliminate deleterious adducts from DNA and maintain genomic stability. Our research is divided into several areas.

DNA Repair Enzymology

We have cloned and overproduced numerous DNA repair proteins, and our work in this area continues. We have used the homogeneous proteins to study their biochemical and enzymatic properties. DNA repair proteins are often associated in complexes to facilitate repair. We have recently identified an interaction between two DNA repair proteins involved in the initial steps of both the base and nucleotide excision repair pathways. This interaction could prove critical in directing repair along both pathways. We are currently developing other methods to study these protein-protein interactions.

Regulation of DNA Repair Genes

In response to DNA damage, DNA repair capacity can increase, decrease, or remain unchanged. We are now investigating the response of DNA repair genes to DNA damage at the mRNA, protein, and activity levels. Alteration of the levels of DNA repair proteins can result in a change in the efficiency of a given DNA repair system to remove adducts. This work will serve as the basis for predicting the outcome of different chemo- and radio-therapeutic treatments.

In vivo Repair of DNA Damage

In addition to the study of individual DNA repair enzymes, we are interested in how these enzymes function in cells to excise DNA damage. We are using genomic sequencing techniques, such as ligation-mediated polymerase chain reaction (LMPCR), to follow DNA repair in vivo. We have shown that the repair of methylated bases via BER at nucleotide resolution in normal human cells is heterogeneous and have identified sites of DNA repair footprints. Damage and repair at nucleotide resolution of bases damaged by oxidation and chemotherapeutic agents have also been studied. We are adapting this technique to study the effects of gene therapy agents on the function of DNA repair in human cells.

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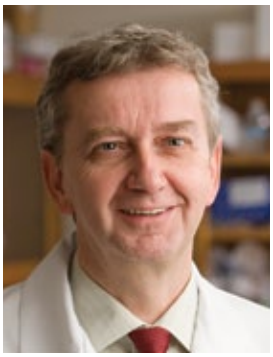

Gerd P. Pfeifer, Ph.D.

Lester M. and Irene C. Finkelstein Chair in Biology
Professor and Chair, Department of Cancer Biology

MOLECULAR MECHANISMS OF CANCER

Our work is focused on determining the molecular mechanisms of cancer initiation. We have been investigating primarily skin cancer and lung cancer, two types of malignancies for which causative agents are suspected. In our earlier work, a method was developed to map DNA damage (adducts) at the sequence level of various genes in carcinogen-exposed or irradiated human cells. The method is sensitive enough to measure repair rates at single nucleotide resolution. We found that induction of UV-induced pyrimidine dimers and excision repair rates are DNA sequence-dependent. The distribution of cigarette smoke carcinogen-induced DNA lesions has been measured along the p53 tumor suppressor gene. The DNA adduct fingerprint of polycyclic aromatic hydrocarbons (PAH) matched well with the distribution of p53 mutations in lung cancers from smokers, providing a molecular link between smoking and lung cancer. The mechanism of this selectivity was found to be a preferential modification and mutagenesis of DNA sequences containing 5-methylcytosine bases at 5'-CpG sequences. These sequences are mutational hotspots in many human cancers and genetic diseases, and we are currently exploring other mechanisms that may produce these mutations.

A major current focus of our research is cancer epigenetics. Epigenetic changes are defined as alterations of genes not involving the primary DNA sequence and include events such as DNA methylation and chromatin modifications. We have cloned a tumor suppressor gene from the 3p21.3 locus, a chromosomal area that is frequently deleted in lung cancer and many other solid tumors. Loss of expression of this gene, which is named RASSF1A, was correlated with methylation of the CpG island promoter sequence and occurred in over 60 percent of small cell lung cancers, breast cancers, prostate cancers, kidney and liver tumors, and many other malignancies. The function of the RASSF1A protein in the mammalian Hippo tumor suppressor pathway is being characterized using biochemical and genetic approaches. We also investigate epigenetic mechanisms of gene silencing in cancer with emphasis on DNA and histone methylation. A new method, the methylated-CpG island recovery assay (MIRA), was developed and applied for the determination of genome-wide DNA methylation patterns in normal and cancer cells. We are investigating the molecular mechanisms of epigenetic changes that occur in cancer using cell culture and animal models.

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MOLECULAR MECHANISMS OF CANCER

Radiation is one of the most effective modalities for the treatment of hematologic malignancies. In order to minimize the toxicity and maximize the efficacy, radioactive materials have been attached to antibodies for targeting hematologic malignancies within patients. Our initial work has focused on B cell non-Hodgkin lymphoma, using antibodies directed against CD20 that are labeled with Yttrium-90. A series of clinical trials, beginning in 2007, were initiated in collaboration with Dr. Stephen Forman and his staff, and significant effort has been spent to extend these trials into Hodgkin lymphoma and leukemia; anti-CD25 and anti-CD33 antibodies will be coupled to Yttrium-90 for the treatment of Hodgkin lymphoma and myelogenous leukemia, respectively.

Radioimmunotherapy for the Treatment of Solid Tumors

Investigators at City of Hope have developed an antibody against CEA, a common molecule on the surface of solid tumors. Recently, they have humanized the antibody using molecular modeling and x-ray crystallography data, building on the pioneering work of Dr. Arthur Riggs on antibody engineering. Clinical trials are now under way with this humanized antibody coupled to Yttrium-90 to treat a variety of solid tumors. We were awarded a five-year Program Project Grant from the National Cancer Institute to support this work. The grant also includes a project on engineering new classes of molecules, built on non-antibody based scaffolds.

Prostate Cancer Imaging

Prostate cancers express a novel antigen, PSMA, on their cell surface. We studied a human anti-PSMA antibody in animal models and found it to localize extremely well to prostate cancers. The antibody has been radiolabeled with a positron emitting isotope that can be imaged, in patient studies, utilizing a PET scanner. This should allow for staging of patients prior to surgery, as well as in assessing response of disease to novel therapies. As part of these studies, a new area of research has been initiated: in vivo tumor imaging, where the antibody is labeled with a molecule that can be seen with a fluorescent light source. We have evaluated this technology in animal models, and efforts are underway to evaluate it in the clinic utilizing the robotic surgery expertise at City of Hope. The anti-PSMA antibody can be used not only for diagnostic purposes, but also as a carrier of drugs and other therapeutic molecules.

We also initiated a collaboration with Caltech to develop external imaging technologies including MRI, CT, PET and optical imaging, to study the immune response to tumors. Thus, the City of Hope/Caltech Biomedical Imaging Group was founded; its initial studies, by Dr. Jensen, evaluated T cells by MRI in animal models. In addition, collaborations have been initiated with the Fred Hutchinson Cancer Research Institute to develop PET tracers to study T cell trafficking. Current studies include evaluating sophisticated MRI procedures to look at the effect of the immunocytokines in mice and to transfer those imaging techniques to planned patient studies. A novel PET/MRI has been constructed at Caltech and the new machine is being used to study the localization of novel immune active proteins, while at the same time image their effect by MRI. These studies will provide the basis for improving human clinical trials.

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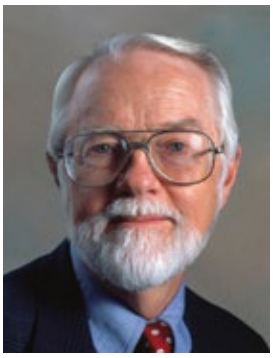

Arthur D. Riggs, Ph.D.

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EPIGENETICS, CHROMATIN STRUCTURE, AND X CHROMOSOME INACTIVATION

Epigenetics is defined as the study of heritable changes in gene function that are not due to changes in primary base sequence. Mammalian development requires stable, somatically heritable epigenetic switches that are dependent on DNA methylation and/or chromatin structure. In 1975, I proposed DNA methylation as one epigenetic mechanism. Since then gene knockout experiments and numerous other experiments have established that DNA methylation is necessary for mammalian development, normal parental imprinting, and X chromosome inactivation. Moreover, some of the changes leading to metastatic cancer and other diseases are epigenetic in nature and involve DNA methylation.

DNA methylation studies are a significant part of my research program; however, methylation is only part of the whole story. There are other epigenetic mechanisms, some involving chromatin structure. Therefore, my research program is broad-based and encompasses other aspects of chromatin structure-function and gene regulation. Current research includes study of epigenetic changes in early mouse development and the differentiation of embryonic stem cells.

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THE BIOLOGY AND APPLICATIONS OF SMALL RNAS

The Rossi lab has as its primary focus studies on the functions and therapeutic applications of small RNAs. Within the past two decades there has been an explosion of new findings about the roles that small RNAs play in biological processes. Ribozymes are RNA molecules with enzymatic activities. There are many different types of ribozymes in nature. Our lab has focused upon the hammerhead motif which is found as part of the self-cleaving domain of small plant viruses. We have exploited these ribozymes for both basic studies of RNA catalysis and for therapeutic applications. Ribozymes developed in our research program have been targeted against HIV and inserted into human blood progenitor cells via a retroviral vector as part of a gene therapy program for the treatment of HIV infection. To date, 10 HIV positive patients have been treated with ribozymes developed in the lab. Efforts to improve catalytic efficiency and co-localization of ribozymes with their targets in mammalian cells are still ongoing in the lab.

The recent discovery of RNA interference in which long double stranded RNAs or RNA hairpins are processed to smaller 21 to 23 base duplexes called small interfering RNAs and microRNAs has revolutionized mammalian cell genetics. These small RNAs can be used to inhibit gene expression by targeting mRNAs with complementary sequences. The lab has been studying the mechanism of RNAi at the biochemical level as well as exploiting this phenomenon for therapeutic applications in AIDS and cancer. We have incorporated short hairpin RNA encoding genes into a lentiviral vector that can transduce hematopoietic progenitor cells, and we find that the expressed small interfering RNAs are potent inhibitors of HIV replication. The goal of this research is to use combinations of shRNAs and other small RNA inhibitors of HIV replication to treat HIV patients combinatorially with gene therapy using their own hematopoietic stem cells to reconstitute their immune systems with HIV resistant lymphocytes and macrophages. An important new finding in the lab is that small double stranded RNAs can trigger transcriptional gene silencing by directing histone methylation and DNA methylation in targeted promoter regions. These small RNAs use components of the RNA interference machinery to target chromatin. A major effort in the lab is to elucidate the active components of this process and to apply transcriptional gene silencing in a therapeutic setting.

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Paul M. Salvaterra, Ph.D.

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MODELING ALZHEIMER TYPE NEURODEGENERATION

Neurodegenerative diseases constitute a major health problem with no effective treatments. The causes of most neurodegenerative disorders are unknown, but certain rare genetic forms provide important clues to what has gone wrong. Alzheimer disease (AD) is the most common form of age-related dementia with an estimated 5 million sufferers in the United States alone. It is estimated that the incidence of AD is more than 50 percent in individuals who live to be older than 85. This obviously presents an enormous medical and economic challenge to society as individuals live longer. First described more than a hundred years ago by the German scientist Alois Alzheimer, to this day the disease is poorly understood, untreatable, and ranked as the number 3 cause of death for individuals in the U.S. (trailing only cardiovascular disease and cancer). Dr. Alzheimer described the neuropathology focusing his attention on characteristic senile plaques and neurofibrillary tangles. Subsequent work revealed the biochemical composition of these brain lesions but not how they are formed or how we can prevent or remove them. In fact, even their contribution to disease phenotypes such as memory loss and dementia is still a subject of controversy. Several decades ago new research led to the identification of several genes that cause a rare type of aggressive early onset AD and provided a strong correlative link with the main protein components of senile plaques, the amyloid beta peptides. No convincing explanation, however, has emerged to link amyloid beta accumulation with the cellular or molecular mechanisms leading to neurodegeneration. Our current work is designed to explore this important relationship.

We use a multidisciplinary approach primarily concentrating on understanding amyloid beta pathogenesis in a *Drosophila* model system. It may at first seem silly to use fruit flies to understand a human disease like AD but there are a number of reasons why we made this choice. First among them are the numerous scientific advantages to be gained by the advanced genetic approaches available in *Drosophila*. Second, flies have a relatively short lifespan, so we can easily design experiments to investigate the relationship between normal aging and AD-like neuropathology and neurodegeneration.

Our initial studies have borne out the usefulness of a fly model for AD type neurodegeneration as a way to understand the human condition. Targeted expression of human amyloid beta 42 in specific types of fly neurons leads to obvious neurological problems and age-dependent neurodegeneration. We have identified the detailed cellular neuropathology that develops specifically in amyloid beta 42 neurons and found that an increase in an abnormal form of autophagy appears to be the key cellular problem. When we undertake similar experiments with the related but non-toxic amyloid beta 40 protein no significant pathology or neurology is observed. Importantly, when we combine amyloid beta 42 expression with pharmacological or genetic changes in autophagy function we can influence the course of neurodegeneration. An increased autophagy activity results in a more severe neurodegenerative phenotype, while down regulation provides a protective benefit to animals expressing amyloid beta 42. These types of experiments suggest new therapeutic approaches that could be considered in the search for AD treatments.

Our current focus is to extend our initial description of autophagy as a central focus of AD neuropathology, begin to link the aging process to amyloid beta 42 induced neurodegeneration, and establish the mechanism for senile plaque formation.

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Dustin E. Schones, Ph.D.

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EPIGENOMICS OF DEVELOPMENT AND DISEASE

Our laboratory is interested in the role of chromatin in gene regulation and how other regulatory elements interact with chromatin. The human genome consists of over three billion base pairs of DNA. This information is packaged into each nucleus in the human body by wrapping DNA around collections of histone proteins to form nucleosomes. This packaging must be done in a manner that allows for the precise combinations of genes to be expressed during development to ensure the faithful production of particular cell types. Modifications to chromatin are associated with many important biological processes, and aberrant modifications can be associated with tumorigenesis. Despite their central importance to gene regulation in both normal and disease cells, little is known about the mechanisms responsible for the regulation of chromatin organization.

Our lab utilizes an integrative approach combining experimental and computational approaches to study these problems. We have shown that there is dynamic reorganization of nucleosome positions at regulatory regions of the human genome in response to cellular stimulus. For example, genes that are induced by TCR signaling in CD4+ T cells have a reorganization of nucleosomes in their promoter regions. Additionally, regions that have been shown to be functional enhancers under T cell activation display reorganization of nucleosomes surrounding the enhancer regions. Furthermore, distal transcription factor binding sites display a characteristic pattern of nucleosome depletion at the binding site and well-positioned nucleosomes surrounding the binding site when proteins are bound to DNA. In contrast, when proteins are not bound, nucleosomes occlude the binding site. This evidence points to a fundamental role for nucleosome positioning in gene regulation, but we don't yet know the mechanisms that are responsible for positioning nucleosomes relative to regulatory regions. ATP-dependent chromatin remodeling enzymes, post-translational modifications to histones, and the DNA sequence itself have all been shown to affect the translational positioning of nucleosomes along the genome. We are investigating the role of each of these mechanisms independently, and in concert, in regulating nucleosome positioning in the human genome.

Our goal is to integrate information on chromatin dynamics with networks of transcription factors to study how chromatin context contributes to regulatory networks during development and disease progression.

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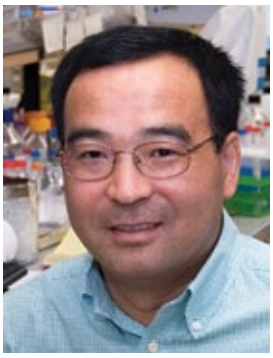

Binghui Shen, Ph.D.

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THE STUDY OF DNA REPLICATION AND REPAIR NUCLEASES IN GENOME STABILITY AND CANCERS

DNA replication and repair are critical for maintaining genome stability. These processes are in part dependent on the activities of an emerging family of structure-specific nucleases. These enzymes, typified by flap endonuclease-1 (FEN-1), possess flap-specific endo- and nick-specific exo-nuclease activities as well as a newly identified gap-dependent endo-nuclease activity. It interacts with 35 different DNA metabolic proteins. The enzyme becomes localized in the nucleus in a cell cycle-dependent manner and in response to DNA damage such as UV and ionized radiation. FEN-1 nuclease is required for the removal of RNA primers during lagging-strand DNA synthesis, DNA damage repair, and apoptotic DNA fragmentation. Post-translational modifications may govern the discrimination of the involvement of the enzyme in different functional complexes and pathways. Dysfunction of Fen1 gene results in a strong mutator phenotype. It was predicted that the deficiency of the gene in mammalian cells will lead to formation of cancers. The focus of our current study is to link the functional deficiency of this critical DNA replication and repair mutator gene to the formation of genetic diseases such as cancers, to clarify the molecular mechanisms of any such pathology, and to provide a solid foundation for the development of new regimens for cancer therapy.

Recently, we found that another major nulcease, DNA2, is dominantly localized into mitochondria and cooperatively processes replication and repair DNA intermediates for ligation and completion of circular mtDNA replication and repair. These novel and exciting observations prompted us (1) to knock out the DNA2 gene in mice to determine if defective DNA2-mediated RNA primer removal causes mitochondrial genomic instabilities and consequently promotes cancers and other genetic diseases and (2) to link functional defects of the DNA2 mutations identified in human mitochondrion-based diseases to pathologic mechanisms. Information made available from these studies should establish a relationship among the functions of these novel mitochondrial genes, unique mitochondrial mutagenic phenotype(s), and pathological mechanisms. The proposed study may also set a good foundation for new treatment regimens to patients with mitochondrion-based cancers and other disorders.

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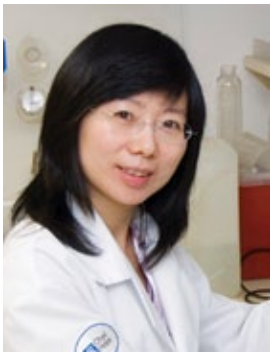

Yanhong Shi, Ph.D.

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NUCLEAR RECEPTORS IN NEURAL STEM CELLS AND ADULT NEUROGENESIS

The finding of neurogenesis in the adult brain led to the discovery of adult neural stem cells. TLX was initially identified as an orphan nuclear receptor expressed in vertebrate forebrains. The brains of TLX-null mice have been reported to have no obvious defects during embryogenesis; however, mature mice suffer from retinopathies, severe limbic defects, aggressiveness, reduced copulation, and progressively violent behavior. We have shown that TLX maintains adult neural stem cells in an undifferentiated, self-renewable state. While TLX-expressing cells isolated from adult brains can proliferate, self-renew, and differentiate into all neural cell types in vitro, TLX-null cells isolated from adult mutant brains fail to proliferate. In vivo, TLX mutant mice show a loss of cell proliferation and reduced neural progenitors in the neurogenic areas of adult brains.

Our primary research focuses on neural stem cells in the adult brain. Specifically, we are interested in characterizing the molecular cascades that program these cells to remain in the stem cell state or that cause them to differentiate and become neurons. An understanding of the molecular basis of stem cell regulation will provide insights into how stem cells are maintained and how they are stimulated to differentiate. Armed with this knowledge, researchers will be able to develop new, targeted therapies for a whole host of neurological disorders, including brain injuries, brain tumors, and neurodegenerative diseases such as Alzheimer's and Parkinson's diseases.

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STRUCTURE, FUNCTION AND REGULATION OF CARCINOEMBRYONIC ANTIGEN GENES

Carcinoembryonic antigen (CEA), a 180 kDa glycoprotein with 50 percent carbohydrate, is probably the best-known tumor marker for colon cancer. Other CEA-related antigens include CEACAM1 (CEA related cell adhesion molecule 1) and pregnancy-specific glycoproteins (PSGs). The antigens have similar domain structures and are all members of the immunoglobulin gene superfamily. Their predicted domain includes a 108-residue N-terminal domain (N) that resembles an Ig-like variable region and multiple copies of Ig-like constant regions (A1, B1, etc.). The C-terminal hydrophobic sequences of CEA and NCA are removed during post-translational processing, resulting in a glycosylphosphatidylinositol (GPI) anchor to the membrane. CEACAM1 is a transmembrane glycoprotein, and the PSGs are secreted by the placenta. CEACAM1 and PSGs have multiple forms based on alternative mRNA splicing, and in the case of the PSGs, multiple genes are present. All of the CEA gene family members are located on the long arm of chromosome 19.

CEA and CEACAM1 transcripts are induced over tenfold by IFN-γ, suggesting a role for CEA and CEACAM1 in immune function. CEA and CEACAM1 have been shown to have cell-cell adhesion properties in vitro. The CEA gene undergoes frequent de novo expression in cancer, while the CEACAM1 gene is frequently downregulated in cancer. We have expressed CEA and CEACAM1 in a variety of cell lines to study their regulation by IFN-γ. The regulation studies have identified an interferon responsive element (ISRE) in the CEACAM1 promoter that is controlled by interferon response factor 1 (IRF1).

We are currently studying the expression and signal transduction of CEACAM1 in breast, colon and activated T cells. CEACAM1 plays an essential role in lumen formation in mammary epithelial cells grown on Matrigel or extracellular matrix. The cytoplasmic domain of CEACAM1 is multiply phosphorylated and interacts with actin, calmodulin and myosin, while it associates with tubulin in activated T cells.

Immunotherapy studies include the engineering of CEA monoclonal antibodies to reduce immunogenicity, to improve biodistributions and to include sites for chemical attachment of radiometal binding chelates. Novel metal ion complexes are based on the macrocycle DOTA. Conjugates are characterized by matrix-assisted laser desorption time of flight mass spectrometry (MALDI-TOF-MS) and electrospray ionization mass spectrometry (ESI-MS). The immunotherapy studies involve a City of Hope-wide effort to offer a new mode of cancer therapy for patients with CEA-expressing tumors of the colon, breast and lung.

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Judith Singer-Sam, Ph.D.

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EPIGENETICS AND DISORDERS OF THE CNS

Although most genes in a cell are expressed from both the maternal and paternal chromosome, there are exceptions. For example, in women, most X-linked genes are expressed from only one of the two X chromosomes, a phenomenon called X inactivation. In addition, there is a class of autosomal genes, termed imprinted genes, for which parental origin determines which allele is expressed. Finally, there are autosomal genes that appear at first glance to be bi-allelically expressed but actually show random monoallelic expression (sometimes termed allelic exclusion) at the single-cell level. These exceptions, examples of epigenetics, have proven to be of great interest for researchers because they shed light on gene regulation, chromatin structure, development, and the pattern of inheritance of certain genetic disorders.

For example, the inheritance pattern of a number of neurogenetic defects, particularly the high rate of discordance (i.e., different phenotypes) between identical twins, suggests the possible involvement of genes that are allelically excluded. Epigenetic differences between the active and silent alleles, such as changes in DNA methylation and replication timing, are known to occur early in development. Thus, identical twins heterozygous for a given gene may develop as mosaics, with critical brain regions differently marked for allele-specific expression. Our long-range goal is to understand the mechanism of random monoallelic expression in the CNS, and to determine if it contributes to CNS disorders in which the twin discordance rate is high.

Towards this goal, we mapped gene-associated DNA sequences that show a dual DNA methylation pattern in the CNS, using a novel microarray-based technique which we term the MAUD assay (Wang et al., 2007; Wang et al., 2010). We annotated the gene map according to known function, expression pattern, and chromosomal location, with particular focus on genes that are expressed in the adult and developing CNS, and that are included within known susceptibility loci for inherited CNS disorders. The specific hypothesis we are testing is that monoallelic expression of key genes in the CNS could be responsible, at least in part, for the twin discordance observed for some inherited disorders.

We selected for in-depth analysis those genes whose chromosomal location, expression pattern, and function were most relevant to our hypothesis. We tested those genes directly for monoallelic expression in clonal neural stem cell (NSC) lines derived from F1 hybrid mice. The "hits" were further assayed to determine whether the allele-specific pattern was preserved in differentiated neurons and astrocytes derived from the NSC lines.

In a continuation of this study, we have probed for monoallelic expression in four of the clonal NSC lines, using next-generation RNA sequencing technology (RNA-seq). Since the lines are derived from F1 female (XX) progeny of the cross *Mus musculus* C57BL/6 and *Mus musculus molossinus* JF1, we first generated a JF1 cSNP library to distinguish C57BL/6 from JF1 alleles prior to transcriptome-wide analysis of the cell lines. We observed monoallelic expression of nearly all of the X-linked genes surveyed, validating the assay. Of ~7000 autosomal genes analyzed, ~180 genes (~2.5% of the total) were expressed from only one allele (at least 85% expression of the predominant allele) in at least two of the cell lines. The genes showing monoallelic expression include Gabrg1, which is implicated in alcohol dependence. In addition, we have found monoallelic expression of several members of the glutathione transferase (Gst) superfamily, which processes xenobiotic compounds as well as carcinogens and cancer therapeutic agents. Monoallelic expression in this group of genes may augment both the specificity and the range of their response to diverse exogenous compounds. Our current goal is to understand the extent and mechanism of monoallelic expression of the most compelling of the genes we have discovered. Our work is being carried out in collaboration with Dr. Min Li (Biostatistics), and Dr. Chauncey Bowers (Computational Biology), as well as the City of Hope Solexa Core.

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Steven S. Smith, Ph.D.

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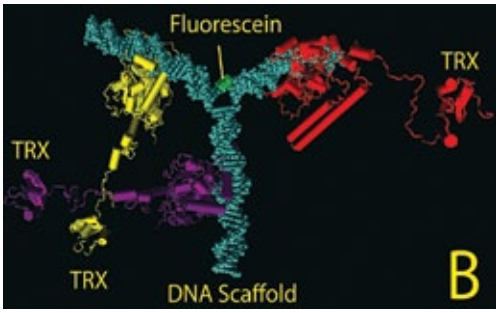
CANCER EPIGENETICS

As cancer cells evolve, the body elicits something akin to a wound response. Cells from the immune system arrive and cells from the surrounding stroma begin to dedifferentiate and behave in unusual ways. This reactive stroma, as it is called, forms a central component of the tumor microenvironment. Together, the reactive stroma and the tumor cells form what is best characterized as an ecosystem that undergoes epigenetic and genetic co-evolution. We have recently opened a window into this system with a nucleoprotein based nanodevice.



Molecular Model of the Nanoparticle (NP-Trx3). Blue, DNA; Purple, Yellow and Red, M•EcoRII-Thioredoxin fusion proteins; Green, fluorescein.

It identifies stromal cells and cancer-associated fibroblasts as key players in this evolutionary process, and opens new avenues for exploration of the epigenetics of this process.

Non-B DNA structures may play a role in this evolution. The geological time point associated with the species divergence node best scales the developmental and therefore epigenetic complexity of a given organism. By adopting this convention one easily sees that the emergence of systems capable of suppressing non-B DNA conformations during normal replication and repair has enabled the proliferation of sequences possessing this potential. In fact, the observed number per base pair of genomic DNA (i.e., the frequency) of G3+N1-7G3+N1-7G3+N1-7G3+ motifs has increased rapidly during eukaryotic evolution.

This increase appears to be under positive selective pressure since it exceeds the frequency expected for a random sequence genome with higher G+C content than any sequenced eukaryotic genome. Positive selection for sequences capable of adopting non-B conformations requires that they are not merely associated with their known harmful effects linked to chromosome damage, but are useful in increasing the number of epigenetic states available to the cell.

In normal cells these non-B conformations are kept under strict control, but in cancer cells the control systems break down. The sum of our work in DNA methylation and our most recent findings in the field strongly suggest that this loss of control can occur in numerous ways, that this breakdown interferes with the normal methylation of DNA and thereby induces chromosomes damage. One of the most interesting questions in the field is whether or not non-CG methylation in stem cells reflects alterations in these processes.

Since chromosome damage, RNA copies made from DNA and changes in DNA methylation patterning are interconnected phenomena in cancer cells, choosing which of them to use in diagnosis becomes a matter of practicality. For this reason we are comparing representative biomarkers of each type. In recently published work we found that testing for two types of damaged chromosomes called the Type III and Type VI TMPRSS2:ERG fusions yielded the best performance in predicting biopsy outcome and severity of the cancer as judged by Gleason score. Current efforts are aimed at identifying new biomarkers with nanotechnology and applying our findings in the epigenetics CG methylation, non-CG methylation, tumor associated unusual DNA structures to non-invasive prostate cancer diagnostics.

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PATHWAYS OF CHROMOSOME BREAK REPAIR IN MAMMALIAN CELLS

While high-fidelity repair of DNA damage is essential for genome maintenance, individual DNA repair pathways can be prone to cause genetic loss, including those pathways that utilize homology. The long-term objective of our lab's research is to understand the factors and pathways that influence the extent of genetic loss during repair of chromosomal breaks in mammalian cells. This objective is important for an understanding of the process of mutagenesis during cancer development, as well as for a mechanistic characterization of potential targets of drugs that could increase the efficacy of cancer treatments that utilize DNA damaging agents.

For one such project, we have developed novel assays for quantifying a pathway for chromosome break repair that is poorly understood: alternative non-homologous end joining (alt-NHEJ). This pathway results in deletion mutations with evidence of small units of homology at the junction point, which mimics a class of mutations observed in cancer cells. We are currently using assays we developed to identify the genetic factors that are involved in this pathway. For example, we have determined that this pathway shares common early mechanistic steps with homologous repair (promoted by CtIP/Nbs1 and inhibited by Ku70/Xrcc4), but is completed by a unique mechanism. Recently, we have begun to use this approach to understand the process of end-utilization during end-joining repair of multiple chromosome breaks. As incorrect end-utilization can lead to loss of genetic information, we are seeking to examine this process in detail.

As an additional project, we are interested in the control of chromosome stability in stem cells during cell division. Incorrect segregation of chromosomes can lead to changes in chromosome number, which has long been shown to correlate with cancer development. By monitoring chromosome dynamics in live cells, we are characterizing the genetic factors and DNA repair pathways that are important to ensure proper chromosome segregation during cell division. For instance, we have recently found that the factor Fbh1 is important for restoration of normal mitotic chromosome segregation following a decatenation stress. More recently, we found that the homologous repair pathway plays a key role in proper anaphase chromosome separation.

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SIGNALING MECHANISMS THAT GUIDE T CELLS

Activation and differentiation of T cells is the first step in the stimulation of our adaptive immune responses. Inappropriate activation of T cells leads to transplantation rejection and autoimmune disorders such as diabetes, arthritis and multiple sclerosis. Prevention of T cell activation leads to immunodeficiency, resulting in the invasion of pathogens such as bacteria and virus infection. The goal of our research is to understand the mechanisms responsible for the regulation of T cell activation so as to develop effective and safe treatments for these immune disorders.

Activation and differentiation of naïve T cells to effector T cells that mediate actual immune responses depend on engagement of T cell receptors (TCR) with antigens. Such engagement of TCR triggers a series of signaling pathways that eventually lead to turn on the program for T cell activation. Manipulation of these critical signaling pathways required for T cell activation allows control of T cell activation. Our research has identified two such signaling pathways, PKC-θ and RORγt. Inhibition of the PKC-θ pathway prevents rejection of transplanted organs as well as autoimmune disorders. Inhibition of the RORγt pathway prevents Th17-dependent autoimmunity such as experimental autoimmune encephalitis (EAE) and collagen-induced arthritis (CIA). However, the precise mechanisms for how PKC-θ and RORγt regulate immune responses still remain unknown. We are actively studying these mechanisms and developing PKC-θ and RORγt-based treatments to prevent autoimmune disorders.

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Associate Professor, Department of Molecular Pharmacology

PHARMACOKINETICS AND BIOMARKERS OF CANCER

As director of clinical pharmacology for both the California Cancer Consortium (CCC) and the SWOG Early Therapeutics Committee (ETC), my laboratory is responsible for the design and conduct of Phase I and II pharmacokinetic investigations. Within the NCI Organ Dysfunction Group (ODG), my laboratory assisted in the design and initiation, and performed the pharmacokinetic analyses, of a new trial of pazopanib in patients with liver dysfunction, a study lead by Dr. Steve Shibata, Department of Medical Oncology & Therapeutics Research. We have also contributed to the design and conduct of multiple City of Hope investigator-initiated pre-clinical and clinical pharmacology studies of novel anticancer agents. One particular highlight has been the clinical application of intracranial microdialysis as a tool for real-time in vivo pharmacologic monitoring. In collaboration with Drs. Jana Portnow and Behnam Badie, we have built the City of Hope clinical intracranial microdialysis program into the largest of its kind in the U.S., with two completed trials, two ongoing, and two studies in development.

In collaboration with Dr. John Termini, Department of Molecular Medicine, we have developed novel assays for the measurement of glycated proteins and DNA in tissues, which have the potential to be important biomarkers for glycolytic tissue damage and mutagenesis. We received a Cancer Center Pilot Project Grant to apply this novel methodology to the determination of glycated DNA in tumors and adjacent normal tissue. The overall objective of this proposal is to determine whether a common DNA adduct observed in non-enzymatic glycation, CEdG, can serve as an effector of tumorigenesis and whether CEdG levels can be correlated with genetic instability.

In another long-standing collaboration with Drs. Susan Kane and Long Gu, Department of Cancer Biology, we developed and validated a mouse model for real-time assessment of in vivo gene regulation. The mouse model consists of firefly luciferase (fLUC) inserted by homologous recombination into the genomic locus of the mouse *mdr*1a gene (the closest counterpart to human *MDR*1). Configuration of fLUC into the *mdr*1a open reading frame is conditional on Cre-mediated recombination. Drs. Kane and Gu have shown that *mdr*1a.fLUC expression is Cre-dependent and fLUC is a faithful in vivo reporter for *mdr*1a transcription in the basal state. We have also shown that *mdr*1a.fLUC imaging can be used to visualize induction and repression of *mdr*1a gene expression in response to environmental and physiologic stimuli.

Finally, in collaboration with Dr. Karen Aboody, Department of Neurosciences, I am investigating the ability of neural stem cells to deliver a pro-drug activating transgene directly to the site of brain tumors in vivo. This collaboration builds on my experience with intracranial microdialysis in clinical trials to assess the neuropharmacokinetics and neuropharmacodynamics of drugs. Preliminary data from this study were instrumental in obtaining an R21 to support the first clinical trial of genetically-modified neural stem cells (Drs. Aboody and Jana Portnow). Additionally, I am the leader of Project 4 (Pharmacology) for Dr. Aboody's recently funded $18 million CIRM Disease Team Concept Proposal.

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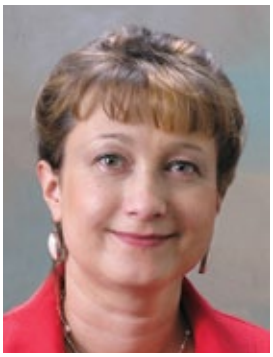

Piroska E. Szabó, Ph.D.

Assistant Professor, Department of Molecular and Cellular Biology

MECHANISMS OF GENOMIC IMPRINTING AND ENVIRONMENTAL REPRODUCTIVE BIOLOGY

Epigenetic marks at imprinting control regions (ICRs) are inherited from the gametes and determine the parental-allele specific expression of imprinted genes in the soma. Our laboratory has been focusing on how allele-specific DNA methylation, chromatin composition and protein factor binding contributes to the mechanism of genomic imprinting. At the *H19/Igf2* locus, proper monoallelic insulation at the ICR is essential for development. Sperm-derived methylation and oocyte-derived hypomethylation determines functional differences at the *H19* and *Insulin-like growth factor 2 (Igf2)* genes. CTCF protein binding in the ICR is required for insulating the promoter of the *Igf2* gene from the shared enhancers in the maternal allele. CTCF is also the single major organizer of the allele-specific chromatin along this imprinted domain.We are also interested in epigenetic remodeling events during mammalian development. One major wave of remodeling occurs after fertilization, when the paternally inherited genome undergoes rapid loss of 5-methylcytosine (5mC). We found that this process involves genome-wide oxidation of 5mC to 5-hydroxymethylcytosine. The third direction of our work is to measure how environmental insults can influence our natural epigenetic programs. Endocrine disruptors (EDs) — synthetic chemicals resembling natural hormones — have profound effects on development and fertility. We are in the process of identifying epigenetic changes in fetal germ cells after in utero exposure to EDs. Epigenetic aberrations that occur in germ cells are of great concern because they have the potential to affect future generations.

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John Termini, Ph.D.

Professor, Department of Molecular Medicine

MUTAGENESIS AND CARCINOGENESIS

DNA damage to the nucleoside bases can impact cellular metabolism by inducing copying errors during replication and by increasing genomic instability. These events play a critical role in driving the initiation, progression, and metastasis of human cancers. Our laboratory is interested in understanding how specific DNA base damage accumulates under physiological conditions and how it promotes mutagenesis and cancer. These studies typically involve measuring DNA damage levels in vivo, determining the mutagenic profile of specific damage products, and examining the ability of cells to repair them. This effort is highly multidisciplinary and involves the application of synthetic DNA chemistry; analytical methodologies such as LC-ESI-MS/MS; protein purification; enzyme kinetics, molecular biology; and mammalian tissue culture techniques. An important goal of these studies is to determine the suitability of these biologically relevant DNA damage products as small molecule biomarkers of potential utility in disease diagnostics and treatment monitoring. Our program offers an excellent training environment for chemistry and biochemistry students seeking to acquire the additional skills required for participating in translational and biomedical research.

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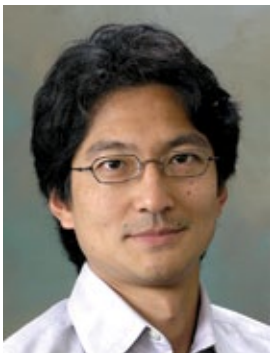

Toshifumi Tomoda, M.D., Ph.D.

Assistant Professor, Department of Neurosciences

ORGANELLE TRANSPORT DURING NEURONAL DEVELOPMENT AND DISEASES

Role of Neuronal Traffic during Development and Diseases of the Nervous System

The neural circuitry develops through coordinated outgrowth of axons and the formation of synaptic connections with their appropriate targets. This neuronal wiring is fundamental to our brain function, which includes the regulation of sensorimotor activities and behaviors, language acquisition, and the development of personality. Neurons are one of the cell types that display most extensive morphological and functional differentiation during development, displaying elaborated dendrites and elongated axons. In order to maintain this highly polarized structure and functionality, neurons must regulate the intracellular traffic of a wide range of cargoes including synaptic components, mitochondria, cytoskeletal elements, and other macromolecular protein complexes. Failure to coordinate neuronal traffic has been linked to many neurological disorders such as Alzheimer's disease and amyotrophic lateral sclerosis. We study the critical role of directed axonal transport in axon/synapse formation as well as its role in axonal regeneration upon injury. In addition, we have evidence that several genes implicated in neuropsychiatric diseases are the components of axonal transport machinery. By using a combination of biochemistry, molecular and cellular biology, live imaging, and mouse/Drosophila genetics, we will further investigate how the neuronal traffic machinery controls axon/synapse formation, and how this machinery maintains neuronal homeostasis during injury or neurological disorders. Through this research, we also hope to better understand the pathophysiology of neuropsychiatric diseases, including schizophrenia, bipolar disorder, and autistic spectrum disorders.

Role of Autophagic Machinery in the Pathophysiology of Neurofibromatosis type 2

Another focus of my lab is to study neurofibromatosis type 2 (NF2) that is characterized by the development of brain tumors of peripheral nervous system origin. We have evidence that deregulated autophagy may play a role in the pathogenesis of NF2. Autophagy is a cellular catabolic system responsible for bulk degradation of damaged organelles and misfolded proteins, and critically depends on the intracellular trafficking machinery that is essential for maintaining cellular homeostasis. We are currently studying how deregulated autophagy contributes to or modifies NF2 tumorigenesis, with the hope of developing a novel therapeutic strategy against this disease.

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Nagarajan Vaidehi, Ph.D.

Professor, Department of Immunology

TARGETING G-PROTEIN COUPLED RECEPTORS FOR CANCER THERAPY

G-protein coupled receptors (GPCRs) form one of the largest gene families of membrane bound proteins. They play a critical physiological role in cell-to-cell communication and also in the pathology of many diseases. More than 50 percent of the drugs in the market today target GPCRs. GPCRs are membrane bound and highly dynamic, and therefore it is difficult to obtain three-dimensional structural information for GPCRs. In my laboratory, we develop computational methods to predict the structural models of GPCRs and to further understand the conformational dynamics that are critical for designing drugs with functional specificity.

Of particular interest to us are chemokine receptors (which are GPCRs) since they play a fundamental role in carrying diverse messages that are critical in mediating both innate and adaptive immunity. Activation of chemokine receptors by chemokines can elicit different responses depending on the cell type including chemotaxis, adhesion, proliferation, maturation, differentiation, apoptosis, malignant transformation, and dissemination. Inadvertent or overexpression of chemokines is an underlying cause of many clinically important diseases with an inflammatory component such as cancer, and, consequently, chemokine receptors have attracted much interest as targets for therapeutic intervention. My laboratory is focused on using computational methods to derive the structures of chemokine receptors (CCR1, CCR2, CCR3, CCR5, CXCR2, CXCR3 and CXCR4) and to understand the interaction of chemokines and small molecule antagonists to these receptors. We also study the dynamics of conformational changes in the receptor that would lead to an understanding of the differences between agonists, antagonists, and inverse agonists in studying the pharmacology of these small molecule drug interactions to GPCRs.

Pancreatic cancer is one of the most refractory cancers to conventional chemotherapy. The chemokine receptor CXCR4 has been implicated in progression and metastasis of pancreatic cancer as well as 23 different human cancers. We are designing small molecules that bind to CXCR4 and would aid PET imaging of the cancer to measure its response to chemotherapy. We are also designing small molecule antagonists to CXCR4 that would suppress cell proliferation and metastasis. This project is a translational research project and involves collaborations with experimental research groups at City of Hope.

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Shizhen Emily Wang, Ph.D.

Assistant Professor, Division of Tumor Cell Biology

OUTSMARTING BREAST CANCER

Breast carcinogenesis is a multi-event process that occurs in a dynamic microenvironment. The long-term objective in our lab is to understand the profound crosstalk between cancer and host in disease progression and response to therapy.

Understanding and Fighting Metastasis

Metastasis is the leading cause of mortality in breast cancer patients. Understanding the molecular mechanisms that influence distant metastasis of breast cancer and identifying biomarkers associated with risk of metastasis will enhance our ability to optimize and individualize anti-cancer treatment at a non-metastatic stage to prospectively prevent metastasis and protect the target organs. The recently discovered microRNAs (miRNAs) play a crucial role in multiple cellular functions by regulating expression of their target genes, and are frequently dysregulated in breast cancer. Cancer-secreted miRNAs, encapsulated in microvesicles (exosomes) shed by cancer cells, are stably present in the blood of cancer patients. Our studies using breast cancer cell and animal models indicated that cancer-secreted exosomes were internalized by various types of cells in the pre-metastatic niche where breast cancer metastases develop. As such, miRNAs that are characteristically secreted by metastatic cancer cells, upon entering the circulation, can influence expression of genes by the niche cells at pre-metastatic sites, which is a major direction in the lab.

Predicting Treatment Response and Overcoming Chemoresistance

Many chemotherapy drugs act against cancer cells by causing damage to the DNA. Resistance to chemotherapy is a major clinical obstacle in cancer treatment. The mechanisms of chemoresistance in cancer patients are not fully understood, leading to urgent needs for determining factors that control drug response and developing novel therapies to enhance the treatment efficacy. Signaling from transforming growth factor (TGF) beta, a tumor suppressor in normal cells, is hijacked in cancer to promote disease progression. In breast cancer, TGF-beta is linked to poor clinical outcomes and chemoresistance through mechanisms that remain largely unknown. Our studies indicate that in breast cancer cells, TGF-beta induces microRNAs (miR-21 and miR-181) that target the DNA damage sensors ATM and MSH2, and may therefore regulate cancer response to genotoxic chemotherapy. Our current research aims to dissect the molecular mechanism of TGF-beta-mediated chemoresistance, and to explore potential therapies to enhance drug efficacy.

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Jeffrey N. Weitzel, M.D.

Director, Division of Clinical Cancer Genetics
Professor, Department of Medical Oncology & Therapeutics Research;
Department of Population Sciences

GENETIC PREDISPOSITION TO CANCER

The major themes of my multidisciplinary clinical and basic research programs are translational research in cancer predisposition, genetic cancer risk assessment, genetic epidemiology, chemoprevention, and clinical outcomes, with a focus on women's cancers and research in underserved minorities.

One theme is molecular oncology and the investigation of molecular genetic changes associated with women's cancers, both inherited and acquired. Hereditary Breast Cancer and Novel Hispanic *BRCA* Mutations is a project to obtain a comprehensive dataset on the prevalence and nature of *BRCA* mutations among Hispanics. We documented that the Jewish founder mutation, *BRCA1* 185delAG, is prevalent in Hispanic populations; we discovered a novel *BRCA1* rearrangement, the result of an Alu repeat-mediated recombination event, that accounts for a substantial proportion of high-risk Hispanic families. We have now established that this mutation is a founder mutation, estimated to have arisen ~1,500 years ago in an Amerindian population. We developed a panel of recurrent *BRCA* mutations on the Sequenom platform for prospective screening of high-risk clinic cohorts. This will represent the largest series of Hispanics with *BRCA* mutation information and clinical annotation.

We have accrued >8,000 high risk patients to an IRB-approved hereditary cancer research registry that enables participation in the multi-institutional consortia required to assemble enough hereditary cases for epidemiological studies, such as the demonstration of significant hormone receptor concordance in *BRCA* carriers with bilateral breast cancer. This collaborative approach has yielded important observations on the effect of oral contraceptive use and of pregnancy on penetrance of breast cancer in *BRCA* mutation carriers, as well the identification of genetic modifiers such as a RAD51 variant, the first known genetic modifier of breast cancer penetrance in *BRCA2* carriers. The registry includes one of the largest existing sets of *BRCA* carriers (>600 families) at a single institution.

In collaboration with Dr. O'Connor, we are developing a translational therapeutics program targeted to hereditary cancers, to bring DNA repair targeted agents from the bench to the bedside. BRCA deficiency in tumor cells is associated with genomic instability due in part to impaired homologous recombination repair (HRR). Inhibitors of poly(ADP-ribose) polymerase (PARP) have shown promising results in pre-clinical studies and early phase clinical trials. We demonstrated pre-clinical activity of a novel PARP inhibitor (ABT-888) obtained from NCI/CTEP in BRCA-deficient breast cancer cell lines, and I am the national PI for a CTEP-approved multi-center randomized phase II trial (ABT-888 +/- carboplatinum) in advanced BRCA-associated breast cancer. An NCI R21 grant is enabling prospective study of molecular mechanisms of resistance. These studies constitute a paradigm shift and proof of principle that complementary DNA repair pathways can be inhibited, resulting in specific tumor killing and a very mild toxicity profile.

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John C. Williams, Ph.D.

Assistant Professor, Department of Molecular Medicine

STRUCTURAL BIOLOGY AND BIOPHYSICS

Our group applies diffraction and other biophysical methods to design, quantify and optimize novel therapeutics for cancer treatment. We also apply these methods to understand the mechanism of signaling pathways, cytoskeletal structure and intracellular transport. Ongoing projects include:

1) *Antibody design*: Often referred to as "magic bullets," monoclonal antibodies (mAbs) preferentially target diseased tissue and are generally better tolerated than traditional chemotherapy. In most cases, therapeutic mAbs bind to an antigen that is "self" but overexpressed in the tumor, such as the Erbb family members (e.g., EGFR, Her2). The mAbs, Erbitux and Herceptin that are currently used to treat neck and colon cancers and breast cancer, however, give rise to acniform eruption, gastrointestinal irritation and cardiotoxicity. To address these adverse side effects, we have recently designed and tested an "antibody prodrug" that uses tumor-specific properties to activate the modified mAb at the disease site. Current efforts include new designs and creating full IgG mask for animal and clinical studies.

2) *Stat3 inhibition*: The protein STAT3 upregulates genes associated with cell survival and proliferation. STAT3 is active in multiple cancers, enabling tumor cells to divide uncontrollably and resist eradication by chemotherapy. Inhibiting STAT3 signaling suppresses those signals and stimulates cancer cells to undergo cellular suicide. Thus, developing reagents that directly inhibit STAT3 constitute a viable therapeutic approach. We have shown that a region of the STAT3 protein is susceptible to irreversible chemical modification by reactive molecules and that that modification blocks STAT3's ability to activate genes, and inhibits growth of STAT3-positive tumor cells in culture and in animal models. While these results are promising, we have found that these compounds can interfere with the activity of other proteins, and possibly produce side effects. Guided by these preliminary studies, we have modified our approach to target a different region of the Stat3 protein using irreversible STAT3 inhibitors. We are currently identifying new lead compounds primarily using methods to evaluate protein structure. We will also create novel cell lines to allow us to distinguish specific STAT3 inhibition from potential non-specific effects. Ultimately, these investigations will provide groundwork necessary for development of potent irreversible STAT3 inhibitors and pave the way for clinical trials.

3) *Dyenin*: Dynein participates in a number of processes including vesicle and organelle transport, cytoskeletal structure and cell division. Not surprising, defects in dynein and its partners are implicated in a number of diseases. These include cancer (hepatocellular carcinoma, breast cancer, etc.), neurological disorders (ALS, SMBA, Huntington's, etc.) and viral infection (HIV, rabies, adenovirus, etc.). While central to many of these diseases, regulation of its activity and ability to participate in so many different processes remains poorly understood. With this in mind, we are applying structural and biochemical methods to understand its pleiotropy at the atomic level. We have also developed a novel method to rapidly sequester endogenous targets (<5 mins), and used these inducible traps to dissect the role of the LCs, kinetically, in different dynein mediated processes.

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Jiing-Kuan Yee, Ph.D.

Professor, Department of Virology

VECTORS FOR GENE THERAPY

My work focuses on the development of safe viral vectors to deliver gene into stem cells for cell-based therapy and gene therapy. We are also evaluating the potential of induced pluripotent stem cells (iPSCs) as in vitro models for human diseases, and their application in drug screening and cell/gene-based therapy.

(1) Vector modifications for site-specific gene insertion. One inherent problem with retroviral and lentiviral vectors is their random integration into host genome that can induce insertional mutagenesis. In two gene therapy trials to treat X-linked severe combined immunodeficiency (X-SCID), retroviral integration near a proto-oncogene Lmo2 resulted in the development of leukemia in several patients. Random vector integration may also contribute to frequent transcription silencing of the delivered gene due to the position effect. Site-specific gene insertion to genomic regions with predictable safety profiles and persistent gene expression should relieve both of those problems caused by random vector integration. We are evaluating strategies to physically modify lentiviral virions to direct the delivered gene to pre-designated genomic regions for insertion. Our approach is based on the incorporation of the site-specific Cre-loxP recombination system or sequence-specific zinc-finger proteins into lentiviral virions to guide site-specific gene insertion. If successful, these strategies are expected to significantly improve the safety of using lentiviral vectors to treat human diseases.

(2) Using iPSCs to study spinal muscular atrophy (SMA) in vitro. SMA is one of the most common autosomal recessive disorders and is also the most common genetic cause of infant mortality. It is caused by the loss of the survival of motor neuron (SMN) protein, resulting in the degeneration of motor neurons in the spinal cord. The underlying disease mechanism for selective destruction of motor neurons remains unclear and currently there is no cure for this disease. We used retrovirus-mediated gene transfer to reprogram the fibroblasts of a type 1 spinal muscular atrophy (SMA) patient and establish five iPSC lines. Motor neurons derived from these iPSC lines exhibit abnormal phenotypes including delayed neurite outgrowth and a reduction in motor neuron generation. These phenotypes mimics those phenotypes observed in animal models and patients. Currently, we are carrying out further studies to determine whether those iPSC-derived motor neurons can recapitulate a whole spectrum of disease phenotypes observed in animal models and patients. We are also using these SMA iPSCs to study the molecular mechanism which leads to selective motor neuron destruction in this disease.

Our goals are to validate the strategy of applying iPSCs for the study of disease pathogenesis and to uncover new molecular targets for drug intervention.

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Yun Yen, M.D., Ph.D.

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NOVEL MOLECULAR-TARGETED CANCER THERAPIES

The long-term goal of Dr. Yen's research is to investigate therapeutic agents and their target for cancer. The major emphasis is the development of novel molecular-targeted cancer therapies. The research focus is on drug mechanisms in DNA damage, repair, signal transduction, and DNA synthesis, and the inhibitors of these targets, such as ribonucleotide reductase. Furthermore, we have enhanced the clinical research in pharmacodynamic, and, recently, pharmacogenomics studies. The translational research further designed the preclinical evaluation and assay to lead the innovative findings for clinical investigators to initiate hypothesis-driven clinical trials. The research goal is to understand our cancer therapeutics targets and agents to bridge innovative cancer research into the personalized medicine.

Ribonucleotide Reductase And Inhibitor Development

Dr. Yen's laboratory has established a ribonucleotide reductase (RR) that is an important molecular target for cancer therapy. Inhibition of RR using genetic approaches demonstrated suppression of growth of human colon, pancreas, liver, lung, breast, renal, ovary, skin, brain, and prostate tumors in xenograft mouse models. In addition, Dr. Yen demonstrated the physiological difference between two small subunits of RR and further explored the interaction with p53 protein under genotoxic stress conditions. Under the sponsorship of NCI/CTEP, early phase trials with the new RR inhibitor, Triapine, and the antisense oligo against RR, GTI2040 are being conducted. Validation of RR targets with these new agents is being studied by real-time PCR, dNTP pool analysis, and proteomic analysis. Furthermore, Dr. Yen's group expressed three RR subunits, RRM1, RRM2, p53R2, by an in vitro expression system following crystallization. The RR activity can be measured through the combination of these subunits, and this protein has currently been used for screening of inhibitors. The lead compound has been selected from hydroxysemicarbazide analog and further characterized. In addition, inhibitors selectively inhibiting individual subunits have been synthesized based on structural differences between each subunit. In collaboration with Drs. Richard Yip, Yate-Ching Yuan, John Williams, and David Horne, the current development of small-molecule COH29 has been done along with development of more potent RR inhibitors.

Development of Nanoparticles in Cancer Therapy

Nanoparticles represent a promising new approach for improved delivery of small-molecule drugs. In collaboration with Dr. Mark Davis at Caltech, we have developed novel cyclodextrin polymers (CDPs) for delivery of chemotherapy drugs. These studies have demonstrated the preclinical efficacy of a nanoparticle polymer conjugate with camptothecin in mouse xenograft models. Results indicate that this polymeric drug conjugate has good tolerability and anti-tumor activity against a wide range of tumors. Based on these studies, the first-in-human clinical trial with nanoparticles for cancer therapy, using CDP conjugates with CPT-11, has been opened at City of Hope. In addition, nanoparticles represent a promising non-viral delivery vehicle for gene therapy approaches including siRNA which are currently under collaboration with Dr. John Rossi and UCLA.

Translational Research in Liver Cancer and Multiple Myeloma

Dr. Yen's laboratory has identified GADD45b gene under-expression as an important marker that is strongly associated with aggressive human hepatocellular cancer. The current investigation in signal transduction of GADD45b in liver cancer is an ongoing study. The preclinical drug therapeutic research for liver cancer by studying sunitinib in combination with a Src/STAT3 (Signal Transducer and Activator Transcription-3) inhibitor dasatinib to block VEGFR and STAT3 pathways is currently investigated. In human myeloma research, the STAT3 expression associated with FGFR3 and VEGFR has also been identified. The functional meaning and the mutation of FGFR3 is currently under investigation. Study of anti-angiogenesis inhibitors such as sunitinib and combination with dasatinib in treating myeloma is also a current project.

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Hua Yu, Ph.D.

Tim Nesvig Lymphoma Research Fellow at City of Hope
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STAT3 AND THE TUMOR MICROENVIRONMENT

A tumor's ability to proliferate, resist apoptosis, invade and thwart the immune system is the essence of cancer. Although many anti-cancer therapies show promise, most are aimed at the tumor cell as an independent entity and ignore the importance of the many cell types that constitute the tumor microenvironment. An emerging picture of the tumor as an organ highlights the role of multiple tumor-associated cells, including fibroblasts, endothelial cells, hematopoietic cells/immune cells and stem cells, that interact intimately with the transformed cells in modulating the oncogenic process. My group has shown that STAT3, which is persistenly-activated in tumor cells, is also activated in normal cells associated with the tumor. We have further demonstrated that STAT3 signaling coordinates multiple levels of crosstalk between tumor cells and their microenvironment, regulating tumor growth, apoptosis, angiogenesis, inflammation and immunosuppression. Our work, along with other studies, has established that STAT3 in tumor cells regulates a large array of genes important for proliferation, survival, angiogenesis, invasion/metastasis and immune suppression. Its central role in organizing the tumor microenvironment makes STAT3 a promising target both in tumor cells and in the normal cells that constitute the tumor organ. The goal of my program is to use novel technologies to target STAT3 in the entire tumor, thereby inducing its collapse through multiple mechanisms, while sparing cells in the normal organs.

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John A. Zaia, M.D.

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GENETIC AND OTHER ANTI-HIV THERAPY

Treatment of AIDS with genetically modified blood stem cells hypothesizes that genetic modification of cells containing inhibitors of genes important for HIV-1 replication will prevent the progression of HIV-1 in these cell populations and slow the progression to disease. Of the various ways that this could be tested, one approach currently tests whether genetically transduced blood stem cells could repopulate in the host after autologous transplantation. City of Hope investigators have developed a platform for testing of new vectors in the setting of autologous blood progenitor cell transplantation (HCT) for high-risk AIDS lymphoma. Using a lentivirus vector (LV) developed at City of Hope, four AIDS lymphoma patients have been treated with LV-modified HCT progenitor cells. Evidence exists for continued expression of anti-HIV RNA, including siRNA and anti-CCR5 ribozyme, for more than two years post-transplant.

A second approach using this same lymphoma model addresses whether a transient, but permanent genetic modification of these autologous stem cells could result in outgrowth of progeny blood cells with resistance to HIV. Here zinc finger nuclease, targeted to the gene for CCR5, a protein important for HIV infection, is in the developmental stages. Using the same LV, a safety study has been approved by the FDA for start in 2010 in AIDS patients who have failed conventional antiviral therapy. Because of the early success with this clinical model, other genetic approaches to HIV treatment are being studied in collaboration with A. Krishnan, J. Rossi and D. DiGiusto.

Finally, the AIDS Laboratory, under the supervision of S. Li, supports research on campus related to development of new cellular targets for HIV therapy, including small ubiquitin-like modifier protein (SUMO) in collaboration with Y. Chen and J-K Yee, and others.

Immunologic Control of Cytomegalovirus Infection (CMV)

In collaboration with the Department of Hematology/HCT and the Division of Translational Vaccine Research, our laboratory has been observing patients undergoing HCT who are at risk for CMV infection and questioning what elements of immunity are most important for protection from CMV infection in the HCT recipient.

Published data suggests a prominent role for donor KIR genotype in protection from CMV infection and disease. However, multiple cytokine responsive effector T cells are contributed by the donor's prior T cell immunity and are important in control of infection. The activation of the co-stimulatory molecule (PD-1) and the presence of killer immunoglobulin receptors (KIR) appear to be biologic markers for risk of CMV complications. It is the expression of PD-1 in CD4 and CD8 T cells, a measure of T cell exhaustion, which correlates with the development of CMV disease. This clinical model serves as a platform to other very interesting studies at City of Hope, which include studies on the role of KIR in control of CMV Infection and the development of vaccines for protection of patients from CMV. Dr. Zaia is the principal investigator of a phase I study of a novel CMV peptide vaccine developed by D. Diamond at City of Hope.

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Defu Zeng, M.D.

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TRANSPLANTATION IMMUNE TOLERANCE

Allogeneic hematopoietic cell transplantation (HCT) is a curative therapy for hematological malignancies and hereditary disorders as well as refractory autoimmune diseases. Induction of mixed chimerism via allogeneic HCT is also one of the most reliable approaches for induction of organ transplantation tolerance. However, graft versus host disease (GVHD) remains a major obstacle in classical HCT, in which recipients are required to be conditioned with total body irradiation (TBI) or high dose chemotherapy in order to allow donor stem cell engraftment. Recent studies have shown that tissue damage and activation of tissue dendritic cells caused by conditioning TBI or chemotherapy plays a critical role in induction of GVHD.

One of the research projects in the Zeng lab is to understand the pathogenesis of GVHD, in which donor T cells infiltrate the target tissues and mediate damage. Based on the clinical features, GVHD can be divided into acute and chronic GVHD. New immuno-suppressants have been effective in preventing acute but not chronic GVHD. The latter remains the major cause of morbidity and mortality of long-term survivors of classical HCT, and there has been no improvement in treating chronic GVHD over the past three decades, due to the poor understanding of its pathogenesis.

We have recently developed new mouse models of chronic GVHD. We are currently dissecting the role of allo- and auto-reactive CD4+ T (Th1, Th2 and Th17), Treg cells, APCs (dendritic and B cells) as well as autoantibodies in the pathogenesis of chronic GVHD. These studies will provide new insights into chronic GVHD pathogenesis and lead to the development of new therapies.

We observed that anti-CD3-preconditioning led to depletion of activated CCR7+ DCs in draining lymph nodes that imprint donor T expression of tissue-specific homing and chemokine receptors and prevention of donor T migration into epithelial GVHD target tissues (e.g., gut and skin) in the recipients conditioned with TBI or chemotherapy, so that donor T cells mediate graft versus leukemia (GVL) effect in the host lymph-hematopoietic tissues without causing GVHD in the epithelial tissues. We are now dissecting the cellular and molecular mechanisms of depletion of CCR7+ DCs and the separation of GVL effect from GVHD via anti-CD3 preconditioning.

Another project is to develop a radiation-free GVHD preventative conditioning regimen for induction of mixed chimerism for the therapy of autoimmune diseases (e.g., type 1 diabetes and lupus). We have recently shown that a radiation-free anti-CD3 mAb-based conditioning allows donor CD8+ T cells to facilitate engraftment and mediate graft versus autoimmunity (GVA) effect without causing GVHD. We also observed that induction of mixed chimerism results in reversal of autoimmunity, elimination of insulitis, and beta cell regeneration in overt diabetic NOD mice. We are currently exploring the cellular and molecular mechanisms of separation of GVA activity from GVHD and mechanisms of reversal of autoimmunity in anti-CD3-conditioned recipients. Our studies will provide new insights into transplantation biology and promote the application of HCT as a curative therapy for not only patients with hematological malignancies but also patients with variety of refractory autoimmune diseases.

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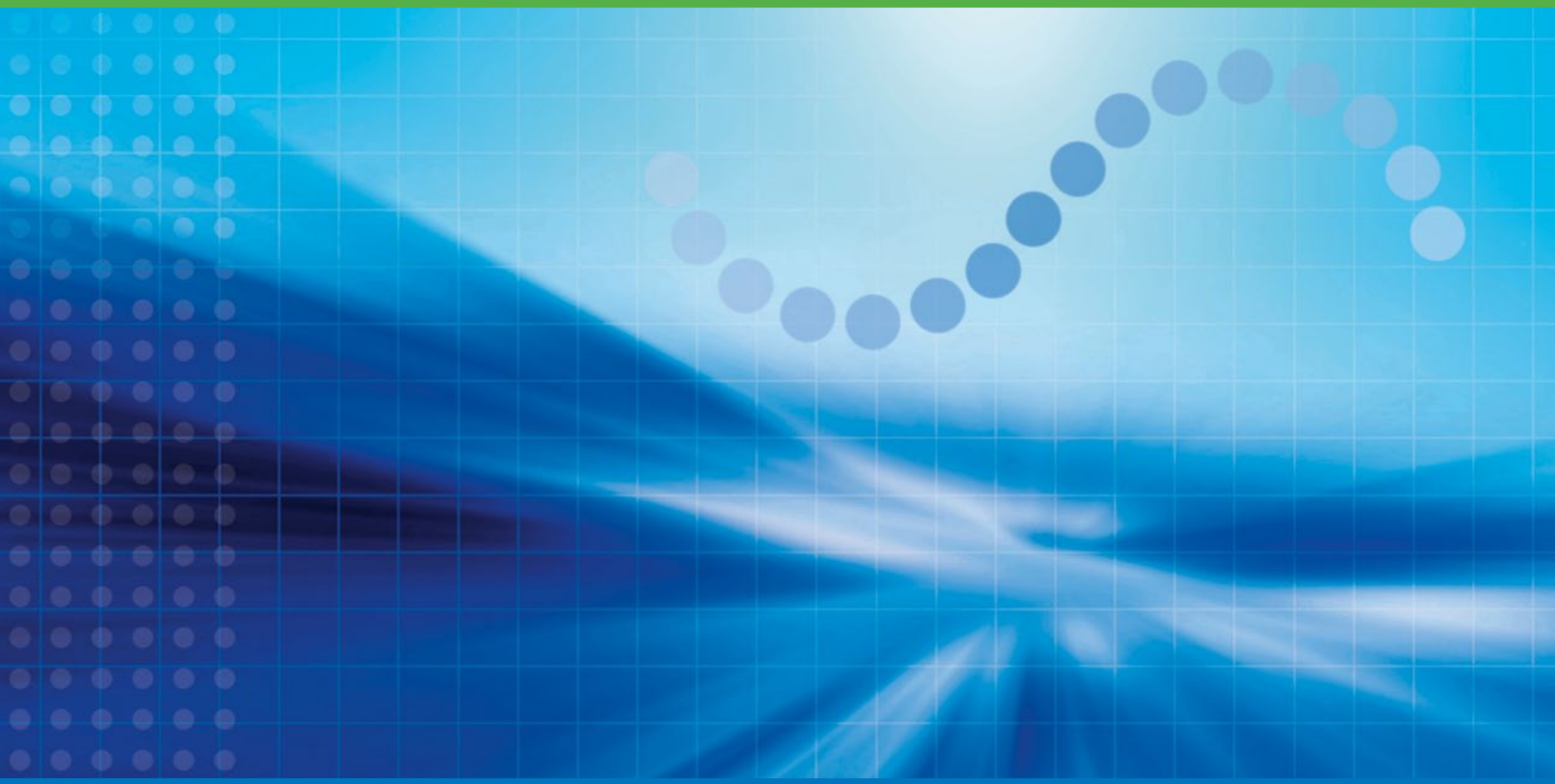
NOTES



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