DEPARTMENT OF DEVELOPMENTAL AND MOLECULAR BIOLOGY

The department consists of the laboratories of Drs. Nick Baker, Teresa Bowman, Dianne Cox, Ana Maria Cuervo, Sofia de Oliveira, Antonio Di Cristofano, Andreas Jenny, David Loeb, Anne Müsch, Michael Ross, Nicholas Sibinga, Richard Stanley, Amit Verma, Duncan Wilson, Fajun Yang, and Liang Zhu. Research interests in the Department cover four major areas: (1) Cell determination and regulation in blood, heart, kidney, brain, muscle, nervous and reproductive systems development (Drs. Bowman, Jenny, Ross, Sibinga, Stanley and Verma); (2) Signal transduction pathways regulating cellular function or developmental interactions (Drs. Jenny, Müsch, Stanley, Verma, Yang and Zhu); (3) Cell cycle regulation in normal and neoplastic cells (Drs. de Oliveira, Di Cristofano, Loeb, and Zhu); and (4) Protein synthesis, processing, targeting, intracellular vesicle trafficking and autophagy (Drs. Cuervo, Müsch and Wilson). The faculty frequently collaborate on projects within and between these areas.

The strength of the Department derives from our outstanding group of PhD and MD/PhD graduate students, who have been encouraged to, and accepted the challenge of, working along side of our faculty in all aspects of department life. We have weekly departmental work-in-progress (Fridays at noon with lunch) and theme-focused journal clubs (in the morning with breakfast) that all graduate students participate along with post-doctoral fellows and our faculty. In these meetings, we enjoy not only superb presentations but also probing questioning-answering periods in an atmosphere of learning, appreciating, and being critical and caring at the same time. A highly cherished asset of the department is our spirit of cooperation, which makes being a member of the department a fruitful and pleasant experience that you will take with you on completion of your Degrees.

The department's outside speakers’ seminar program contains regularly scheduled “student-invited speaker” seminars. Students poll and vote on their most favorite speakers every year and the students serve as hosts at the seminars and restaurant dinners.

The department retreats are another highlight of our department life. The retreat locations have included a mountain lodge in the Shawangunk Mountains in upstate New York, Mystic Seaport in Connecticut, Montauk on the east tip of Long Island and Long Island Aquarium and Exhibition Center, Riverhead, NY. In addition to formal presentations of research findings and strategies, we discuss ways of improving the function and atmosphere of the department as well as finding time to socialize!

Besides the scientific events, we are just as proud of our recreational activities, including BBQ and picnic at Glen Island at the beginning of the school year, and the joyful but competitive presentations of skits by the students and the faculty at our Holidays parties, when students and faculty try to outwit each other in making the most fun of each other!
Regulatory genes that control translation, cell competition, and neuronal development

One of the unresolved questions in biology is that of how organs grow. It is thought that cancer and neurodegenerative diseases involve defects in the regulation of cell growth and survival that are fundamental to organ growth. Genetic studies can be used to uncover new genes controlling tissue growth and maintenance, and to characterize their roles in vivo. Our current research uses both *Drosophila* and mice to address newly discovered mechanisms of growth regulation. These involve the mechanisms and functions of ‘cell competition’ in development and pathology, the regulation of ribosome biogenesis and translation, and the regulation of neural cell fate and reprogramming by transcription factors.

**Cell competition** When organs contain mixed cell genotypes, for example because of somatic mutation during aging, ‘cell competition’ can eliminate less fit cells through selective apoptosis of only these cells. Our studies show that cell competition is a mechanism that can remove cells that have become aneuploid, or acquired other large-scale genetic changes, and may be important in preventing birth defects and cancer. Our current goals include the molecular characterization of the cell competition pathway using *Drosophila* and its conservation and roles in aneuploidy cancer and aging in mice.

**Regulation of ribosome biogenesis** Ribosomes are essential for growth. Their biogenesis and assembly are regulated, both during growth and in neurodegenerative disease. Our laboratory has discovered novel signaling pathways that are activated when defects occur in ribosome assembly, and that also affect cell competition (see above). Ribosomal proteins are affected in several human diseases and also appear to act as tumor suppressors for multiple cancers. How ribosomal proteins act as tumor suppressors is not yet clear or affect neurological disease is not yet clear. We are studying the molecular signaling mechanisms activated by ribosomes, and their potential roles in mammalian diseases such as Diffuse Large B-Cell Lymphoma (DLBCL).

**Neural cell fate determination**

Proneural bHLH proteins are the transcriptional master regulators for most neuronal differentiation and important in neuronal reprogramming strategies. Their activities appear to be highly regulated. Our studies use genetic screening in
*Drosophila*, modern genome resequencing methods and multidisciplinary studies to characterize how proneural bHLH proteins are regulated in neuronal development.

**Selected recent publications**


Bowman Laboratory
Hematopoietic stem cells (HSCs) are one of the most widely utilized stem cell populations in the clinic today. Our research focuses on identifying the regulation of normal and malignant HSCs. Our studies combine the advantages of zebrafish and mammalian models to explore the development and genetic regulation of HSC formation and regeneration. Zebrafish offer powerful genetic pliability, easily accessible in vivo imaging, numerous transplantation assays, and screening capabilities. We aim to identify factors that are critical in the HSC regenerative response, which can be used to inform therapeutic strategies to improve treatments for patients with hematologic diseases.

RNA regulation during HSC specification and regeneration: Proper mRNA processing is critical to HSC function, but which components are involved is largely unknown. Recent identification of somatic mutations in spliceosomal components in patients with myelodysplastic syndrome (MDS) demonstrated how deregulation of splicing could lead to a disease state. We are combining zebrafish genetics and biochemical techniques to determine how mutations in spliceosomal factors result in aberrant hematopoiesis. Zebrafish is well-suited for this project as mutants in a subset of splicing factors display hematopoietic stem cell or differentiation defects. Additionally, we have performed chemical modifier screens for factors that enhance or suppress defects in a zebrafish spliceosomal mutant, identifying tissue-selective roles for spliceosomal components in DNA damage and inflammatory signaling. Determining the cellular and contextual specificity of interactions between splicing and these processes will help reveal how mutations in such "general" machinery could elicit such specific phenotypic outcomes in vivo.

Deciphering the development of HSC regeneration capacity: Rapid hematopoietic recovery following myeloablative treatments or post hematopoietic cell transplantation is critical to minimize complications from infection, bleeding, or anemia. In order to find additional pathways involved in HSC regeneration, we plan to take advantage of the optical transparency and screening capabilities afforded in the zebrafish. We have developed numerous novel approaches to examine self-renewal and differentiation capacities in developing zebrafish and into adulthood via hematopoietic cell transplantation, targeted cell ablation, and lineage tracing. The ultimate goal is to identify novel regulators of HSC regeneration using genetics and chemical screening in the zebrafish. One of the advantages to chemical screens is the ease at which chemicals can be tested in multiple assays. Thus, once confirmed in the zebrafish, compounds will be tested for functional conservation in murine and human models of hematopoietic regeneration. The identified compounds could have direct therapeutic potential in treating patients following myeloablative regimens.

Lab website: https://sites.google.com/site/thebowmanlaboratoryeinstein/
Selected Publications


Selected Reviews


* Equal author contribution. # Co-Corresponding authors.
Macrophage Phagocytosis and Motility

Macrophages play important roles in host defense against invading micro-organisms and they are also key players in initiating and maintaining an immune response. However, macrophages can also play negative roles, such as in chronic inflammatory disease. Also, tumor-associated macrophages (TAMs), which are present in large numbers in many tumors, appear to play an important role in promoting the progression of solid tumors to an invasive, metastatic phenotype. Macrophages are therefore a prime target for therapies, but it is important to elucidate the mechanisms by which they are recruited to and activated in tissues.

Studying the molecular mechanisms of phagocytosis

Among their many roles, macrophages are best known for their striking ability to engulf a large number of big (>0.5µm) particles that are very diverse in size and shape in a process called phagocytosis. Phagocytosis is important in many situations such as the clearance of pathogen and particles (bacteria, yeast, pollen and pollutants). Crucially, phagocytosis may also be a major player of cancer immunity by mediating the engulfment and killing of cancer cells. Phagocytosis requires actin assembly, pseudopod extension, and phagosome closure (Figure 1). Actin polymerization in response to particle binding requires the activation of members of the Rho GTPases, either Rac or Cdc42 for Fc gamma Receptor-mediated phagocytosis or Rho in the case of CR3-mediated phagocytosis. Both Rac and Cdc42 regulate the cytoskeleton in part through the activation of the Wiskott Aldrich Syndrome/WASp verprolin-homologous (WASP/WAVE) family of proteins. RhoG, another family member, is also important for phagocytosis but the precise role of RhoG is currently unknown. We are exploring the roles of these signaling pathways as well as those regulating the myosin family of molecular motors in phagocytosis. We are employing a novel technique called traction force microscopy to understand the roles of these factors in the protrusive forces needed to engulf the diverse particles of various sizes and stiffnesses found in nature.

Studying the molecular mechanisms of chemotaxis

The directed movement of cells in response to chemoattractants involves several complex, interrelated processes, including directional or chemotactic sensing, polarity, and motility. These processes are mediated by complex, interacting signaling pathways that appear to
have many similarities but yet have distinct characteristics depending on the chemoattractant and receptor. Many of the signaling cascades utilized for phagocytosis are also required for chemotaxis yet they result in the appearance of different structures (Figure 2). We are currently dissecting the signaling pathways required for macrophage chemotaxis towards:

1. CSF-1, a growth factor for macrophage survival and differentiation produced by many tumors and found in high concentrations in arthritic joints;
2. Chemokines that direct monocyte recruitment to different tissues.

**Determining the role of macrophages in the tumor microenvironment**

It is now increasingly recognized that the tissue microenvironment plays a critical role in tumor progression, but most studies on tumor metastasis involve the use of endpoint assays or in vitro studies on cell lines. It appears that macrophages and tumor cells participate in a paracrine interaction, with the tumor cells secreting CSF-1 and macrophages secreting EGF, but the precise roles of this paracrine interaction in tumor metastasis are unknown. We have developed a number of in vitro assays that reconstitute paracrine interaction between macrophage and carcinoma cells that mimic in vivo interactions of macrophages and tumor cells in the tumor microenvironment that have been observed by intravital imaging using multiphoton microscopy (Dovas et al., *J. Microscopy* 2012). We are currently using these assays to understand the roles of both soluble factors and direct interaction between macrophages and tumor cells through tunneling nanotubes to mediate long distance signaling to promote tumor metastasis (Figure 3).

**Development of tools for live cell imaging**

In order to determine the role of individual molecules in macrophage functions it is essential to understanding the timing and localization of specific protein involved. We have been actively involved in the generation of new probes for single live cell imaging including photoconvertible actin probes that label various structures in live cells. We have also developed and employed biosensors to monitor localized protein activity in live cells, including the recent work in collaboration with Dr. Louis Hodgson on the development of isoform specific single chain RhoGTPase biosensors.

**Selected Publications** (Students in bold)


The main focus of our laboratory is on understanding how cytosolic proteins are transported into lysosomes for their degradation (autophagy) and how impaired autophagy contributes to aging and age-related diseases.

A common feature of most cells in aging organisms is the accumulation of abnormal or damaged proteins in their cytosol that, undoubtedly, impairs cellular function. Protein accumulation results, at least in part, from impaired protein degradation with age. Among the different systems that participate in the intracellular degradation of proteins, lysosomes are the most affected by age. We have previously identified in many tissues of aged animals a decrease with age in the activity of a selective pathway for the degradation of cytosolic proteins in lysosomes known as chaperone-mediated autophagy (CMA). The main goal of our research is to identify the defect(s) that lead to the decreased activity of chaperone-mediated autophagy with age and in age related pathologies, and to analyze if the correction of those defects and recovery of normal proteolytic activity in old cells leads to an improvement in cellular function.

Among the different types of cellular autophagy, chaperone-mediated autophagy is responsible for the degradation of as much as 30% of cytosolic proteins, and it is mainly activated under conditions of stress, such as nutrient deprivation and oxidative stress. Substrate proteins are selectively recognized by cytosolic chaperones (hsc70 and cochaperones) that stimulate their binding to a glycoprotein receptor in the lysosomal membrane (LAMP-2A). The transport of the cytosolic proteins into lysosomes for their degradation requires also the presence of another chaperone in the lysosomal matrix (lys-hsc70).

Autophagic pathways in mammalian cells

Our efforts are currently directed to the:

1. Characterization of the different components involved in chaperone-mediated autophagy and identification of new players for this pathway.

We can isolate intact lysosomes from several tissues (liver, kidney and spleen) of rodents. For the identification of new CMA components we are using different immunochemical approaches and a
global proteomic and lipidomic approach. We have also developed a photoswitchable reporter which allows us to identify changes in CMA in intact cells. We are currently using this reporter to perform RNAi screenings in order to discover novel unknown regulators of this pathway.

2. Understanding the consequences of the age-related defect in chaperone-mediated autophagy. We have generate conditional and inducible transgenic mouse models incompetent for CMA in different tissues and have started to investigate possible tissue-dependence differences in the requirements for functional CMA. We are also analyzed the cellular response to CMA blockage and the different compensatory mechanisms elicited. We have found that blockage of CMA leads to important alterations in cellular quality control and cellular metabolism and deficiencies in the response to different stressors.

3. Consequences of impaired autophagy in age related-disorders. We have been analyzing changes in autophagy in three main groups of age-related diseases: 

   - neurodegeneration
   - metabolic disorders
   - cancer

By combining metabolic assays, cellular fractionation procedures and our in vitro lysosomal transport assays in different animal and cellular models of these diseases, we have found that autophagy malfunctions in conditions such as diabetes, obesity and also that the age-related decline in autophagy could be an early step in oncogenic transformation. We have identified a primary defect in CMA in some familial forms of Parkinson’s disease and of taupathies such as Alzheimer’s disease. We are interested in identifying the primary defect, the compensatory mechanisms elicited by the cell and developing approaches to modulate autophagy with therapeutic purposes in age-related disorders.

References:


CMA in lipid droplets metabolism
Reviews:


- Tekirdag KA, Cuervo AM. Chaperone-mediated autophagy and endosomal microautophagy: joint by a chaperone. J. Biol. Chem. 293:5414-5424, 2018


Western-type diet triggers inflammation in organs and tissues that leads to the development of non-communicable diseases such as diabetes, cancer, cardiovascular and gastrointestinal diseases. In liver, western diet induces fat accumulation in hepatocytes, which triggers the development of nonalcoholic steatohepatitis (NASH), a severe inflammatory form of nonalcoholic fatty liver disease (NAFLD). NAFLD/NASH can progress to liver cancer, such as Hepatocellular Carcinoma (HCC). A major gap remains in understanding how the innate immune cell networks modulate the liver tumor microenvironment (TME) and contribute to the progression of liver cancers in vivo, particularly in presence of western diet. In addition to liver diseases, the epidemic incidence of diet-induced chronic diseases is a major burden for healthcare systems worldwide. The field has been neglecting the impact of diet on the innate immune system and its consequences at a systemic level. The overarching goal of my laboratory is to understand how diet fuels inflammation and impacts disease progression in different inflammatory contexts. In our research, we are using zebrafish as an animal model to overcome the major limitation in the fields of inflammation and liver disease, which is the lack of good animal models amenable to large-scale non-invasive live imaging of immune responses and cell-cell interactions in a whole animal context.

I. Diet triggered immune mechanisms involved in liver disease progression. The in vivo immune mechanisms modulated by diet involved in HCC progression are poorly studied and unclear. Using zebrafish, I started to tackle some of these issues and have recently shown that diet-induced inflammation stimulates hepatocarcinogenesis by exacerbating the innate immune response and reducing T cell infiltration to the liver in a NAFLD/NASH-associated HCC model (de Oliveira et. al.; 2018, J. Hepatology). Innate immune cells are a therapeutic target for combinatorial therapies and might be a valuable resource to overcome resistance mechanisms to other therapies, including immune checkpoint therapy. In my lab we are interested in using zebrafish to 1) study if high fat diet effects on T-cells is mediated by neutrophils and macrophages in liver cancer, and 2) address if inflammasome activation mediates the effects of high fat diet on T-cells in liver cancer progression.

II. Effect of diet-induced systemic inflammation on innate immune response. Western diet induces systemic inflammation but also results in increased immune aging. Patients that suffer from diet-induced pathologies such as obesity or diabetes are more likely to develop secondary acute and chronic inflammatory diseases. There are increasing evidences that show that western diet can induce innate immune cell reprogramming and trained memory. However, it is unclear how diet-induced systemic inflammation affects the immune response to secondary local inflammatory stimuli. To study the impact of diet in a whole animal context, I have developed a zebrafish model of diet-induced systemic inflammation. We found that a short-term high fat diet promotes
meta-inflammation in zebrafish causing innate immune cell priming, which ultimately leads to the establishment of an exacerbated immune response towards a secondary inflammatory stimulus like tissue damage (manuscript in preparation). In our lab, we are interested in to 1) address the systemic effect of different diets on neutrophils and macrophages, and 2) study the mechanisms involved on the diet modulation of innate immune cells response towards secondary local inflammation such as tissue damage or infection.

Selected Publications|


A complete list of my published work is available in My Bibliography:

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Dr. Antonio Di Cristofano

Dissecting And Targeting Critical Pathways in Tumor Progression

The main focus of my laboratory is the PI3K signaling pathway, and the role it plays in epithelial transformation and tumor progression. We utilize genetically engineered mouse models to identify and deconstruct the signaling cascades controlled by PI3K and altered during early neoplastic transformation, to understand through which signaling nodes the PI3K cascade intersects with other growth-promoting pathways, and to design and test in vivo innovative, rationally designed therapeutic approaches for aggressive cancer.

As a model system, we utilize the thyroid gland. The thyroid is a central endocrine hub where a complex array of signals in the form of hormones and growth factors are integrated to govern thyroid growth and activity. As such, the thyroid represents an ideal model system to study the interaction between these signals in normal homeostasis as well as in the context of neoplastic transformation.

Beyond AKT: additional PI3K-regulated pathways in thyrocyte proliferation

PI3K pathway activation, via mutation/amplification of PIK3CA, or PTEN mutation/silencing, is a frequent event in thyroid cancer. However, our data suggest that the linear PI3K-PDK1-AKT pathway is not sufficient to drive thyroid transformation, and that additional PI3K-dependent pathways, including a novel one leading to activation of SGK1, are absolutely required for tumor development and thus may represent valid therapeutic targets.

Metabolic rewiring upon PI3K activation

We have found that PI3K activation initiates a coordinated rearrangement of the expression of many metabolic genes, favoring aerobic glycolysis at the expense of TCA/OXPHOS, through the inhibition of AMPK. Ongoing studies are dissecting mechanisms and biological consequences of this metabolic rewiring.

Crosstalk between PI3K and RAS

Thyroid tumors often harbor mutations leading to the constitutive activation of the PI3K and RAS signaling cascades. We have shown, using mouse models, that while each of these two pathways, alone, is unable to transform thyroid follicular cells, their simultaneous activation is highly oncogenic, leading to invasive and metastatic follicular carcinomas. Ongoing studies are aimed at understanding and targeting the molecular mechanisms of this synergy.

Rationally-designed therapies for advanced thyroid cancer

Anaplastic thyroid carcinoma (ATC) represents the most aggressive type of thyroid cancer and is almost invariably lethal. The dismal prognosis of ATCs reflects their intrinsic resistance to any current therapeutic approach. We have generated the first mouse model of ATC, a key tool that will allow us to design and test innovative, targeted therapies based on the alterations detected by interrogating the ATC transcriptome and phosphoproteome.
Overcoming adaptive resistance to PI3K inhibitors in thyroid cancer

Intrinsic, acquired, and adaptive resistance are common consequences of cancer cell exposure to targeted inhibitors. Indeed, we have found that while PI3K inhibitors reduce the growth rate of ATC cells in vitro and in allografts, this effect is short-lived and these cells invariably become resistant. Our objective is to characterize in vivo the cell autonomous and non-autonomous mechanisms through which PI3K-active thyroid tumor cells develop adaptive resistance to PI3K inhibitors, and to test the efficacy of combination therapies targeting key mediators of resistance.

Recent representative publications:
Key Words: autophagy, cell polarity, development, Drosophila, genetics, cancer, RNAi, kinases, cytoskeleton, Wnt signaling

Canonical and non-canonical Wnt signaling: patterning and cell polarization

Wnt/Wingless (Wg) growth factors commonly signal through either the canonical Wnt (Wg)-Frizzled (Fz)/\(\beta\)-catenin pathway or through non-canonical Wnt pathways such as the Wnt/Fz-planar cellular polarity (PCP) pathway, resulting the polarization of cells within the plane of the epithelium. These two pathways are highly conserved between humans, mice, fish, and flies. Canonical Wnt/\(\beta\)-catenin signaling is essential for many aspects of development. For example in vertebrates, it controls the specification of the dorsal-ventral (D-V) embryonic axis, cell proliferation in many tissues, and the maintenance of stem cells and during vascularization. In addition, aberrant canonical Wnt signaling in humans causes cancer. Our lab studies the function of Wnk kinases, for which we have identified a novel role in Wnt signaling in addition to their well-known role in the regulation of ion homeostasis in the kidney, where their misregulation causes hypertension (Gordon syndrome).

Non-canonical Wnt signaling established polarity within the plane of an epithelium, commonly referred to as epithelial planar cell polarity (PCP) and allows a cell to form structures that require not only positional, but also vectorial information. Examples of PCP in vertebrates can be very obvious, as in the ordered arrangement of scales on fish or hairs of mammalian skin. Less visible examples are the cilia of the respiratory tract and oviduct as well as the stereocilia of the sensory epithelium of the organ of Corti in the vertebrate inner ear. Aberrant PCP can lead to left/right asymmetry defects, open neural tubes, deafness and kidney disease. PCP signaling is, however, best studied in *Drosophila melanogaster*, mainly because of the versatility of the fly as model system. Our lab is particularly interested in how Rho kinase (Rock) is required for the migration aspect of PCP establishment which will help to understand tumor cell migration.

A genetic model for Endosomal Microautophagy

Autophagy delivers cytosolic components to lysosomes for degradation and is thus essential for cellular homeostasis and to cope with different stressors. As such, autophagy counteracts various human diseases and its reduction leads to aging like phenotypes. Macroautophagy (MA) can selectively degrade organelles or aggregated proteins, but selective degradation of single proteins has only been described for Chaperone-mediated autophagy (CMA) and endosomal Microautophagy (eMI). These two autophagic pathways, described to date only in mammals, are specific for proteins containing KFERQ-related targeting motifs. In collaboration with the Cuervo lab, we have developed a fluorescent reporter to characterize an eMI or CMA-like process in *Drosophila in vivo*. Our data provide evidence for a novel, starvation inducible catabolic process resembling endosomal microautophagy in a non-mammalian species. We are thus for the first time able to perform genetic screens for regulatory components of eMI, this only recently identified form of autophagy about which barely anything is known.
It is our goal to use *Drosophila* as model system to address fundamental questions that are relevant for development and disease in general.

**Lab homepage:** [http://jenny-lab.org](http://jenny-lab.org)

**Selected References:**

**Deregulated PAM trafficking and pancreatic beta cell function.** Diabetes affects about 10% of US population and almost 80 million adults are prediabetic. Approximately one third will progress to beta cell failure. This ‘natural history’ is known to relate to obesity and loss of beta-cell function. In order to not miss the early years of this natural history when the disorder may be easiest to treat, we need to understand the molecular details that define an ‘overworked’/failing beta cell.

Insulin storage and release to meet demand is the job of specialized organelles, the secretory granules (SGs). The importance of recent observations is that newly synthesized SGs are remodeled during maturation, a process that includes the removal of specific granule membrane proteins. These proteins, if not removed from immature secretory granules (ISGs), may acquire access to the nucleus. Thus, in addition to their recognized role as SG components, their new role may be to influence gene expression. Such dual participation implies that obesity and beta cell response to overcome this insulin resistant state by meeting elevated demand through the use of unmodified, immature granules may have important consequences for beta cell function.

Increased proinsulin secretion is an early, clinically observable marker of progression to diabetes/hyperglyceremia. The mechanistic explanation is that the unabated demand for hormone drives an untimely release of proinsulin-rich, immature granules (ISGs). Albeit peptidylglycine-α-amidating-monooxygenase (PAM) has not been well studied in beta cells, PAM is an ISG protein whose expression patterns suggest a relationship between obesity and granule to nucleus signaling (references of PAM characterization in neurons and pituitary shown below). Thus, to test the hypothesis that ISG exocytosis sets the stage for deregulated ISG protein trafficking, potentially initiating altered transcriptional programs, we selected PAM as a marker. Our current work demonstrates that

- In healthy β cells, PAM is a temporary resident of newly made, immature granules (ISGs).
- With obesity and disease, PAM accumulates in mature granules and stimulated delivery of SGs to the plasma membrane, initiates PAM routing to processing/degradative compartments.
- Changes in cellular lipid content affect PAM’s routing within endosomal structures rather than degradation, favor the production of the stress inducible form that enters the nucleus.
- Increased abundance/mislocalization of PAM is associated with attenuated glucose responsiveness.

**Goals:** Characterize PAM trafficking in beta-cells and determine which manipulations (cellular content/PAM localization /catalytic activity) depend on PAM/lipid co-regulation. Determine which targeted parameters also impair glucose responsiveness.


Dr. David Loeb – Research Summary

The primary focus of our laboratory is sarcoma metastasis. Sarcomas are malignant tumors arising from bone, muscle, and other connective tissue. Refinements in the use of chemotherapy, radiation, and surgery have resulted in significant improvements in the survival of children, adolescents, and young adults diagnosed with localized osteosarcoma and Ewing sarcoma, the two most common sarcomas in this age group. In contrast, patients diagnosed with metastatic sarcomas have the same grim prognosis today as those diagnosed 30 years ago. Since most cancer-related deaths are due to metastatic disease, future improvements in patient survival depend upon a deeper understanding of the biology of metastasis.

Our group developed a novel animal model of sarcoma metastasis involving orthotopic implantation of patient-derived xenografts followed by amputation of the tumor-bearing limb. Mice treated in this manner develop spontaneous distant metastases and ultimately die from metastatic disease. This model more faithfully reflects the clinical course of sarcoma patients than traditional models of metastasis, such as tail vein injection. We have leveraged this model to fuel a number of research projects.

The Wnt signaling pathway has been implicated in a variety of processes in normal development and in cancer biology, including processes vital to metastasis such as invasion, motility, and stem cell function. Utilizing pharmaceutical reagents being developed for clinical use, we have investigated the role of this important signaling pathway in sarcoma metastasis. Interestingly, we found that activation of Wnt signaling promotes the differentiation and inhibits metastasis of osteosarcoma, while inhibition of Wnt signaling inhibits Ewing sarcoma metastasis. Ongoing work is dissecting the molecular details on Wnt signaling in these tumor types, as well as investigating the seemingly contradictory roles of this pathway in these related tumors.

In related work, we have used both our mouse models and patient blood samples to begin to explore the biology of circulating tumor cells, based on the hypothesis that these cells, the presumed agents of metastasis, have a distinct biology that can be understood at a molecular level and targeted therapeutically. In partnership with a biotech company from Maryland, we have developed a low pressure filtration procedure to isolate circulating tumor cells with minimal manipulation, allowing us to quantify these cells and analyze them at the molecular level. Preliminary data from single cell RNA-seq analysis of these cells has revealed important signaling pathways that will be the subject of future investigations.

Our mouse model also revealed an important role for the tumor microenvironment in regulating sarcoma metastatic potential. We observed that patient-derived xenografts implanted in an orthotopic location undergo spontaneous distant metastasis, while the same xenograft implanted subcutaneously does not. This difference in metastatic potential correlated with a difference in the nature of the macrophage infiltrate in the tumors, suggesting a possible role for the immune system in mediating sarcoma metastasis. Our laboratory is currently engaged in a number of collaborative efforts to understand the interactions between sarcomas and the immune system, as well as exploring ways in which this interaction might be modulated pharmacologically for therapeutic benefit.
Also, based on our longstanding interest in radiopharmaceuticals, we are also exploring how radiation affects the interaction between sarcomas and the immune system.

Finally, another area of active investigation is the role of a specific RNA helicase, DDX3, in sarcoma biology. We discovered that DDX3 is expressed at high levels in a variety of sarcoma subtypes, including Ewing sarcoma, that DDX3 expression is important to the survival and proliferation of these cells, and that genetic or pharmacologic inhibition of DDX3 is toxic to Ewing sarcoma. Interestingly, proteomic analysis of cells after inhibition of DDX3 implicated this molecule in DNA damage repair. This observation led to our discovery that a small molecule DDX3 inhibitor potentiates the effect of ionizing radiation on DDX3-expressing sarcomas, but not on sarcomas with low levels of DDX3. Ongoing work is focused on understanding this phenomenon at the molecular level, as well as exploring other mechanisms by which DDX3 plays a role in sarcoma growth and metastasis, including DNA damage repair and regulation of stress granule formation.

**Selected Publications**


We are using cell biological approaches and stem cell-derived epithelial organoid cultures to understand signaling mechanisms that govern the establishment and maintenance of epithelial cell polarity and their relevance for morphogenesis and cell transformation.

1. Mechanisms for the establishment of the two distinct epithelial phenotypes in the liver

The liver is our largest metabolic organ. It produces proteins, lipids, clotting factors and glycogen while dispensing bile and detoxifying xenobiotics. In order to transport these different substances, a sophisticated network of liver venules, capillaries and interstitial conduits has evolved. An essential feature of this network are the lumen-forming epithelia that give rise to two major liver cell populations: (1) mature hepatocytes - the main parenchymal cell type, and (2) bile duct cells. Hepatocytes form single-cell cords with a capillary-like luminal network (bile canaliculi) running between them. In contrast, bile duct cells form tubules, each with a central lumen that receives the content of the bile canaliculi that has formed next to hepatocytes. During initial liver development and bouts of regeneration, both hepatocytes and bile duct cells are derived from a common epithelial precursor. How hepatocytes and biliary epithelia obtain their unique morphological and functional phenotypes from this common precursor is poorly understood. Indeed, because bile canaliculi are not readily visible by conventional H&E tissue stain, the study of epithelial polarity in the liver has largely been neglected. The resulting gap in our knowledge has greatly hindered our ability to better understand the molecular basis of common liver diseases, which typically present with changes in lumen organization. It also severely limits our ongoing efforts to engineer hepatic tissue that can be used for transplantation, toxicology and gene therapy studies. To tackle these issues, we have developed a unique tissue culture model in which we polarize an epithelial cell line with either hepatocytic or ductal polarity. It allowed us formulate hypotheses on how these two major epithelial phenotypes arise, implicating cell adhesion signaling and protein trafficking as key determinants. We are testing our hypotheses and elucidate their molecular details in primary and organoid-derived liver cells.

2. Identification of Par1b signaling pathways in epithelial cells - The role of Par1b in *H. pylori* pathology

Par1 isoforms are serine/threonine kinases that have emerged as "core determinants" of cell polarity in different contexts, including in mammalian epithelial cells. In collaboration with the microbiologist M. Stein (then Univ Edmonton, Alberta) we found that Par1b is inactivated by the cytotoxic protein CagA of *Helicobacter pylori*. *H. pylori* is a human adapted bacterium that colonizes the gastric mucosa of an estimated half of the worlds’ population and destroys the epithelial lining of the stomach in a fraction of infected carriers, resulting in gastritis and gastric ulcers. *H. pylori* induced gastric atrophy can progress to gastric cancer, and CagA has been classified as a bacterial oncogene by the WHO. We are elucidating CagA signaling mechanisms that are mediated by Par1b inhibition. In addition to the effects on epithelial morphology and cell-cell junctional integrity we identified a contribution of Par1b-inhibition to the accumulation of DNA doublestrand breaks in H.p. infected gastric epithelium. Since repair of these lesions is error-prone, they likely contribute to the transformation risk. To identify relevant Par1b- substrates we have conducted an unbiased screen that yielded more than 70 putative novel Par1b substrates and their Par1b phosphorylation sites in polarized epithelial cells. We are investigating their roles in *H. pylori* infected gastric epithelial monolayers derived from organoids, which we establish from human tissue that is leftover from bariatric surgery.
The major focus of research in the Ross laboratory is to identify novel mechanisms of kidney injury occurring in HIV-positive persons and his laboratory uses in vitro and murine models to generate new strategies to prevent and treat kidney diseases. Dr. Ross also works with members of the International Network for Strategic Initiatives in Global HIV Treatment (INSIGHT) to perform research on kidney disease in the context of large international HIV treatment trials.

**Ongoing NIH-funded projects:**

**Mechanisms by which antiretroviral medications protect kidneys from HIV- and diabetes-induced injury:**

Though antiretroviral therapy is efficacious in preventing and treating HIV-induced kidney disease, the mechanisms by which these medications protect the kidney from the deleterious effects of HIV are poorly understood. We have exciting data demonstrating that HIV protease inhibitors protect the kidneys from HIV via affects that are independent of blocking HIV protease and recent data from our lab demonstrates that these medications protect mice from diabetic kidney disease, the most common cause of kidney disease in developed countries. We are performing studies using transgenic animal models and molecular and genomic techniques to identify novel pathways by which antiretroviral medications protect the kidneys from injury and disease.

**The role of APOL1 polymorphisms in promoting HIV-induced kidney injury:**

Polymorphisms in the APOL1 gene account for most of the excess risk of African-Americans to non-diabetic kidney disease and HIV-associated kidney disease in particular. In our studies, we are using genetically modified human kidney cells to perform innovative proteomic, and genomic studies to identify novel mechanisms by APOL1 genetic variants predispose to HIV-induced kidney injury.

**Relevant recent publications:**


CONTROL OF VASCULAR CELL GROWTH, DIFFERENTIATION, AND INJURY RESPONSE

Vascular disease, the greatest single cause of morbidity and mortality in developed societies, results from interactions between circulating inflammatory cells, the endothelium that lines the vasculature, underlying vascular smooth muscle cells (SMCs) that comprise most of the arterial wall, and systemic metabolism. We seek to identify new factors and pathways that regulate disease-associated functions of relevant cell types and tissues.

The underlying pathogenesis of vascular disease is complex: it includes reactivation of developmental pathways, innate and acquired immune responses, and changes in cell function and cell fate (including transdifferentiation) that result from physical stresses, perturbed blood flow, and biochemical stimuli. Our general approach is to characterize such responses at the molecular level, in cell culture, and in mouse models that reflect specific types of vascular injury, including atherosclerosis, restenosis after angioplasty, vein graft disease, transplant-associated arteriosclerosis, and integrated metabolism. One project focuses on FAT1, a member of the ancient Fat cadherin subfamily – in Drosophila, these proteins are important regulators of growth and planar cell polarity. Our work in mammalian SMCs shows that FAT1 interacts with and limits the transcriptional activity of beta-catenin, the major downstream mediator of Wnt signaling, a core developmental pathway that regulates many aspects of metazoan embryogenesis. Interestingly, beta-catenin-mediated signaling in SMCs is required for vessel wall assembly during vascular development, while FAT1 controls SMC growth, metabolism, and differentiation in injured vessels in the adult. A second project involves the allograft inflammatory factor-1 (AIF1, aka Iba1), a 17kD EF-hand protein expressed primarily in activated macrophages and microglia. We have found that AIF1 promotes macrophage migration, phagocytosis, survival, and selected cytokine production. We are currently evaluating how AIF1 contributes to integrated immune responses and metabolism, and how loss of AIF1 function in the mouse affects the pathogenesis of multiple inflammatory diseases, including atherosclerosis, obesity, and diabetes. In collaboration with the Stanley lab, we have characterized a role for colony stimulating factor-1 (CSF1), the main regulator of macrophage survival, proliferation, and differentiation, in control of transplant-associated arteriosclerosis, the major barrier to long-term success of solid organ transplants. Our preclinical studies suggest that pharmacologic inhibition of CSF1 signaling can limit neointimal formation and vascular obstruction of transplanted arteries.

Ongoing work in these areas involves defining the molecular bases for these effects. By identifying such novel mechanisms, we hope to find new targets for therapeutic intervention to regulate SMC and inflammatory cell activities and improve vascular disease prevention and treatment.

Selected References:


Growth Factors and Signaling in Development: The colony stimulating factor-1 receptor

The major focus of my laboratory has been to identify colony stimulating factor-1 (CSF-1), its 3 isoforms and its receptor (CSF-1R), and to define their developmental and physiological roles using biochemical, cell biological and mouse genetic approaches. We have shown that CSF-1 and the CSF-1R regulate the production of macrophages, osteoclasts, microglia, Langerhans cells, Paneth cells and neural progenitor cells and play an important role in the development of leukemia and of several inflammatory diseases. We have shown that CSF-1 and the CSF-1R, via their regulation of tumor-associated macrophages, enhance solid tumor progression. We have developed novel biochemical and genetic approaches to CSF-1R signal transduction to analyze CSF-1R structure/function, identifying and elucidating the function of several downstream signaling molecules, most recently, MAYP/PSTPIP2, in which we have described mutations that lead to an autoinflammatory disease in mice, involving macrophages and osteoclasts. In work relevant to our recent focus on the nervous system, we have shown 1) that a novel ligand for the CSF-1R, interleukin-34 (IL-34), similarly activates the CSF-1R, but has a different tissue and cellular expression pattern from CSF-1, 2) the complete dependence of microglial development on the CSF-1R, 3) that via the CSF-1R, CSF-1 and IL-34 directly suppress neural progenitor cell (NPC) self-renewal and enhance NPC survival and differentiation, 4) that IL-34, but not CSF-1, also acts via an additional receptor, protein tyrosine phosphatase zeta, which is also expressed on NPC, but not on microglia, 5) that miR-21 is a novel CSF-1R pTyr-721–induced molecule that suppresses the macrophage M1 (inflammatory) phenotype and enhances the M2 (trophic) phenotype and 6) that the Csf1r+/- mouse is a model of the adult onset leukoencephalopathy with axonal spheroids and pigmented glia (ALSP), a dementia in man caused by dominant inactivating mutations in the kinase domain of the Csf1r gene. Our recent studies have elucidated the mechanisms underlying ALSP development in the Csf1r+/- mouse.

Selected Recent Publications:


Key Words: cytokines, hematopoiesis, epigenetics, mds, leukemia, esophageal cancer, pancreatic cancer

1. Targeting signal transduction in hematologic malignancies: Cytokines play important roles in the regulation of normal hematopoiesis and a balance between the actions of hematopoietic growth factors and myelosuppressive factors is required for optimal production of different hematopoietic cell lineages. We study the role of MAP kinases in the regulation of hematopoiesis and have shown that the p38 MAPK signaling pathway is the dominant cytokine regulated inhibitory pathway in human hematopoiesis and is overactivated in MDS. The myelodysplastic syndromes (MDS) are collections of heterogeneous hematologic diseases characterized by refractory cytopenias due to ineffective hematopoiesis. These preleukemic disorders are common causes of anemia in the elderly and are rapidly increasing in incidence. We have also demonstrated that the TGF-β pathways is overactivated in MDS. We are trying to study the molecular mechanisms that lead to the activation of these pathways in MDS and are using small molecule inhibitors in mouse models to target these pathways.

2. Epigenomic analysis of tumors: We are using high throughput assays to analyze DNA methylation across the genome and have optimized these assays to work with low amounts of DNA. We have successfully used these assays in conducting an integrative analysis of epigenetic and genetic alterations in MDS and esophageal cancer. We are now exploring alterations in DNA methylation in pancreatic cancer, myeloproliferative neoplasms and renal cell cancer.

3. Clinical studies in Myelodysplastic syndromes: We have a “center of excellence” clinic for patients with Myelodysplastic syndromes. We offer a variety of clinical trials for these patients (www.mdstreatment.com).

Selected References:


Understanding the role of sumoylation during spermatogenesis

Post-translational modification by Small Ubiquitin-like Modifiers or SUMO proteins has been identified as an important regulatory event that is implicated in several cellular processes, seemingly based on the cell type. Recent findings from our laboratory suggest diverse and potentially multiple roles of SUMO in testicular function and spermatogenesis however, SUMO targets remained uncharacterized in the testis due to the complex multicellular nature of testicular tissue, the inability to maintain and manipulate spermatogenesis in vitro, and the technical challenges involved in identifying low-abundance endogenous SUMO targets. My laboratory performed cell-specific identification of sumoylated proteins using concentrated cell lysates prepared with de-sumoylation inhibitors from germ cells and sperm. Hundred and twenty proteins were uniquely identified in specific germ cell fractions. The identified proteins are involved in the regulation of transcription, stress response, microRNA biogenesis, regulation of major enzymatic pathways, nuclear-cytoplasmic transport, cell cycle control, acrosome biogenesis, and other processes. Several proteins with important roles during spermatogenesis were chosen for further characterization by co-immunoprecipitation, co-localization and in-vitro sumoylation studies. Many of these proteins have a unique role in spermatogenesis, particularly during meiotic progression. Interestingly, several kinases have been identified as sumoylated targets. This research opens a novel avenue for numerous further studies of SUMO at the level of individual targets. Studies are currently underway to understand how sumoylation affects activity of several targets, in particular the activity of kinases and phosphatases important for spermatogenesis.

Characterization of new regulators of WNT signaling in lung diseases

Wnt signaling is required for airway epithelial differentiation and healing. A decrease in Wnt target genes and an increase in Wnt inhibitors was reported in bronchial epithelial cells of smokers and COPD (Chronic obstructive pulmonary disease) patients, and in experimental mouse models of emphysema. Notably, preventive and therapeutic WNT/β-catenin activation led to a significant reduction of experimental emphysema. Therefore, targeting the Wnt pathway is a promising approach to the development of therapies for lung diseases; however, the question of where and how to target Wnt pathway remains open. CDK14 was identified by our group as a gene significantly down-regulated by tobacco exposure in vivo in testes and lungs. It has recently been discovered that Wnt signaling is activated by Cdk14/ cyclin Y complex through phosphorylation of LRP6 co-receptor, a key regulator of the WNT pathway. We have recently shown that CDK14 is expressed in bronchial and alveolar epithelium and that treatment of lung cell lines including primary normal human bronchial cells (NHBE) with CS decreased the level of CDK14; COPD-derived human bronchial cell (DHBE) showed reduced CDK14 levels versus NHBE. Furthermore, preliminary observations suggest a lower number of CDK14 positive cells in emphysema versus normal lungs. The research in our laboratory currently focuses on 1) performing a screening and quantitative analysis of COPD samples as well as smokers versus non-smokers for the levels of CDK14/ Cyc Y and Wnt pathway activity; 2). Testing whether down-regulation of CDK14 and/or CycY gene expression inhibits Wnt signaling in NHBE and small airway epithelial cells (SAEC), and whether restoration of the normal level of CDK14/CycY in COPD-derived cells would activate Wnt signaling.
comparative analysis of quantitative changes in phosphoproteome of NHBE before and after the down-regulation of CDK14 and/or CycY in order to identify CDK14 /CycY downstream targets and molecular pathways. The results from this research will lead to a better understanding of the molecular pathology of COPD in order to use the WNT pathway as a diagnostic and therapeutic target for lung diseases.

Lab homepage: http://einstein.yu.edu/labs/margarita-vigodner/research-projects/

Selected Publications

Viruses and the nervous system

Humans are infected by at least eight different species of herpes viruses. These pathogens cause several forms of cancer, severe birth defects or miscarriage, and are a leading cause of organ transplant rejection. Our interests are focused on the neurotropic herpes viruses, including herpes simplex (HSV).

**HSV: a pathogen of the nervous system (see our References on last page)**

Herpes simplex virus (HSV) is a leading cause of blindness, fetal mortality and severe neurodevelopmental and other birth defects. It is also increasingly recognized as a causative agent in perhaps the majority of cases of Alzheimer’s. All these diseases are a direct result of the ability of the virus to invade, manipulate and traffic within the human nervous system. Our laboratory is dissecting the molecular machinery that HSV uses to achieve assembly and transport within neurons (Fig. 1).

**Fig. 1: HSV makes a break for it.** Left hand image: HSV initially assembles clusters of capsids in the cell nucleus. These then fill with viral DNA, visible as a dense core (arrow at right). They then traffic to, and bud into, the nuclear membranes (left hand arrow) to enter the cytoplasm and cell body. Right hand image: Similar to left hand but at higher magnification, showing a DNA-containing HSV capsid punching through the nuclear membranes.

Once in the cell cytoplasm HSV capsids dock with, and become enveloped by, cytoplasmic organelles to assemble the mature infectious virus. These then traffic along neuronal microtubules to travel within the nervous system (Fig. 2).

**Fig. 2: HSV steals motors and traffics down the axon.** HSV particles (labeled with red fluorescent protein fused to a viral structural subunit) recruit molecular motors (kinesins) then stream down the axon of neurons (white arrow) to invade adjacent epithelia and spread to...
The events of virus assembly, and the detailed molecular structure of assembly and trafficking intermediates are very poorly understood. In collaboration with the analytical imaging facility (AIF) here at the Albert Einstein College of Medicine we have pioneered the application of Correlative Light and Electron Microscopy to the study of HSV assembly in the neuronal cell body (Fig. 3).

**Fig. 3: What to do when more light doesn’t help you see better.** Correlative light and electron microscopy makes it possible to observe HSV assembly simultaneously by fluorescence microscopy and electron microscopy. Paired images A-B, C-D, E-F and G-H show HSV capsids assembling onto cellular organelles in infected cells. For each pair a scanning electron microscopy image is shown on the right (e.g. B), and an alignment of the same structure with its fluorescent light microscopic image is shown on the left (e.g. A). Red light is being emitted by HSV capsids engineered to contain molecules of red fluorescent protein. Green light is coming from organelle-bound forms of green fluorescent protein. Scale bars: 200nM.

**Fig. 4: HSV goes for a walk.** Using the same technology as in Fig. 3 we image viruses (red) attached to microtubules (green). Boxed region in (A) is expanded in (B). Combining this structural approach with genetically manipulated viruses and fluorescently-tagged kinesins and dynein’s enables us to dissect the mechanism of motor-recruitment by these viruses.
Selected Recent References


Key Words: gene expression, lipid metabolism, metabolic syndrome, obesity

Dysregulation of lipid homeostasis is common in major human diseases, including type 2 diabetes, obesity, fatty liver diseases, atherosclerosis and some types of cancer. Our laboratory is interested in the transcriptional control of lipid metabolism. In addition to DNA-binding transcription factors, gene expression in eukaryotic cells often requires a series of transcriptional cofactors. We use biochemical and genetic approaches to study the physiological and pathophysiological functions and regulation of the network of transcription factors and their cofactors in the liver (hepatocytes) and adipose tissues (white, brown and beige adipocytes). A focus of our work is the multi-subunit Mediator complex, which is a highly conserved transcriptional cofactor. The mammalian Mediator is one of the cofactors for the sterol regulatory element -binding proteins (SREBP), the master regulators of both fatty acid/triglyceride and cholesterol biosynthesis. SREBP proteins are synthesized as inactive precursors that are tethered to the endoplasmic reticulum membrane. When lipid biosynthesis is demanded, SREBP precursors are proteolytically processed to mature forms of transcription factors that migrate into the nucleus, interact with cofactors including the Mediator complex, and activate transcription of target genes, which encode the rate-limiting enzymes in synthesizing fatty acids/triglycerides and cholesterol. Through the protein-protein interactions of different subunits with SREBP as well as other transcription factors, the Mediator complex critically regulates lipid metabolism. Moreover, dynamic remodeling of the Mediator complex conveys the metabolic signals to transcriptional outputs, providing another layer of regulation of gene expression. With the ultimate goal of identifying novel targets for preventing or treating metabolic syndrome (including type 2 diabetes, obesity, fatty liver disease, hyperlipidemia and hypertension), our research will advance our understanding of how lipid homeostasis is regulated at the molecular level.

Selected Publications:


**OVERVIEW**

A common feature shared by cancer and obesity is deregulation of cellular homeostasis, including cell proliferation, cell growth, cell metabolism, and cell death; while liver regeneration involves robust proliferation without tumorigenesis. Tumor suppressors such as pRb and p53, oncoproteins such as Ras, Myc, and Pten, and organ size regulators such as Yap, are the major regulators of cellular homeostasis. We aim to understand how these regulators function with the goals to improve clinical success in treating cancer, treating obesity, and cell therapy with hepatocyte transplantation.

In the cancer field, we are identifying treatment strategies for cancers that have genetically inactivated pRb and p53. pRb and p53 are the two major tumor suppressors. Their functions are activated by most oncogenic events and they implement most and best cells’ antitumor mechanisms. When prostate cancers progress, RB1 and TP53 (genes encoding pRb and p53) are more and more frequently inactivated together (ref 11). On the other hand, 99% of small cell lung cancer (SCLC) co-inactivate RB1 and TP53. These findings explain why advanced prostate cancer and SCLC are among the deadliest cancers in modern medicine. Since it is not feasible to reintroduce RB1 and TP53 back into all cells in a cancer, these frequent features have not led to targeted therapies. Chemotherapies are the only treatment for these cancers but they quickly lose effectiveness. SCLC was designated as one of the two “recalcitrant cancers” by the 2012 US Congress.

We generated mouse prostate cancer and SCLC models by deleting Rb1 and Trp53 in the respective organs to identify mechanisms that can still inhibit these cancers. We showed that combining deletion of Skp2, which is a target of repression by pRb, completely blocked tumorigenesis in the absence of pRb or both pRb and p53 (refs 2, 5, 7, 8). Mutating p27T187 to an alanine, which prevents recognition of p27 by Skp2 but does not harm mouse development and life span (ref 9), also significantly inhibited pRb and p53 doubly deficient prostate tumorigenesis and in cancer organoids (ref 11). SCLC originates from neuroendocrine cells. Using mouse embryonic brains, we found that Skp2 deletion and Rb1 deletion induces synthetic lethal apoptosis that remains effective when p53 is additionally deleted (ref 10). Ongoing studies aim to target functions of Skp2 in regulating p27 degradation, regulating cancer cell metabolism, and regulating epithelial-to-mesenchymal transition to inhibit pRb and p53 doubly deficient prostate cancer and SCLC. At the same time, we are translating mouse model findings to patients on the organoid platform side by side, in order to increase the predictive values of human cell experiments to better rationalize clinical trials.

In the obesity field, we are studying the function of pRb in hypothalamus neurons. These neurons form circuits to positively and negatively regulate energy balance, and it has been suggested that high fat diet could directly disrupt homeostasis of these neurons, leading to diet induced obesity, explaining why dieting commonly fails. We discovered that high fat diet can activate kinases in these neurons to phosphorylate and functionally inactivate pRb. In this context, inactivation of pRb induces degenerative changes in these neurons. When we deleted Rb1 in POMC neurons, the mice increased their food intake and become obese, demonstrating that pRb functions to maintain POMC neuron homeostasis to suppress obesity. To our surprise, in AGRP neurons, which reside together with POMC neurons in hypothalamus and functionally antagonize POMC neurons to reduce the desire to feed, deletion of pRb did not harm their homeostasis. Based on these findings (ref 6, in collaboration of with Dr. Chua of the Einstein Diabetes Center), we are identifying the kinases that phosphorylate pRb in POMC neurons after high fat feeding, and establishing techniques to prevent pRb phosphorylation following high fat feeding. Through these studies, we aim to prevent and treat diet induced obesity.
The liver has remarkable ability to regenerate when 70% of it is resected. This regenerative capability has important implication to tumorigenesis and regenerative medicine. Since the supply of donor liver is far smaller than the demand, hepatocyte transplantation is the best approach to treating liver failure and liver defects. We showed that by deleting the cyclin-dependent kinase inhibitor p27, hepatocytes were stimulated to proliferate more in the host liver to save mice from liver failure (ref 1). Unfortunately, deleting p27 also increased liver cancer burden during liver carcinogenesis by chronic HBV or carcinogen DEN (ref 3, 4). In collaboration with Dr. Shafritz of the Einstein Liver Center, we are now studying the liver size regulator YAP to determine its ability to increase hepatocyte proliferation following transplantation and its associated liver cancer risk.

**Selected Publications:**


