DEPARTMENT OF CELL BIOLOGY
2019-2020

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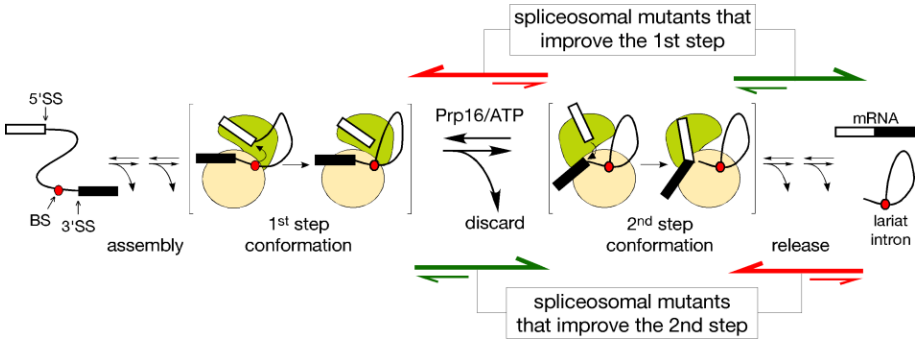

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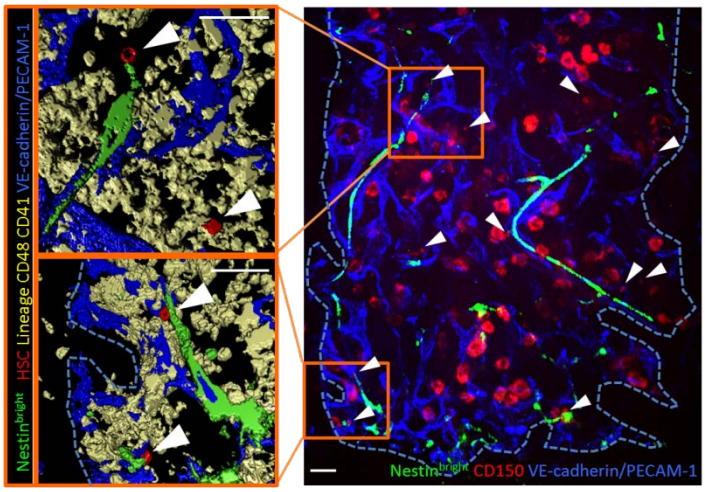

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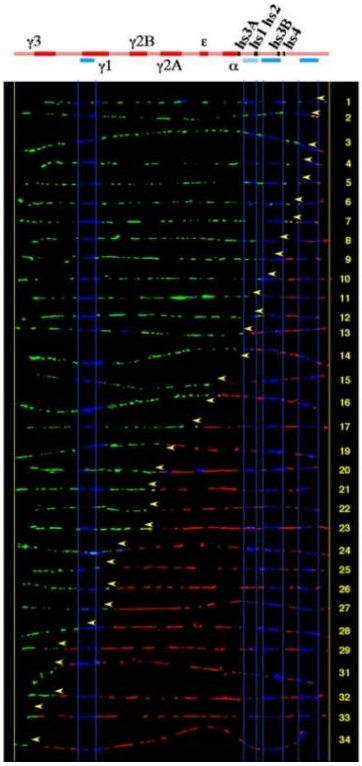

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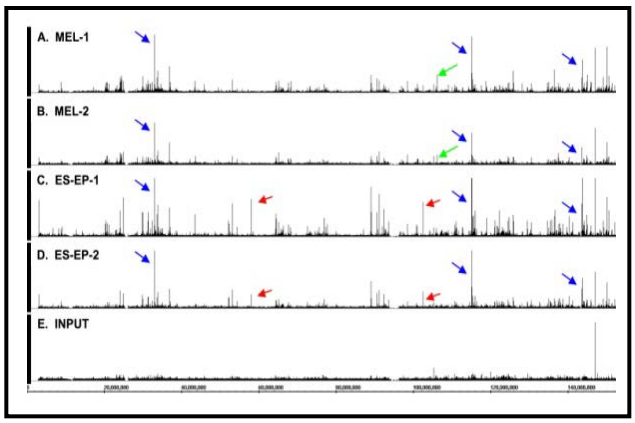

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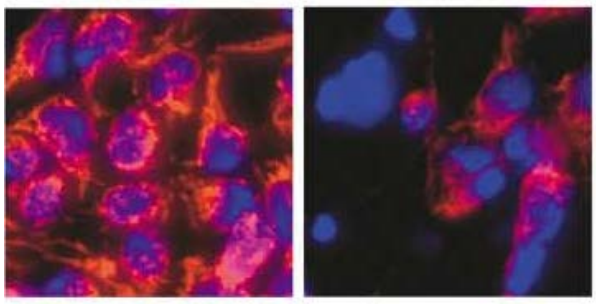

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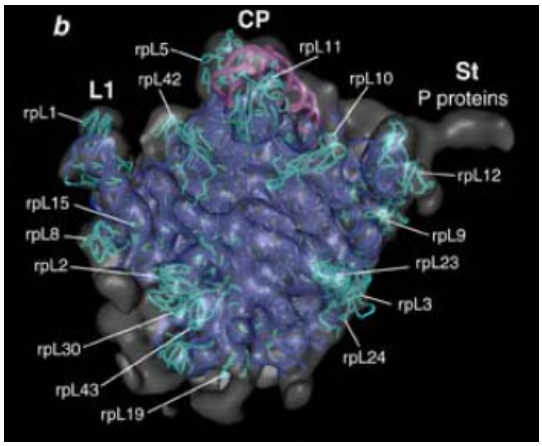
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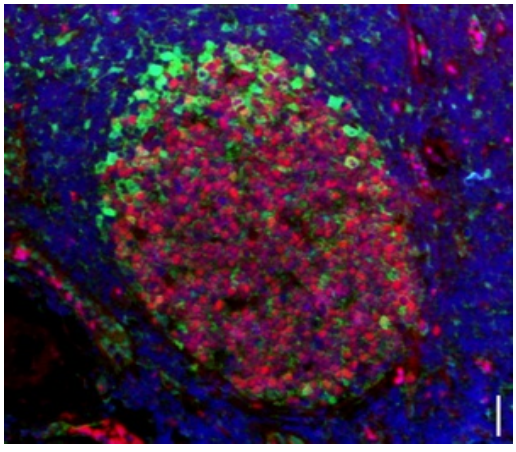

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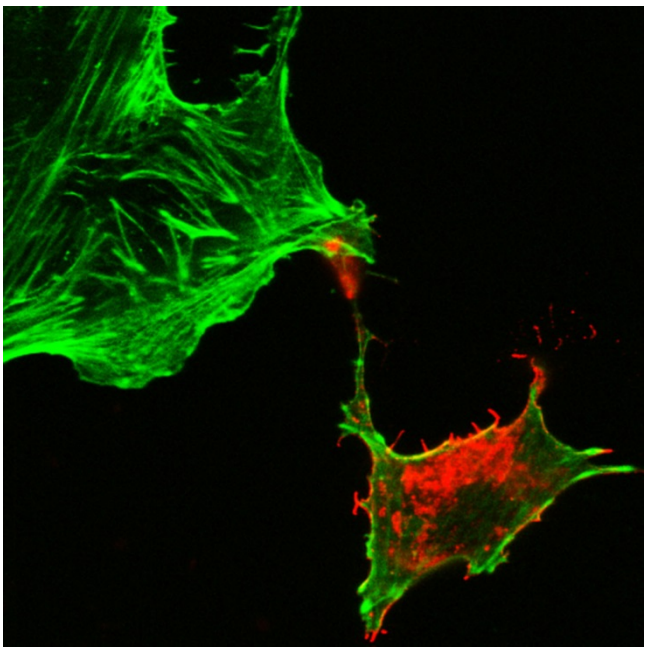

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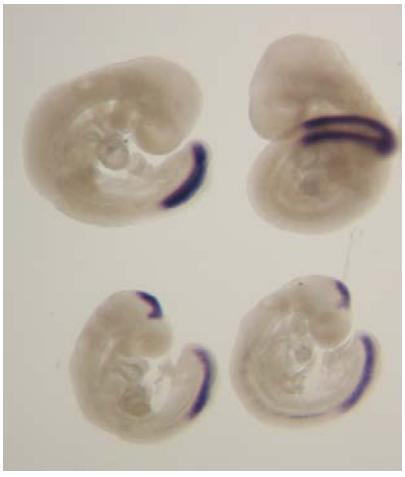

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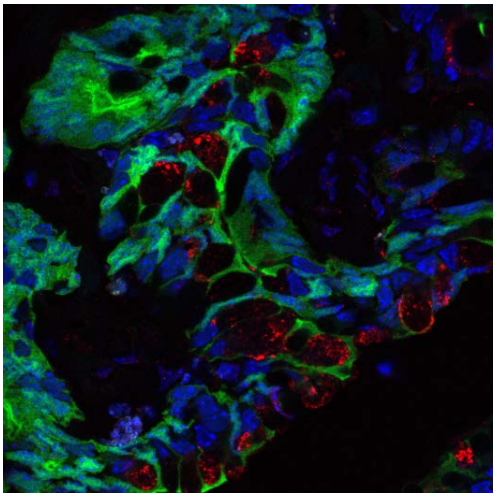

Picture Legends

1. **Query Lab**: Scheme for progression of pre-mRNA splicing, highlighting the different conformations required for the first and second catalytic steps; modulation of transition between the two catalytic steps by spliceosomal mutations results in altered splicing fidelity and splice site choice.

2. **Fyodorov & Skoultchi Labs**: Drosophila melanogaster, a model system to study biochemistry and genetics of chromatin.

3. **Frenette Lab:** A whole-mount images of the bone marrow niche with 3D reconstruction. Arrowheads denote hematopoietic stem cells.

4. **Schildkraut Lab**: Fluorescent antibodies showing replication fork direction in single DNA molecules labeled with halogenated nucleotides.

5. **Skoultchi Lab**: ChIP-Seq – Chromatin immunoprecipitation followed by massive parallel sequencing reveals differences in the DNA binding patterns of transcription factor PU.1 in normal red blood cells (ES-EP) and malignant erythroleukemia cells (MEL).

6. **Kitsis Lab**: Cell death. Healthy (left) and dying (right) HEK293 cells. Blue - Hoechst 33342 staining of nuclei. Red-tetramethyl rhodamine ethyl ester reflecting electrical potential difference across the inner mitochondrial membrane.

7. **Warner Lab**: The structure of a yeast ribosome.

8. **Ye Lab**: Immunofluorescence staining reveals that transcription factors BCL6 (red) and STAT3 (green) are expressed in separate populations of B cells within the germinal center, a dynamic microenvironment critical for T-cell dependent antibody response.

9. **Kielian lab**: Alphavirus infection induces the formation of actin and tubulin-positive intercellular extensions that emanate from infected cells and form stable contacts with neighboring cells, mediating cell-cell transmission of infection. This figure is a confocal image of alphavirus-infected Vero cells, with the red channel showing the virus glycoproteins and the green channel showing phalloidin staining of F-actin.

10. **Stanley Lab**: The Notch ligand Dll3 is upregulated in mid-hind brain of E8.5 mouse embryos lacking *O*-fucose glycans on Notch receptors.

11. **Kielian Lab**: The structure of the alphavirus membrane fusion protein, which mediates virus infection of host cells.

12. **Edelmann Lab:** Lgr5+ and Paneth cells form a stem cell niche in MMR-deficient intestinal tumors

Department of Cell Biology

Welcome to the Albert Einstein College of Medicine and the Department of Cell Biology. Our department is focused on molecular mechanisms in many important areas of cell biology, ranging from stem cells to viruses, DNA replication to RNA processing, gene expression to immunology, glycobiology to cancer. We share many common interests and enjoy an interactive and scientifically stimulating atmosphere that makes the Cell Biology Department a great place to work.

Graduate students in Cell Biology participate in a variety of departmental activities. The department meets every Friday for a "work-in-progress" seminar in which post-doctoral fellows and graduate students describe the progress of their current research and discuss future directions. The department hosts a bi-weekly seminar program of invited outside speakers, with many opportunities for students and postdocs to meet the speaker for discussion and lunch. There is a departmental journal club series in which students present original articles and discuss over dinner. A Friday afternoon get-together encourages scientific interactions as well as social connections. Every few years, our departmental retreat takes us all to the seashore or mountains for a chance to talk about the big picture of our research, to enjoy poster presentations from students and postdocs, and to try to solve the zany puzzles organized by the skit committee.

On the following pages you will find information about the research programs of the individual faculty members, as well as listings of the current students and postdocs in the department. You can also find out more about the department on our web page at http://www.einstein.yu.edu/cellbiology . Feel free to contact any of us for further discussions.

Enjoy your first year!

Cell Biology Faculty

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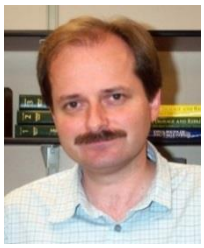
Eric Bouhassira

Nicholas Chiorazzi



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Matthew Gamble

Kira Gritsman

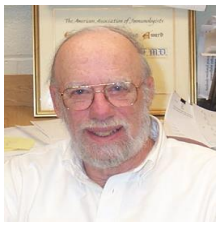
Wenjun Guo

Keisuke Ito

Margaret Kielian

Richard Kitsis

Charles Query



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Carl Schildkraut

Robert Singer

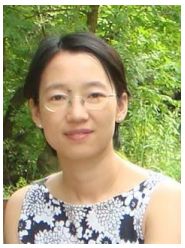
Arthur Skoultchi

Pamela Stanley

Ulrich Steidl

Jonathan Warner

Britta Will



Hilda Ye

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Key Words: *intestinal homeostasis and cancer, diet, mouse models, inflammation, stem cells*

Our research is on the cellular and molecular mechanisms that maintain intestinal homeostasis, and perturbations that disrupt this causing intestinal and colonic disease - in particular, tumor development. This has involved development of mouse genetic models targeting intestinal cell maturation and lineage specific differentiation, and the impact of environmental alterations which in human populations are linked to altering probability of tumor development.

We demonstrated an orchestrated reprogramming of intestinal epithelial cells as they migrate from the progenitor cell compartment in the intestinal crypt and undergo maturation, eliminating cell cycling and promoting cell differentiation. We determined where key regulators of proliferation are active during this traverse of cells along the crypt-luminal axis, their oscillation along this axis, and effects of their disruption on mucosal homeostasis and tumor development.

The Profound Role of Diet in Both Genetically Initiated and Sporadic Tumorigenesis:

Colon cancer incidence in human populations is strongly linked to long term dietary patterns. For the mouse, this can be modeled using a purified rodent diet (NWD1). NWD1 incorporates changes of a number of key nutrients to mimic the level of intake of each to its level consumed in western populations with a much higher incidence of colorectal cancer. Feeding NWD1 accelerates and increases tumorigenesis in mouse genetic models of intestinal cancer, regardless of etiology, mechanism, or altered genetic drivers. For example, we collaborated with Winfried Edelmann on a mouse he engineered to be a faith-full genetic model for Hereditary Non-Polyposis Colon Cancer (HNPCC, or Lynch Syndrome). Feeding these mice NWD1 caused a major increase of tumor development in the large intestine, which is the site-specificity characteristic of individuals in human pedigrees who are carriers of inherited genetic mutations causal for Lynch Syndrome.

However, the vast majority (~80%) of human colon tumors are sporadic, developing without known inherited genetic factors, and arising after 5-6 decades of life with incidence largely determined by long term dietary patterns. Importantly, therefore, feeding NWD1 to wild-type C57Bl6 mice for 1-2 years caused sporadic small and large intestinal tumors with a lag, incidence, frequency and pathology similar to that of *sporadic* colon cancer in the human. This is the only mouse model of common sporadic tumors. Long before tumors developed, the mucosa, though seeming to function normally, exhibits distinct alterations at the cellular and molecular levels: there is macrophage infiltration into the mucosa, elevated serum cytokine levels, decreased secretory and increased absorptive cell lineage marker expression; and elevated Wnt signaling and ectopic Paneth cell marker expression throughout the small intestinal and colonic mucosa.

These discoveries led to our current research on interaction of genetic and nutritional factors in the function of $Lgr5^{hi}$ intestinal stem cells. In summary, feeding the NWD1 (higher in fat, lower in vitamin D_3, calcium, methyl donors and fiber), decreases the ability of $Lgr5^{hi}$ intestinal cells to function as stem cells in both intestinal homeostasis and in tumor development. An important role of lowered Vitamin D signaling in this was established in recapitulating the phenotype by targeted inactivation of the vitamin D receptor targeted to $Lgr5^{hi}$ cells. The dietary effect is linked to extensive transcriptional reprogramming of the $Lgr5^{hi}$ cells. For example, expression of the DNA mismatch repair pathway iss elevated by lower vitamin D_3 and/or calcium in the diet, paralleled by reduced accumulation of relevant somatic mutations detected by single $Lgr5^{hi}$ cell whole exome sequencing. In compensation, NWD1 also reprograms a second cell population - Bmi1+ cells - to function and persist as stem-like cells in mucosal homeostasis and tumor development. There is also a key role of compromised mitochondrial structure and function in inhibiting ability of $Lgr5^{hi}$ cells to function as stem cells, linked to down-regulation of Ppargc1a, a master regulator of Ppar signaling and mitochondrial biogenesis.

Key questions raised by the data are under investigation: **a)** What are the signals, and how are they transmitted, that recruit the Bmi1+ cells to function as stem cells? **b)** Is there a specific subpopulation of the heterogeneous Bmi1+ cell population that is mobilized, and what is its molecular phenotype (under investigation by single cell RNAseq). **c)** Since human diets vary considerably, how does this impact which and how different potential intestinal stem cell populations function in the population and can give rise to tumors? Further, are genetic, epigenetic and clinical phenotypes of colon tumors a function of this nutritional impact on stem cell populations? And what is the implication of nutritionally linked genetic and epigenetic alterations in relatively long-lived stem cells for effective approaches for prevention? **d)** Finally, experimentally induced damage to $Lgr5^{hi}$ cells can recruit multiple different intestinal cell populations to function as stem cells; our data demonstrate that this plasticity of function can also be marshalled by nutritional alterations. In this context, *"adaptive radiation"* – the rapid expansion of species and higher taxa to occupy new niches – is driven principally by ability of organisms to adapt to and utilize new sources of food. Therefore, an interesting hypothesis is that the plasticity of intestinal epithelial cells to function as stem cells may have arisen to provide flexibility to organisms to function in new ecosystems.

Selected Publications:

Yang, K., Newmark, H., Rigas, B., Bancroft, L., Corner, G., Livote, E., Lesser, M., Edelmann, W., Velcich, A., Lipkin, M. and Augenlicht, L.H. (2008). Dietary induction of colonic tumors in a mouse model of sporadic cancer. Cancer Research 68, 7803-7810.

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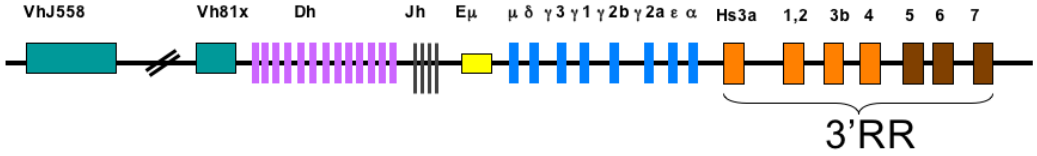
Regulation of Antibody Heavy Chain Gene Rearrangements and Expression by the 3' Regulatory Region

The immune system is our spacesuit for life in an environment containing enormous numbers of infectious agents. An essential part of the immune system is the B cell, which is the only cell type that produces antibodies. Antibody (Ig) genes are constructed via a series of DNA rearrangements. Occasionally, mistakes occur during antibody construction, which activate oncogenes and lead to cancers. My long term goal has been to understand the mechanisms that initiate and control antibody gene rearrangements within the 3 megabase heavy chain gene (IgH) locus.

Our experiments have focused on a complex 3' IgH regulatory region (3' RR) that lies immediately downstream of the antibody heavy chain gene cluster in mouse and humans. This region has recently been identified as a "super-enhancer" by Richard Young's laboratory, which activates the myc oncogene when it comes under its control as a result of chromosomal translocation. The ~50 kb 3' RR containing multiple regulatory elements, a 1 kb intronic enhancer, and two small elements in the diversity DH gene region are the only currently known long-range regulatory regions for the IgH locus. We have identified an extension of the 3' RR (hs5, 6, 7, 8), which like the other 3' enhancers, has binding sites for Pax5, a transcription factor essential for B cell development. In addition, the 3' RR extension contains insulator activity, i.e. prevention of communication between an enhancer and its target promoter. This is associated with multiple binding sites for CTCF, a protein identified in all mammalian insulators. Chromatin analysis shows that the 3' RR extension is likely to be active throughout B cell development while other 3' RR segments become progressively and stage-specific active.



Murine germline Igh locus, with variable (VH), diversity (DH), joining (JH) and constant region genes (□u, □elta, □amma, □psilon, □lpha). Regulatory elements, Eμ and the 3' regulatory region (3'RR), are depicted. Two CTCF sites in the DH segment are important for VDJ joining.

The 3' RR has been shown to be critical both for class switch recombination that affects expression of virtually all antiboody classes and for somatic hypermutation of the heavy chain variable region. In addition, the 3' RR regulates high levels of expression of antibodies in fully differentiated plasma cells. A major goal of our laboratory has been to understand the mechanisms by which the 3' RR functions normally in both mouse and human, both by acting on the Igh locus and by insulating the locus from its non-Igh neighboring genes. CTCF binding sites are anticipated to play a major role in these activities by promoting interactions between distal DNA sequences through loop formation.

We have been analyzing the extent of B cell-specific regulation of the IgH locus, by studying DNA demethylation, histone modifications of and the binding of CTCF and Pax5, and other factors to 3' RR sequences during B cell development and class switching.. Furthermore, we have examined the interaction of the 3' RR with target sequences within the IgH locus, as assessed by the

chromosome capture conformation technique (3C). Together, these approaches have been designed to understand how the 3' RR facilitates molecular acrobatics necessary for the immunoglobulin heavy chain locus without their intrusion on downstream non-IgH genes, or resulting in chromosomal translocations involved in malignancies.

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Key Words: Red Blood Cells, Sickle Cell Disease, Epigenetic, gene regulation, stem cells, reprogramming.

CULTURED RED BLOOD CELLS:

We have developed a system to produce genetically engineered human red blood cells by differentiation of human induced-Pluripotent Stem Cells. We have several ongoing projects aiming at producing large amount of genetically homogenous, genetically modified red blood cells that will be used as reagent red blood cells and for transfusion of allo-immunized sickle cell disease patients, and as carrier for replacement therapy of a variety of diseases including TTP and several coagulation blood disorders.

GENE THERAPY:

We are studying the biology of hematopoietic stem cells in the context of sickle cell disease and are developing methods to genetically modify these cells for gene therapy using a safe harbor approach.

HUMAN EMBRYONIC STEM CELLS, EPIGENETICS AND REPROGRAMMING:

Epigenetic is the study of mitotically or meiotically heritable changes in gene function not associated with changes in DNA sequence. Epigenetic regulations are mediated by changes in chromatin structure that alter access of transcription factors to their cognate binding sites, and therefore, expression levels of genes and transgenes. Understanding these regulations is critical for gene therapy, cancer therapy and generally to gain a greater ability to modify mammalian genomes. We have several ongoing basic science projects to study these questions in human iPSCs and hematopoietic cells.

FOR MORE DETAILS: http://cellbio.aecom.yu.edu/Lab/Bouhassira/

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Genomic Instability and Cancer in Murine Models

The maintenance of genomic integrity in all organisms requires multiple DNA repair pathways that are involved in the processes of DNA replication, repair and recombination. Perturbations in these pathways can lead to increased mutation rates or chromosomal rearrangements that ultimately result in cancer. MMR is one of the repair systems that mammalian cells employ to maintain the integrity of its genetic information by correcting mutations that occur during erroneous replication. Mutations in MMR genes are linked to one of the most prevalent human cancer syndromes, Lynch syndrome and a significant number of sporadic colorectal cancers. At the molecular level tumors that develop in these patients display increased genomic mutation rates as indicated by increased instability at microsatellite repeat sequences (termed microsatellite instability, MSI). MMR in eukaryotes is complex and involves several homologs of the bacterial MutS and MutL proteins. In mammals, the initiation of the repair process requires two complexes formed by three different MutS homologs (MSH): A complex between MSH2-MSH6 for the recognition of single base mismatches and a complex between MSH2-MSH3 for the recognition of insertion/deletions. The repair reaction also requires a complex between the two MutL homologs MLH1 and PMS2 that interacts with the MSH complexes to activate subsequent repair events which include the excision of the mismatch carrying DNA strand and its re-synthesis. In addition to correcting DNA mismatches, the MMR system mediates an apoptotic response to DNA damage and both of these functions are thought to be important for genome maintenance and tumor suppression. We have generated gene targeted mouse lines with inactivating mutations in all the different MutS and MutL homologs, and also in genes that function in the later MMR steps to study their roles in genome maintenance and tumor suppression. In addition, we have generated knock-in mouse lines with missense mutations and conditional knockout mouse lines that inactivate specific MMR functions and/or model mutations found in humans. Our studies indicate that specific MMR functions play distinct roles in maintaining genome stability and that defects in these functions have important consequences for tumorigenesis. These studies have also revealed that MMR proteins play essential roles in class switch recombination and somatic hypermutation during antibody maturation and the control of meiotic recombination in mammals. We are currently studying the functions of MMR in intestinal stem cells (ISCs) and cancer stem cells (CSCs) in preclinical mouse models and how loss of MMR in stem cells affects tumorigenesis and the response of tumors to anticancer treatment.

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Our laboratory has three areas of interest:

A) We are interested in the biology of hematopoietic stem cells (HSCs) with a focus on microenvironment cues that promote their survival, differentiation and self-renewal. We have identified novel niche constituents and novel regulatory mechanisms using genetically engineered mice and whole-mount imaging of the bone marrow.

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B) We have ongoing projects on the mechanisms of vaso-occlusion in sickle cell disease. We have identified activated neutrophils as a key promoter of vaso-occlusion by interacting with circulating sickle erythrocytes.

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C) Based on our finding of an important role of neural signals in regulating hematopoiesis, we are investigating the role of peripheral nerves in hematologic malignancies and prostate cancer.

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BIOCHEMISTRY AND GENETICS OF CHROMATIN TRANSITIONS IN *DROSOPHILA*

Hundreds of millions of base pairs of nuclear DNA are packed into chromosomes. Chromatin, the nucleoprotein filament of a chromosome, has many organization levels. It is the natural state of DNA in the nucleus and the native substrate for DNA-directed reactions, such as DNA replication, recombination, repair and transcription. The assembly of DNA into chromatin and dynamic conversion between its different forms are critical steps in the maintenance and regulation of the eukaryotic genome. The goal of our research is to understand how chromosomes are assembled and how this process regulates the structure and activity of eukaryotic chromosomes. The crucial first step in this direction is a systematic study of factors that mediate this process. To this end, we use biochemical approaches to analyze mechanisms of chromatin assembly by histone chaperones and ATP-driven enzymes. We also dissect their function *in vivo* by methods of *Drosophila* genetics. Thus, we are trying to uncover the network of chromatin assembly factors and to elucidate their roles in hierarchical organization of the chromosome.

1. Molecular mechanisms of nucleosome assembly

ACF (ATP-utilizing chromatin assembly factor) was identified on the basis of its ability to facilitate reconstitution of chromatin *in vitro*. It consists of two subunits, a SNF2-like ATPase ISWI and a polypeptide termed Acf1. In conjunction with a core histone chaperone NAP-1, ACF mediates deposition of histones onto DNA and forms arrays of regularly spaced nucleosomes. We study ACF as a prototype factor to elucidate molecular events that take place during ATP-dependent formation of nucleosomes. During assembly, ACF commits to the DNA template and forms nucleosomes as a processive, ATP-driven, DNA-translocating motor. Multiple conserved domains of Acf1 and ISWI are required for this activity.

2. Biological functions of chromatin assembly factors

ACF is the major ATP-dependent assembly factor in *Drosophila*. To expose its biological functions, we produced fly mutants that do not express ACF. ACF-deficient animals have multiple defects of chromatin organization. However, ACF is not essential for viability due to the presence of redundant ACF-like factors. We discovered novel ISWI-containing complexes ToRC (comprising Tou, ISWI and CtBP) and RSF (Rsf1 and ISWI) that can functionally substitute ACF *in vivo*. Our genetic and cytological analyses implicate the network of ATP-dependent, ISWI-containing chromatin assembly factors in diverse, partially redundant pathways of regulation of chromatin structure and activity.

SNF2-like protein CHD1 is another ATP-dependent nucleosome assembly factor. We disrupted *Chd1* in flies and discovered that CHD1 is required for replication-independent deposition of histones into chromatin *in vivo*. Specifically, CHD1 is essential during early embryonic development for deposition of replacement histone H3.3 into paternal chromatin.

3. Higher-order chromatin forms

To reconstitute higher-order chromatin structures, we supplement the *in vitro* assembly system with modified core histones, histone variants, linker histone (H1) and heterochromatin proteins, such as *Drosophila* HP1a. Chromatin vectors can turn into useful tools in research and therapy. These studies will also eventually lead to the discovery of techniques to reconstitute functional metazoan chromosomes.

In collaboration with A. Skoultchi, we began to examine flies in which H1 is depleted by RNAi or genetic approaches. We discovered that H1 is the major component of heterochromatin and is required to establish its biochemical identity and functional properties. For instance, H1 recruits HMT Su(var)3-9, which mediates methylation of lysine 9 of histone H3 (H3K9), a signature heterochromatin-specific epigenetic mark. We have also demonstrated that H1 is essential for the faithful regulation of DNA endoreplication timing in *Drosophila* larval cells. We are now extending these studies to normal, mitotically dividing cells.

A prevalent view of heterochromatic silencing is that its physical compaction results in steric exclusion of regulatory proteins, such as RNA polymerases. In collaboration with G. Karpen (LBNL), we have recently shown that the formation of heterochromatin domains is also mediated by liquid-liquid phase separation that gives rise to a non-membrane-bound nuclear compartment. We demonstrated that HP1a and H1 undergo demixing *in vitro* and nucleate into foci that display liquid properties during

heterochromatin domain formation in early *Drosophila* embryos. We propose that biophysical properties associated with phase-separated systems are critical to understanding the behavior of heterochromatin and, potentially, other chromatin forms that regulate essential nuclear functions.

4. Sperm chromatin assembly and remodeling

In sperm, DNA is compacted with cysteine-rich protamines and protamine-like sperm nuclear basic proteins (SNBPs) to form enzymatically static sperm "chromatin". We have begun to analyze protein factors that mediate SNBP deposition during spermatogenesis and their removal from DNA after fertilization. It turns out that sperm chromatin assembly and remodeling is mediated by a group of factors that are similar to core histone chaperones.

Upon deposition on sperm DNA, protamines/SNBPs are extensively crosslinked via interchain disulfide bonds. After fertilization, the egg has to reverse the crosslinks for efficient eviction of SNBPs. This nuclear reaction is mediated by specific thioredoxin (TRX) and thioredoxin reductase (TRXR) molecules. Thus, we are investigating biological roles of the evolutionary conserved thioredoxin system in sperm chromatin metabolism and female fertility. A number of chemical compounds are known to specifically inhibit the function of TRX and TRXR proteins. We are studying their ability to suppress fertilization in the egg *in vivo* and testing their utility as novel, non-hormone agents for female contraception.

5. Non-coding RNA

Epigenetic regulation is dependent in part on non-coding RNAs that affect gene expression post-transcriptionally or by interfacing chromatin via tethering DNA- and histone-modifying enzymes in a sequence-specific manner. This tethering involves a formation of RNA-DNA hybrids (R-loops), which encompass a large fraction of eukaryotic genomes. Despite their apparent importance, biochemical details of R-loop formation and the identities of factors that mediate this reaction remain enigmatic. We have designed an *in vitro* assay to look for enzymes that mediate sequence-specific RNA-DNA pairing in an ATP-dependent fashion *in trans*. By chromatographic fractionation of *Drosophila* extracts, we purified to near homogeneity a novel, evolutionary conserved factor termed R-loop enzymatic complex, RLEC. We are now characterizing biochemical properties and biological functions of *Drosophila* RLEC.

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MacroH2As, histone variants with diverse roles in gene expression and DNA damage responses – The macroH2A-type histone variants (which include macroH2A1.1, macroH2A1.2 and macroH2A2) have roles in tumor suppression, cellular senescence, activation and repression of transcription, promotion of DNA repair and suppression of the reprogramming of differentiated cells into stem cells. MacroH2As are typified by a histone H2A-like region fused by a flexible linker to a C-terminal macrodomain, a ligand-binding domains whose functions is modulated by binding to poly(ADP-ribose) produced by a family of poly(ADP-ribose) polymerases. MacroH2A1 regulates the expression of genes found within its large chromatin domains which can span hundreds of kilobases. Through changes in its expression and/or alterations in its genomic localization, disruption of macroH2A1's tumor suppressive functions are common in cancer; alterations of macroH2A transcription and splicing occur in a variety of cancers including those of lung, breast, colon, ovaries, endometrium, bladder, testicles, and melanocytes. Consistently, macroH2A1 loss in primary cells is sufficient to trigger an oncogenic gene expression profile. We are interested in many aspects of macroH2A biology. 1) How are macroH2As targeted to specific regions of the genome? 2) How does macroH2A1.1 in collaboration with PARPs regulate gene expression? 3) How does macroH2A1 regulate chromatin accessibility at enhancers? 4) How does macroH2A participate in DNA repair? 5) What regulates macroH2A1's alternative splicing?

Chromatin dynamics during oncogene-induced senescence and cancer – Oncogene-induced senescence (OIS) is an important tumor suppressive mechanism whereby a cell harboring an oncogenic mutation enters a stable proliferative arrest. At the same time the senescent cell secretes a host of inflammatory cytokines, chemokines and metalloprotease called the senescence-associated secretory phenotype (SASP), which serves to recruit immune cells to clear the senescent cells from tissues. The histone variant macroH2A1 plays a critical role in the transcriptional regulation of SASP genes during senescence. We are currently studying the mechanism by which macroH2A regulates the SASP response. We hypothesize that changes in macroH2A1 expression, seen in many cancers, allows these cells to bypass senescence and proceed on the pathway towards transformation.

Interplay between transcriptional elongation rates and alternative splicing – Alternative splicing is a crucial aspect of gene expression, allowing a gene to yield functionally distinct products, the abundance of which are regulated by cellular cues. Splicing dysregulation is central to several cancers and developmental diseases. Alternative splicing can be regulated through the recruitment of splicing factors which promote or repress distinct splicing events. Splicing largely occurs co-transcriptionally, and so, splicing outcomes are also affected by aspects of the transcription process and chromatin environment. The local elongation rate of RNA polymerase II is one aspect of transcription with important consequences on splicing outcomes. A barrier to progress in the field has been the lack of a high-throughput assay to measure splicing rates in mammalian cells. To address this, we have developed SKaTER-seq (Splicing Kinetics and Transcript Elongation Rates through sequencing). With this assay, we are exploring a myriad of factors that regulate splicing, including elongation rate, gene architecture, binding sites for RNA binding factors, chromatin structure and histone modifications. With this powerful approach we will determine the underlying causes of spicing alterations in disease.

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The PI3 kinase Signaling Pathway in Adult Blood Development and Leukemia

My lab studies the signal transduction pathways that affect the early fate decisions of adult hematopoietic stem cells (HSCs) as they progress from an undifferentiated multipotent state to the generation of differentiated blood cells. When these early fate decisions go awry, this can lead to the formation of leukemic stem cells, which can initiate leukemia and contribute to relapse after treatment.

Roles of the PI3 kinase isoforms in adult blood development

PI3 kinase (PI3K) is a lipid kinase that is important for the regulation of metabolism, the cell cycle, apoptosis, and protein synthesis. In hematopoietic cells, there are four isoforms of the catalytic subunit of PI3K, each encoded by a separate gene. Emerging evidence suggests that these isoforms have unique functions in normal and cancer cells, but may substitute for each other in some contexts. We have generated a series of mouse knockout models that allow us to study the roles of each of these isoforms individually in adult hematopoiesis. For example, we have found that the p110alpha isoform is most important for red cell development, but is not required in normal blood stem cells. We have now also generated compound knockout mice to determine the redundant roles of the PI3K isoforms in blood development. We are studying how deletion of PI3K impacts normal HSC function, including self-renewal, proliferation after infection or chemotherapy treatment, and differentiation along different blood lineages.

Roles of the PI3 kinase isoforms in leukemia

Acute myeloid leukemia (AML) is a genetically diverse disease, but activation of the PI3K pathway has been reported in up to 80% of cases. A subset of AML cell lines and AML patient samples respond to PI3K pathway inhibitors, but it is unclear how patients should be selected for potential response to these inhibitors. We found that RAS-mutated myeloid leukemias are particularly dependent on the p110alpha isoform of PI3K, and that pharmacologic inhibition of p110alpha can be used to treat both RAS-mutated cell lines and RAS-mutated leukemia in mice. We are now using several different mouse models of AML to examine the roles of individual PI3K isoforms in leukemic stem cells, which is the cell population that has been implicated in relapse. We are also studying the roles of PI3K in the normal and leukemic bone marrow microenvironment.

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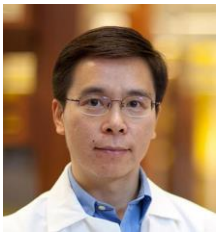

Key Words: ***mammary stem/progenitor cells, breast cancer stem cells, drug resistance, metastasis***

My lab is interested in two interlocking areas of stem cell biology and cancer biology: the molecular pathways that regulate the normal stem-cell fate in the mammary gland, and the role of stem cell fate/pathway dysregulation in breast cancer pathogenesis.

Role of mammary stem cells in cancer initiation

We have developed sensitive and specific in vitro and in vivo mammary stem cell assays. Using these assays, we have identified novel unipotent stem cells that are responsible for the development of distinct mammary epithelial cell lineages. We are investigating whether these unipotent stem cells are the cell-of-origin for different breast cancer subtypes. In addition, we are elucidating the key cell fate determinants of these distinct stem cells, and investigating oncogenic mechanisms causing the dysregulation of stem cell fates during tumorigenesis.

Function of stem-cell pathways in breast cancer progression and metastasis

Emerging evidence suggests that normal stem-cell pathways often get activated aberrantly in cancers and contribute to aggressive cancer behaviors. Identification of key normal stem cell regulators provides us a framework to understand how breast cancer stem cells are regulated. We are particularly interested in understanding the role of stem-cell factors in regulating metastatic colonization, a rate-limiting step of the metastatic cascade that involves cancer stem cells. In addition, we are interested in how cancer stem cells are regulated by the tumor microenvironment.

Selected Publications

Wang C, Christin JR, Oktay MH, **Guo W**. (2017) Lineage-biased stem cells maintain estrogen-receptor-positive and -negative mouse mammary luminal lineages. ***Cell Reports*** 18: 2825 – 2835.

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Key Words: Hematopoietic stem cell, Leukemia, Myelodysplastic syndrome

The central research goal of the Ito Lab is the expansion of our understanding of the regulatory pathways controlling the equilibrium of stem cells, with a special focus on the development of novel therapeutics for hematopoietic disorders. At the core of our work is the process of stem cell division, and the resulting balance between self-renewal or differentiation, which directly impacts tissue homeostasis. We are also devoting increased attention to targeting cellular metabolism as a therapeutic strategy, and have cut a path along the leading edge of research into the role of epigenetic-microRNA crosstalk, including physiologically relevant Ten-eleven translocation, in the pathogenesis of myelodysplastic syndrome. We believe our expertise in stem cell biology, hematology, and the bone marrow microenvironment, combined with our development of new single-cell approaches to tracking stem cell fate in animal models, will together facilitate a major contribution to the improvement of transplantation efficiency and the development of new therapies and treatments, and potentially even cures, for many forms of hematologic pathology.

Selected Original research and Theoretical treatises;

1. Morganti C, Bonora M, **Ito K**. Improving the accuracy of flow cytometric assessment of mitochondrial membrane potential in HSPCs through the inhibition of efflux pumps. **J Vis Exp**. in press.
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5. Song SJ, **Ito K** et al. The oncogenic microRNA miR-22 targets the TET2 tumor suppressor to promote hematopoietic stem cell self-renewal and transformation. **Cell Stem Cell.** 2013 Jul 3;13(1):87-101.
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Molecular Mechanisms of Virus Entry and Exit.

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All enveloped viruses use the essential steps of membrane fusion to enter a host cell, and membrane budding to exit. Molecular information on the entry and exit processes is critical to understanding the lifecycle of enveloped viruses and how they exploit the host cell machinery, and as a key model for cellular membrane fusion and budding reactions.

Our research focuses on the molecular mechanisms of virus entry and exit using alphaviruses and the closely related virus Rubella virus, and flaviviruses such as dengue virus. The flaviviruses and alphaviruses include many important human pathogens such as dengue, Zika, and Chikungunya viruses, which cause millions of human infections each year. There are no vaccines or antiviral therapies for most of these viruses, and new strategies are urgently needed.

Alphaviruses, Rubella virus and flaviviruses enter cells by endocytic uptake and then fuse their membrane with the endosome membrane in a reaction triggered by the low pH of the endocytic vesicle. The membrane fusion proteins of these viruses are structurally related proteins and refold during fusion to a homotrimer conformation that mediates virus fusion and infection. Recent studies have also shown that structurally similar proteins are expressed in plants and in many animals, where they mediate cell-cell fusion of gametes and during development.

Many important questions on the molecular mechanism of membrane fusion remain for both viruses and cells. Little is known about the mechanism and structural features of fusion protein insertion into the target-membrane. We are also investigating the pH-dependent control mechanisms for the Rubella virus fusion reaction.

During alphavirus and flavivirus biogenesis, a companion protein forms a closely-associated dimer with the fusion protein, and protects it from low pH and premature fusion during exocytic transport. This companion protein must then dissociate to permit virus fusion. The pH protection mechanisms for many other viruses are unknown, and we are using Rubella virus as a system to define novel mechanisms of pH protection.

Alphaviruses exit by budding through the plasma membrane of the infected host cell. Little is known about alphavirus assembly and budding, although it is clear that these processes are highly regulated to produce organized virus particles of high specific infectivity. How does this happen and what are the roles of cellular and viral factors? We seek to determine how the internal viral RNA-capsid core is assembled, how the virus excludes host RNAs, and how nucleocapsid assembly can be inhibited by small molecules. We are using a novel capsid protein retrieval strategy to identify and characterize host factors involved in alphavirus nucleocapsid assembly. We have developed fluorescently tagged alphaviruses to follow virus assembly and budding in real time in infected cells. We are investigating how alphaviruses spread from cell to cell, a process that protects the virus from antibody neutralization. The cell plasma membrane and cytoskeletal network are dramatically remodeled during budding and we are defining the mechanisms and signaling pathways that mediate remodeling.

Our lab uses a wide variety of approaches including molecular biology, virus genetics, protein biochemistry, live cell imaging, cell biology, and structural biology.

Potential research projects include: investigation of specific molecules involved in cell-to-cell virus transmission, use of fluorescently tagged viruses to follow steps in virus assembly and budding, characterization of the role of cellular factors in virus assembly and exit, use of virus mutants to characterize specific steps in fusion and pH protection.

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Fundamental mechanisms of cell death; molecular connections among death programs; development of small molecule drugs to manipulate cell death in human disease

Cell Death: Fundamental Mechanisms and Roles in Human Disease

The most basic decision that any cell makes is to grow, differentiate, or die. Our laboratory studies fundamental mechanisms of cell death and the roles of cell death in normal biology and human disease.

Basic science

From a fundamental perspective, we are interested in how different death programs (in particular, apoptosis and necrosis) interconnect at the molecular level, and the mechanisms that determine whether a cell will die through one or another pathway. This is an important issue because different modalities of cell death have dramatically different consequences with respect to collateral damage to surrounding tissue. We have discovered mechanisms that unite apoptosis and necrosis signaling, and therefore may serve as "decision points" between these death programs. One is the cell death inhibitor ARC that antagonizes multiple apoptosis and necrosis pathways (Molecular Cell, 2004; PNAS, 2007; JBC, 2007; Cell Death Differ, 2014; others). Another is the BCL-2 protein BAX, which has been long recognized for its permeabilization of the outer mitochondrial membrane during apoptosis and that we have discovered also plays a critical role in regulating necrosis through distinct mechanisms involving mitochondrial dynamics (fission-fusion) (PNAS, 2012). We hypothesize that these effects are mediated by different conformations of BAX, an area we are currently pursuing. Related collaborative work has focused on understanding conformational events in the activation mitofusins, GTPases that mediate outer mitochondrial events in mitochondrial fusion (Nature, 2016; https://www.biorxiv.org/content/early/2018/04/17/301713; Science, 2018)

Translational research

While we have studied roles of cell death in cancer (Cell Death Differ, 2005; Cell Cycle, 2008; JBC, 2010; Cancer Res, 2011; PLoS One, 2015), diabetes (Diabetes, 2013; Sci Rep, 2017), and pulmonary hypertension (Circulation, 2011), our most important translational accomplishments have focused on cell death in heart disease - specifically in the most common and lethal cardiac syndromes: myocardial infarction (heart attack) and heart failure. Our lab was one of the founders of the cardiac cell death field and has played a major role in its development (reviewed in Annu Rev Physiol, 2010), including the first demonstrations that regulated forms of cell death play central roles in the pathogenesis of myocardial infarction (Circulation, 2000; J Mol Cell Cardiol, 2000; others) and heart failure (J Clin Invest, 2003; others). Currently, our translational work is focused on the chemical biology of cell death and, specifically, the development of small molecule drugs to reduce heart damage from myocardial infarction and cancer therapies. While we have employed unbiased phenotypic screening of large chemical libraries (Probe Reports from the NIH Molecular Libraries Program, 2013), our current focus is on the development of the first BAX inhibitors - the rationale being that BAX is a critical mediator of both necrotic and apoptotic cell death. In addition to traditional biochemical/molecular/cellular approaches, the latter work involves

chemistry, structural biology, and small and large animal models. Our recent research, in collaboration with Dr. Evripidis Gavathiotis, has identified allosteric inhibitors of BAX that protect the heart against damage from traditional chemotherapy and targeted cancer drugs (Amgalan et al; Garner et al; both in revision).

I supervise a laboratory of approximately 12, including Ph.D. and M.D./Ph.D. students and postdocs. An important facet of my work is training and mentorship. I have been thesis research advisor to more than a dozen individuals who have received the Ph.D. degree, ~50 postdoctoral research fellows, and ~10 clinical fellows, a significant proportion of whom have gone on to academic faculty positions as independent investigators. My pre- and postdoctoral trainees have included a substantial number of individuals from groups under-represented in science.

Selected Publications

Publications (primary research papers only) 2016-present, and older publications as cited above:

Chen Y, Paulino V, Zheng M, Garcia C, Lee J, Owusu-Ansah E, Li H, Cuervo AM, **Kitsis RN**. Regulation of mitochondrial function by chaperone-mediated autophagy (submitted).

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Mechanisms of RNA Processing

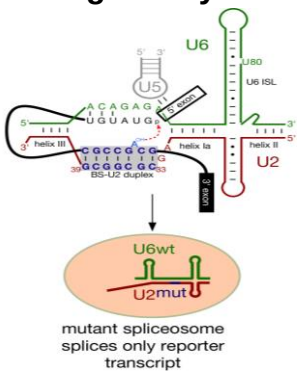
Intron removal, a defining feature of eukarya, is catalyzed by the spliceosome, a 50-60S complex composed of five snRNAs and >100 proteins. Our laboratory investigates spliceosome assembly and catalysis and the mechanisms by which splicoesomal mutations cause disease. We have developed orthogonal (or 'designer') spliceosomes to facilitate investigation of core RNA–RNA interactions, and we have identified new spliceosomal snRNAs in the human genome.

Do variant snRNAs make variant spliceosomes?

One 'black hole' in RNA biology is the identification of 50-300nt RNAs, missing in modern-day sequencing datasets. Vertebrate genome sequences reveal hundreds of snRNA gene loci; with few exceptions, only the most abundant canonical snRNAs have been investigated. We are sequencing the snRNA transcriptome, asking which variants are expressed in different tissues and disrupting variant snRNA loci. We find that variant-snRNA expression changes greatly during development. How does this impact spliceosome function?

snRNA	Mature Length	Annotated Loci
U1	163	143
U2	190	70
U4	143	91
U5	116	32
U6	106	1333
U4atac	127	18
U6atac	126	41

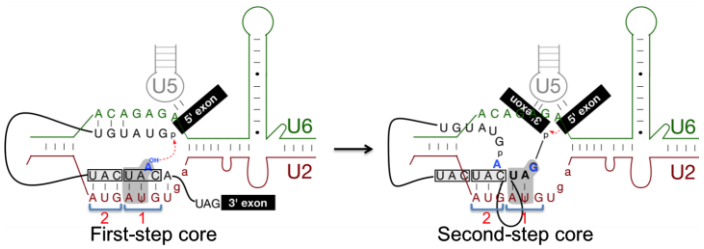
Orthogonal systems for in vivo investigation of catalytic center interactions.

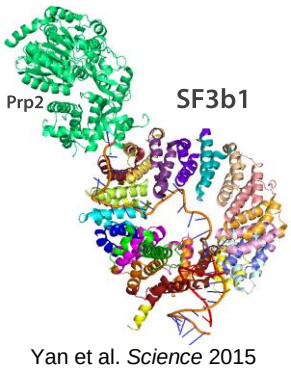


During pre-mRNA splicing, branch site (BS) base pairing with U2 snRNA is essential for spliceosome assembly and first-step catalysis. Investigation of the BS-U2 duplex was previously limited by the deleterious nature of mutations that disrupt BS-U2 pairing. We developed an orthogonal system wherein a second-copy U2 with grossly substituted BS-binding mediates splicing of a cognate reporter gene. This orthogonal BS-U2 pair produces a non-essential second spliceosome that allows in vivo characterization of the BS-U2 helix, first-step nucleophile positioning, and interaction with the spliceosome core. These properties allowed us to demonstrate that the BS-U2 structure exists at the time of first-step catalysis.

What is the second-step catalytic core?

We used our orthogonal systems to elucidate the 3'SS binding site within the second-step core, demonstrating that the branch structure formed in the first step becomes partially unpaired from U2-GUAGUA, allowing the 3'SS to bind on U2 snRNA.





Yan et al. *Science* 2015

Splicing and disease. U2 snRNP protein SF3b1 is highly mutated in myelodysplastic syndromes and is essential for spliceosome function. Using both yeast and mammalian cells, we discovered a second function of SF3b1 in macroautophagy. SF3b1 mutations affect autophagosome biogenesis and fusion into the lysosome/vacuole. An intact macroautophagic pathway is known to be required for proper hematopoietic stem cell (HSC) function (Ho…& Passegué, 2017). Does loss of SF3b1-dependent autophagy play a role in disease? Is there SF3b1-specific cargo?

Recent Publications

Tang, Q.*, Rodriguez Santiago, S.*, Wang, J., Pu, J., Yuste-Rivero, A., Gupta, V., Moldón, A., Xu, Y.-Z., and Query, C.C. (2016). SF3B1/Hsh155 HEAT motif mutations affect interaction with the spliceosomal ATPase Prp5, resulting in altered branch site selectivity in pre-mRNA splicing. **Genes & Development 30**, 2710–2723. PMID28087715 PMC5238730.

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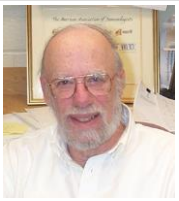

Our laboratory is studying how antibody-forming cells respond to antigen by undergoing somatic hypermutation and class switch recombination so that they can produce higher affinity antibodies with more useful effector functions. The molecular and biochemical mechanisms of antibody variable region hypermutation and class switch recombination is being studied in mice that have mutations in various repair proteins in collaboration with Dr. Winfried Edelmann. In order to examine detailed molecular mechanisms, we are also studying how mutation is targeted to antibody genes and some oncogenes in human Burkitt's lymphoma cell lines which are undergoing variable region mutation in culture. These cell lines and genetically defective mice are being used to study the role of activation induced deaminase (AID), mismatch repair and error prone polymerases in the variable region hypermutation and isotype switching. The analysis of these events also involves the examination of AID activity biochemically and, in collaboration with Dr. Thomas MacCarthy at Stony Brook, computationally by analyzing the human antibody response in vivo using mutation data bases..The highly mutagenic processes required to generate antibody diversity also leads to B cell lymphomas so we are trying to understand how AID causes mouse B cell lymphomas and human Chronic Lymphocytic Leukemia (in collaboration with Dr. Nicholas Choirazzi at Northwell Health Center) and how mismatch repair protects B cells from undergoing malignant transformation while also contributing to the generation of antibody diversity.

We are also the Hybridoma Facility that helps investigators throughout the institution to make their own monoclonal antibodies.

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Our laboratory is a part of the Einstein Center for Human Embryonic Stem Cell Research and the Cancer Center.

Molecular Analysis of DNA Replication and Repair in Alzheimer's Disease and at Cancer-Associated Sites in the Human Genome

A major interest of our laboratory is how the DNA replication program in mammalian cells is organized and regulated. We are currently focused on understanding the role of genomic instability at DNA loci the have been implicated in Alzheimer's disease. We also study human chromosomal fragile sites in cancer (including mechanisms of chromosomal translocations), aging disorders related to trinucleotide repeat expansion, telomere replication and reprogramming of DNA replication in human embryonic stem (ES) cells.

After many years of study there is still no cure for Alzheimer's disease (AD). We plan to take a novel approach to study the impact of DNA replication of certain regions of the genome that have been implicated in causing AD. This would lead to rational strategies that target the replication mechanisms and to therapies that could be

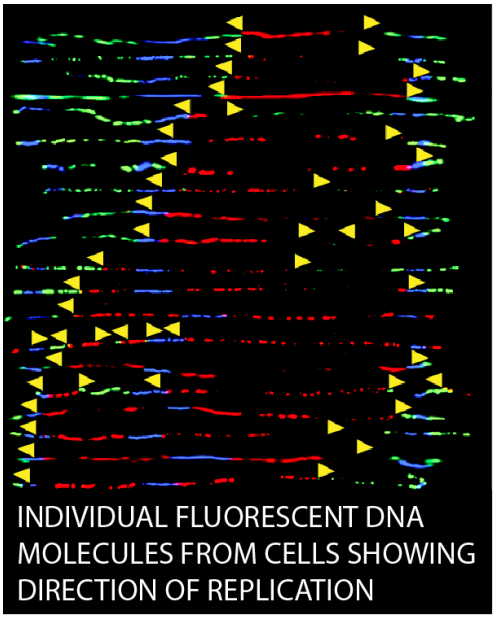
We are studying how aspects of nuclear compartmentalization affect DNA replication. We are developing a novel approach using single cell dynamic imaging to examine replication in real-time in living human cells. We are following the progression of DNA replication forks within telomeres and unusual DNA structures not having the standard double helical such as those present in chromosomal fragile sites.

Long term interests:

- Novel approaches to study regions of the genome that have been implicated in causing Alzheimer's disease.
- Role of common Fragile sites in human cancer and cancer prone disorders such as Fanconi anemia..
- Regulation and reprogramming of DNA replication of human embryonic stem (ES) cells and induced pluripotent stem cells (iPS).
- Triplet nucleotide expansion diseases and aging.

Current projects include a wide range of interests:

- Mechanisms leading to breaks at common fragile sites that result in chromosomal rearrangements frequently detected in Alzheimer's disease and cancer cells.



INDIVIDUAL FLUORESCENT DNA MOLECULES FROM CELLS SHOWING DIRECTION OF REPLICATION

- ♥ Understanding trinucleotide repeat expansion and telomere maintenance to gain insights into aging related disorders
- ♥ Triplet nucleotide expansion diseases. The fragile X premutation expansion to 55 – 200 CGGs affects ~ one in 200 women resulting in serious fertility problems and ataxia.
- ♥ Study of human ES cell DNA replication dynamics. Thorough understanding of replication programs to advance the availability of immunologically compatible hES cells for patients.

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FOLLOWING THE TRAVELS OF RNA

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Our work is focused on the expression and travels of RNA within the cell: from the site of its birth to its ultimate biological destiny in the cytoplasm where it makes proteins in specific locations.

Our new technology, based on in situ hybridization allows us to visualize specific nucleic acid sequences within individual cells. Synthetic nucleic acid probes are labeled with fluorochromes. Subsequently these molecules are hybridized to the cell and detected using high resolution digital imaging microscopy.

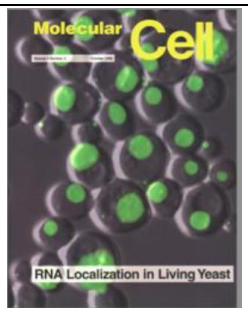
We have developed imaging methodologies and algorithms capable of detecting a single RNA molecule within a cell. This enables the detection of specific nucleic acid molecules for comparison between normal or cancer cells. This method of molecular diagnosis is the clinical application of the technology. As an additional result of this approach, we have found specific RNA sequences located in particular cellular compartments. An example is the messenger RNA for beta-actin, which is located in the periphery of the cell where actin protein is needed for cell motility. These transcripts are not free to diffuse, and appear to be associated with a cellular matrix or skeleton from the moment of their synthesis through translation.

We are investigating how this spatial information is encoded within the gene and how the RNA transcript is processed within the nucleus and then transported to its correct compartment in the cytoplasm, resulting in asymmetric protein distribution.

RNA localization also occurs in yeast. During budding, a nuclear factor represses mating type switching asymmetrically, only in the daughter cell. This is because the factor is synthesized only in the bud because the mRNA was transported there by a motor, myosin. This discovery has provided a model by which to investigate the mechanisms responsible for moving RNA within the cell. For example, we have constructed genetically altered yeast and vertebrate cells in order to elucidate the sequences responsible for mRNA localization. A reporter gene can be "delivered" to a variety of cellular compartments by using specific sequences, or "zipcodes" from the mRNAs found in those compartments.
These "zipcodes" consist of short sequences in the 3' untranslated region of the mRNA.

Recently we have developed technology that allows us to image RNA movement in living cells and tissues and characterize how the motors connect with and drive the RNA. Recent developments have allowed us to visualize transcription and RNA life cycle from birth to death in transgenic mice, including translation of single mRNAs.

Single molecules of mRNA being synthesized and diffusing within the nucleus (Femino et al, 1998)



Detection of mRNA movement in living yeast (Bertrand et al, 1998)

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Key Words: ***Chromatin, epigenetics, transcription, proliferation, differentiation, leukemia***

Our laboratory is interested in understanding the mechanisms controlling mammalian development and cell differentiation. We study the epigenetic functions of chromatin proteins and transcription factors in control of gene expression in embryonic stem cells, in red blood cells, and in *Drosophila*. Our approaches involve directed gene inactivation and transgenesis in mice and *Drosophila.* We also study control of proliferation and differentiation in red blood cell progenitors and in leukemia cells in which normal development is disrupted. Currently there are two major projects underway in the lab.

Role of H1 Linker Histones and Chromatin Remodeling Factors in Chromatin Structure, DNA Methylation, the Histone Code, Gene Expression and Development in Mice and *Drosophila*. Recent studies show that posttranslational modifications of core histones (H2A, H2B, H3, H4) (the Histone Code) play a very important role in control of gene expression. The H1 linker histones are more diverse than the core histones. Mice contain 8 H1 histone subtypes including differentiation-specific and tissue-specific subtypes, whereas *Drosophila* has only one type of H1. H1's are thought to be responsible for the final level of packaging DNA into the compact chromatin structure but we know very little about their role in gene expression and development. We are studying the functional roles of H1 linker histones by inactivating (knocking-out) specific H1 genes in mice and the single H1 in *Drosophila*. We are also reintroducing mutant H1 linker histones into H1 depleted mouse cells and flies, to perform structure-function studies. We have also established a new role for H1 histone in DNA methylation, genomic imprinting and establishment of the histone code. We are also studying the chromatin remodeling factor that assembles H1 histone into chromatin.

Control of Proliferation and Differentiation in Normal and Leukemic Blood Cells: In this project we are investigating how cell proliferation and differentiation are coordinated in normal blood cell development and how this coordination is disrupted in leukemia. We have investigating the molecular mechanisms for the cross talk between these two cellular programs in normal and leukemic blood cells. Our studies are focused on the relationships between the master transcription factors that control blood cell development and the cell cycle regulators (cyclins, cyclin-dependent kinases (cdks), cdk inhibitors and RB) that regulate the cell division cycle proliferation. This project includes genome-wide approaches involving chromatin immunoprecipitation and high throughput sequencing (ChIP-Seq) and gene expression profiling by RNA-Seq.

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Glycan Functions in Development, Spermatogenesis and Notch Signaling

Glycosylation is the most abundant and varied post-translational modification of proteins and is a critical factor in regulating their biological functions. The complement of glycans that may be produced by an organism is called the GLYCOME. Changes in glycans expressed on cell surface glycoproteins occur during development and differentiation. Specific glycans on Notch receptors modulate signal transduction by Notch ligands. This is a novel paradigm of signal transduction whereby the transfer of a single sugar residue alters the ability of Notch receptors to signal. We are using cell-based glycosylation mutants, Notch signaling assays, glycosyltransferase gene knockout mice, and biochemical approaches including MALDI-TOF mass spectrometry imaging (MALDI-MSI), to identify biological functions of growth factor receptor and Notch glycans, and the underlying mechanisms by which glycans mediate biological events.

Notch receptors span the cell membrane. When a Notch ligand like Delta or Jagged on an apposing cell binds to a Notch receptor, it induces cleavage of Notch extracellular domain, followed by a second cleavage that releases Notch intracellular domain. The Notch intracellular domain goes to the nucleus and activates target genes that ultimately lead to a change in cell fate or cell growth control. Using a CHO glycosylation mutant that adds few O-fucose glycans to Notch extracellular domain, we showed that Notch signaling is markedly reduced when fucose is limiting. We are continuing to use Notch signaling assays to define the mechanisms of action of Fringe and other glycosyltransferases that modulate Notch signaling. We are also targeting glycosyltransferase genes that encode enzymes that modify Notch in the mouse, and generating Notch mutants that cannot accept an O-fucose glycan at a specific site in Notch. Mice lacking O-fucose in the ligand binding domain have defective T cell development and are being investigated for other immunological defects. Mice lacking the three Fringe activities are affected in T and B cell development. The most recent modification of Notch is by O-GlcNAc and we are exploring its functions in the regulation of Notch signaling in mammals.

In other work we have found that complex N-glycans that are initiated by the transferase MGAT1 are essential for male fertility and are testing the hypothesis that they play an important role in spermatid/Sertoli cell interactions. We have identified a substrate of MGAT1 that may be the basis of the loss in fertility. We are screening for a small molecule MGAT1 inhibitor which should have potential as a male contraceptive or inhibitor of cancer progression which is facilitated by MGAT1.

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A complete list of referencescan be found at
https://www.ncbi.nlm.nih.gov/myncbi/browse/collection/40358567/?sort=date&direction=ascending

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Molecular Regulation of Pre-Cancerous and Cancer Stem Cells in Hematopoiesis and Leukemogenesis

Hematopoiesis maintains a life-long supply of the entire spectrum of highly specialized blood cells dependent on systemic needs. This process relies on a tightly regulated balance of self-renewal, commitment, and differentiation of a small number of pluripotent hematopoietic stem cells (HSC).

Recent experimental evidence has shown that acute myeloid leukemias (AML) and myelodysplastic syndromes (MDS) arise from transformed immature hematopoietic cells following the accumulation of multiple stepwise genetic and epigenetic changes in HSC and committed progenitors. The series of transforming events initially give rise to pre-leukemic stem cells (pre-LSC), preceding the formation of fully transformed leukemia stem cells (LSC). Pre-LSC as well as LSC are characterized by a relative resistance to chemotherapy and thereby contribute to treatment failure. As a consequence, and despite the use of poly-chemotherapy and newer agents that transiently reduce the tumor burden, relapse continues to be the most common cause of death in most subtypes of AML and MDS. Defining the molecular characteristics and regulatory mechanisms in pre-LSC and their progression to fully transformed LSC is critical to understanding the genesis of leukemia and to developing therapeutic strategies by which these cells can be eradicated.

Recent findings from our own group and others have demonstrated a critical role of key transcriptional regulators, chromatin-remodeling factors, and mediators of aberrant signaling in the genesis and function of pre-LSC and LSC in AML and MDS in mouse and human model systems.

The goal of our research is to delineate critical mechanisms in HSC that drive formation and progression of pre-LSC and LSC. To identify and functionally study implicated pathways we are utilizing rigorously defined stem and progenitor cell subsets isolated by means of multi-parameter high-speed fluorescence-activated cell sorting (FACS). Identified target genes are biochemically and functionally tested. We utilize lentiviral gene transfer allowing for forced expression or shRNA-mediated knockdown, followed by in vitro as well as in vivo assays for stem and progenitor cell functions including murine transplantation models. This allows for assessing the function of candidate genes in normal and leukemic stem cells. We are studying murine genetic models as well as primary human samples from patients with leukemia. Our studies aim at the development of targeted, pre-LSC- and LSC-directed therapies.

Project areas in the lab include:

- Mechanisms of leukemia pathogenesis at the (pre-leukemic) stem cell level
- Identification and study of novel molecular mechanisms and pathways in normal and malignant hematopoiesis (with focus on transcription, and signaling)
- Development and preclinical testing of novel therapeutics targeting aberrant stem cells
- Translational computational biology (e.g. integrated analysis of WGS, RNA-seq, ChIP-seq, RIP-seq data etc.; including from longitudinally sampled, sorted/single stem cells from patients and mouse genetic models)

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MECHANISMS OF STEM CELL AGING AND TRANSFORMATION

Key Words: *Hematopoietic stem cells, cell fate determination, hematopoietic malignancies.*

Hematopoietic stem cells (HSC) maintain multi-lineage blood formation throughout our lifetime. Balancing stem cell regeneration and differentiation commitment to produce mature blood cells is quintessential for a healthy hematopoietic system. Dysregulation of such HSC fate determination processes can lead to loss of immune function, bone marrow failure, and malignant transformation during aging. However, up to date very little is known about the molecular events driving age-related HSC changes and how they contribute to disease.

Understanding age-associated molecular alterations will not only uncover fundamental mechanisms guiding function of HSCs, but may also allow for therapeutic intervention to "rejuvenate" aged hematopoietic systems and possibly even prevent age-associated hematopoietic diseases. **Our mission is to clarify the central mechanisms establishing and guarding sustained hematopoietic stem cell function, particular those that drive leukemogenesis, if disrupted.** We develop innovative genetic mouse models, use *ex vivo* and *in vivo* primary mouse and human stem cell assay systems, exploit lentiviral gene transfer, and apply state-of-the-art molecular biology and next generation sequencing techniques. Currently, we have four major project lines in the laboratory:

The labile iron pool as a rheostat for stem cell function

Our recent work has uncovered a key role of the amount of readily accessible intracellular iron (termed labile iron pool, LIP) in instructing HSC self-renewal (*Kao et al., STM 2018*). We are currently investigating the precise molecular mechanism of action, particularly focusing on metabolic and non-enzymatic molecular pathways relying on iron – a completely uncharted territory for healthy as well as leukemic stem cells.

Gene expression program erosion in aging stem cells and leukemia

Our past work has demonstrated a causative role of even minimal dosage alterations of a key transcription factor instructing hematopoiesis, PU.1, observed in hematopoietic stem cell aging to myeloid leukemia evolution (*Will et al., Nat Med 2015*). Our current efforts focus on understanding (1) how such slight deviations from optimal PU.1 dosage lead to the erosion of PU.1-dependent gene expression programs, and (2) in which way a slightly altered PU.1 gene expression network can functionally cooperate with age-associated inactivation of epigenetic regulators (TET2 and DNMT3A).

Identification of molecular safeguards of cancer stem cells

Teamed-up with Dr. Ana Maria Cuervo (Dept. of Developmental & Molecular Biology), the discoverer of a highly precise protein degradation pathway named chaperone-mediated autophagy, we are investigating the role of this stress-related molecular defense mechanism in leukemic stem cell evolution and maintenance.

Improving stem cell-directed therapies

We are actively engaging with commercial research partners to test and evaluate novel therapeutic options for patients with hematologic malignancies. We have uncovered a beneficial role of eltrombopag, a small molecule thrombopoietin receptor mimetic and iron chelator, for enhancing platelet production (*Will et al., Blood 2012*) as well as healthy HSC function (*Kao et al., STM 2018*). Our current work looks into how

eltrombopag can efficiently be combined with standard-of-care treatment regimen for patients suffering from treatment or disease-initiated thrombocytopenia.

Selected Publications

Full list of publications can be found at:
https://www.ncbi.nlm.nih.gov/myncbi/browse/collection/47490382/?sort=date&direction=descending

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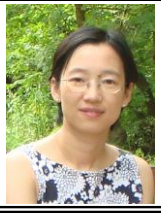

Transcription Regulation and Cell Signaling Control in Normal B/T Cells and Lymphomas

Molecular pathogenesis of lymphomas situates at the crossroad of lymphocyte development, cancer genetics, transcription regulation, and cell signaling. Thus, we constantly draw upon the most recent advances in these fields to address mechanism questions that are related to lymphoma initiation and development. As each lymphoma entity often corresponds to a specific B/T cell activation/differentiation state that is phenotypically "frozen" by the malignant transformation process, our lymphoma-related studies also provide valuable insights to the regulatory mechanisms that govern the normal immune system. Our research has three major goals: to better understand mature B and T cell development in molecular terms, to decipher how this process is perturbed during lymphomagenesis, and to develop mechanism-based novel therapies to improve patient outcome.

The germinal center (**GC**) response is a very important B cell development stage that has the unique property of generating high affinity antibodies and B cell memory. Because dysregulated GC responses contribute to the development of B cell lymphomas and autoimmune diseases, in-depth understanding of the control mechanisms governing the GC response has both immunological and clinical implications. GCs are dynamic and specialized structures in the secondary lymphoid organs where the B cell genome is subject to two types of genetic alterations catalyzed by AID (activation induced cytidine deaminase), e.g. Ig class switch recombination and somatic hypermutation. Prior to their GC exit, B cells bearing mutated surface Ig molecules undergo positive and negative selections through interaction with two other types of cells in the GC, e.g. follicular dendritic cells follicular T helper (**Tfh**), and T follicular regulatory (**Tfr**) cells. Only the fittest B cells are licensed to terminally differentiate into memory or plasma cells. At the single cell level, the acquisition and termination of GC phenotype is the coordinated transcriptional response to various extracellular and intracellular stimuli; yet the precise sequence and nature of events that orchestrate this process is incompletely understood. We are particularly interested in the roles of two transcriptional factors, BCL6 and STAT3, both are known to play pivotal roles in fate specification and function of GC B cells, Tfh, and Tfr cells.

Our studies over the past 25 years have revealed novel mechanisms that govern the expression and activity of BCL6, and demonstrated the importance of functional interactions between BCL6 and several cell signaling pathways including RhoA, NF-κB, and Jak/STAT3. In recent years, a central focus of our B cell lymphoma work was on the IL-6/Jak/STAT3 signaling pathway. With the help of our collaborators, we characterized expression regulation of STAT3, cause and consequences of its aberrant activity in diffuse large B-cell (**DLBCL**) development and therapeutic response. Functional contribution of this pathway to normal plasma cell maturation was also investigated.

Since three years ago, our work has taken on a new focus, i.e. the pathogenesis and immunologic features of adult T-cell leukemia/lymphoma (**ATLL**). ATLL is a rare CD4 T cell neoplasm, endemic in the Japanese, Caribbean and Latin American populations. It arises in HTLV-

1 carriers and is an extremely aggressive cancer with a dismal outcome and lack of effective therapies. In a recently study published in Blood, we demonstrate that ATLL patients diagnosed in North American (**NA-ATLL**) have a distinct genomic landscape compared to the Japanese cohort (**J-ATLL**). In particular, NA-ATLL is characterized by a much higher frequency of prognostic epigenetic mutations and is targetable preclinically with DNA de-methylation drugs. Taking advantage of the fact that the Montefiore Medical Center follows one of the largest groups of ATLL patients in the U.S, we have built up a NA-ATLL Biobank, generated and characterized a number of novel NA-ATLL cell lines and PDX models to support our laboratory studies.

We are currently pursuing the following research questions:

1. Can the therapeutic outcome of the ABC subtype of DLBCLs be improved by manipulating the endogenous redox homeostasis of the lymphoma cells?
2. How does BCL6 contribute to the pathogenesis and transcription programs of NA-ATLL?
3. What is the genetic and clonal evolution basis that underlies the profound chemo-resistance of ATLL?
4. Explore novel, targeted therapies for NA-ATLL.

Selected Publications:

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