DEPARTMENT OF CELL BIOLOGY
2023-2024

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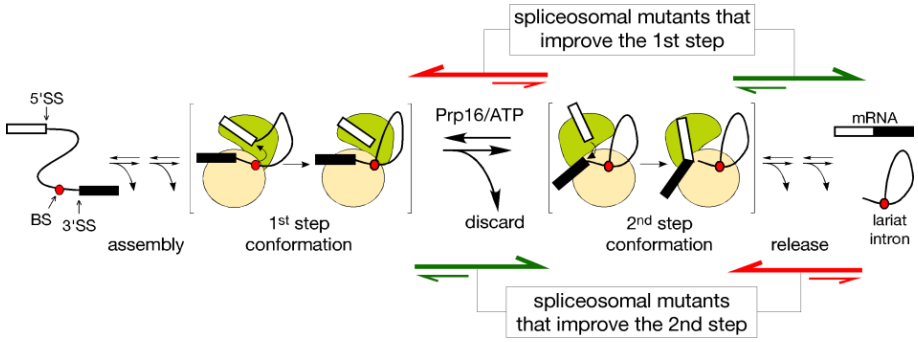

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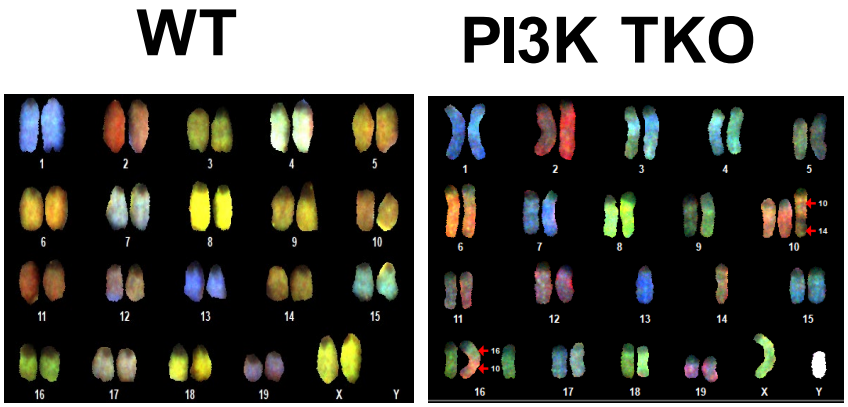

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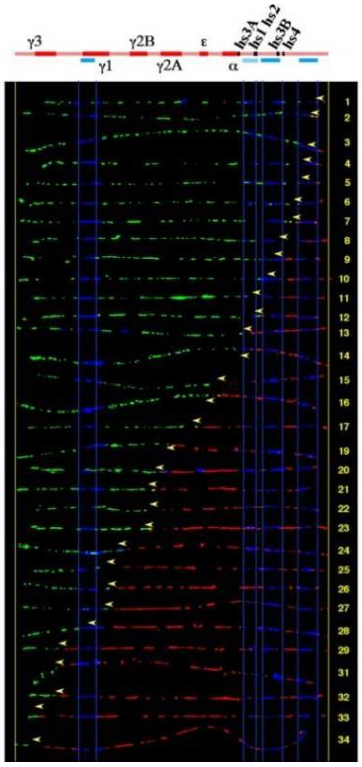

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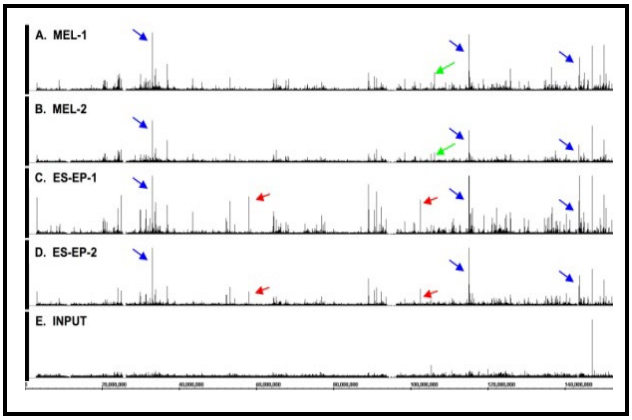

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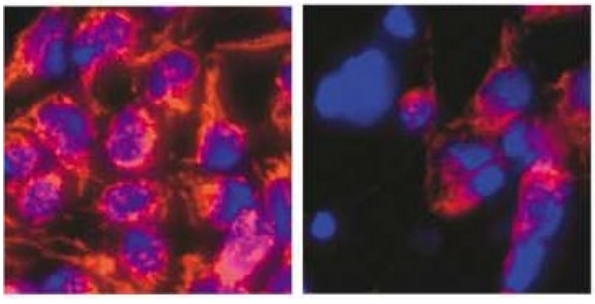

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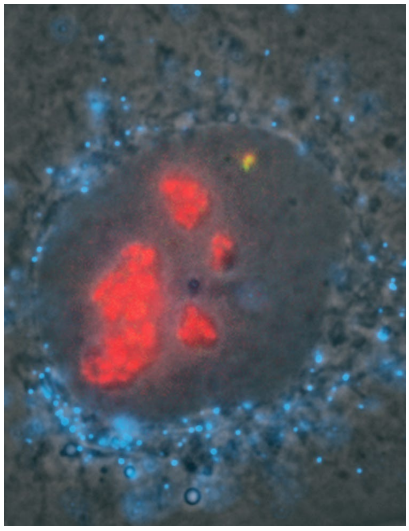

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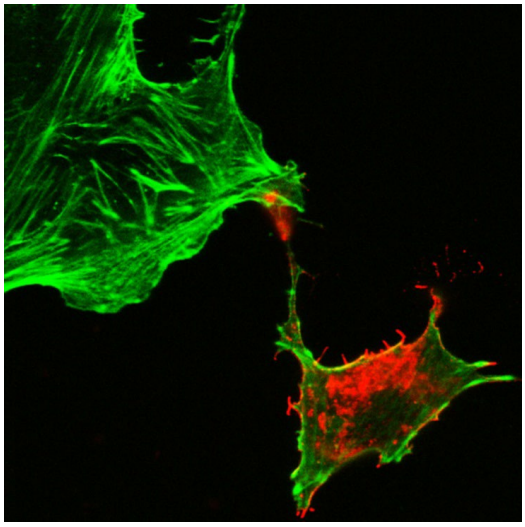

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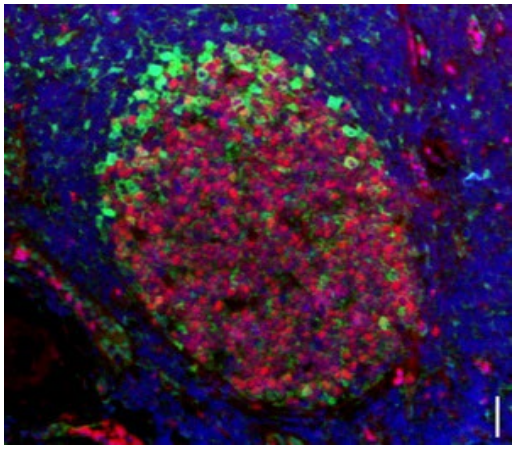

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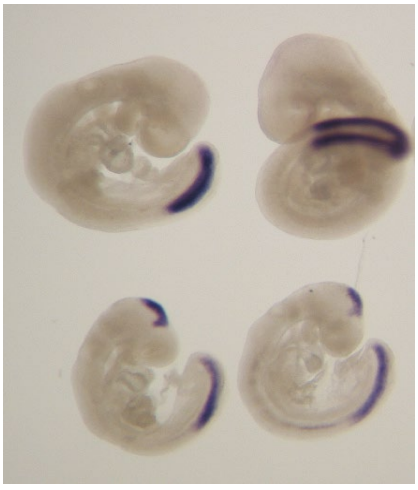

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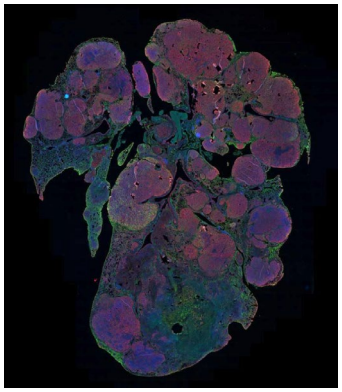

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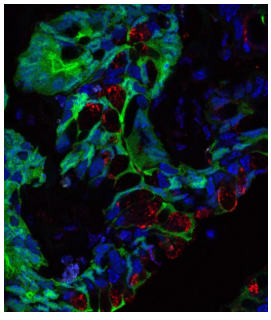

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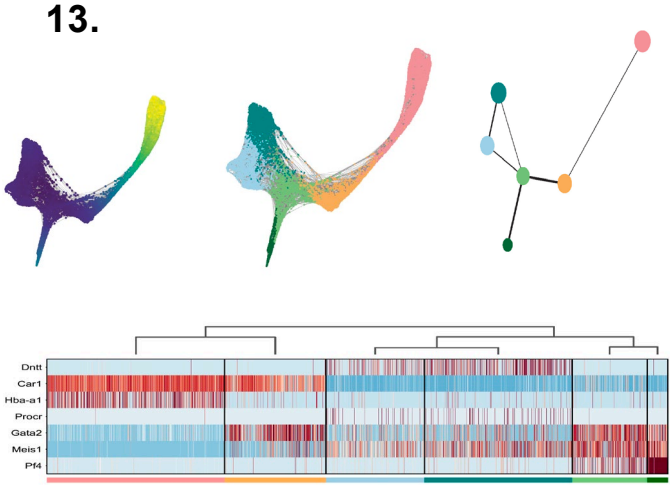


Picture Legends

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. **Gritsman Lab:** Spectral karyotyping analysis revealing multiple chromosomal abnormalities in hematopoietic cells from a PI3 kinase (PI3K) triple isoform knockout (TKO) mouse

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. **Meier Lab**: Fluorescence image overlayed on a phase contrast image of an enlarged nucleus surrounded by cytoplasm. This stable cell line expresses a cyan fluorescent protein (CFP) with a peroxisomal targeting signal (blue) and a small nucleolar RNA (snoRNA, red) from an intron (green). In this fluorescence in situ hybridization (FISH) of RNA, the snoRNA is visualized in nucleoli (red) and together with the intron at the site of transcription (green/yellow). The spliced mRNA is exported to the cytoplasm and the translated CFP translocated into peroxisomes (blue).

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-infected cells showing intercellular extensions that mediate virus cell-to-cell transmission. Confocal microscopy image of alphavirus-infected Vero cells, with virus glycoproteins shown in red and F-actin shown in green.

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. **LaFave Lab:** Multiplexed immunohistochemistry in a murine model with advanced lung adenocarcinoma. Whole tumor-burdened lungs were stained for Alveolar type 2 (AT2) (OPAL 520, green), Alveolar type 1 (AT1) (OPAL 690, red), and nuclear (DAPI) markers. Image generated by the LaFave lab.

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

13. **Steidl Lab**: Single-cell RNA-sequencing of mouse hematopoietic stem and progenitor cells reveals a continuum of cells differentiating into megakaryocytic and erythroid lineages. (Left) A force-directed graph colored by pseudotime. (Middle) Community detection via the Louvain method. (Right) Partition-based graph abstraction (PAGA) provides an interpretable graph-like map of the arising data manifold. (Bottom) Cell-type-specific markers aid in cluster identification. [Emily Schwenger, MSTP student, Steidl Laboratory]

Department of Cell Biology

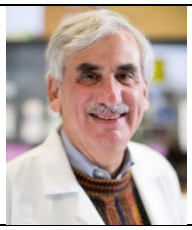
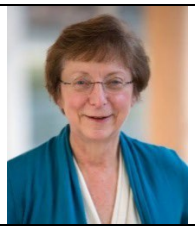
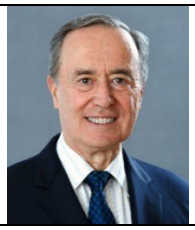
Welcome to the Albert Einstein College of Medicine and the Department of Cell Biology. Our department is focused on molecular mechanisms in many important areas of cell biology, ranging from stem cells to viruses, DNA replication to RNA processing, gene expression to immunology, glycobiology to cancer. 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 and discuss original articles. An informal weekly get-together on Friday afternoons encourages scientific interactions as well as social connections. Our yearly departmental retreat takes us all to the seashore or woods 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 https://www.einsteinmed.edu/departments/cell-biology/ . Feel free to contact any of us for further discussions.

Enjoy your first year!

Cell Biology Faculty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	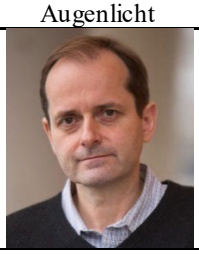				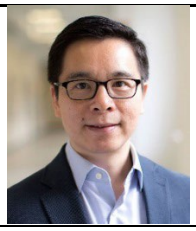
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		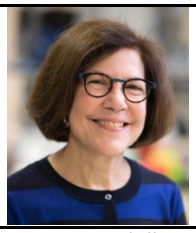			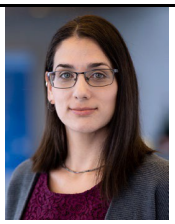
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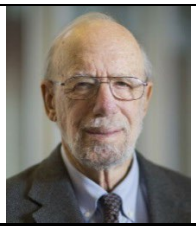
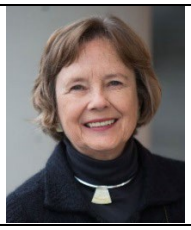
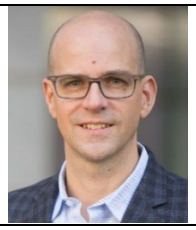
					
U. Thomas Meier	Satish Nandakumar	Charles Query	Matthew Scharff	Carl Schildkraut	Robert Singer
					
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*MD/PhD Student

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Rose C. Falkenstein Chair in Cancer Research
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Co-Leader Tumor Microenvironment and Metastasis Program, AECC

Price BLDG. – ROOM 220

CANCER DORMANCY: UNDERSTANDING THE BIOLOGY OF METASTASIS

The major challenge faced by physicians is the prevention and treatment of metastasis, the main reason for cancer mortality. Often, cancer patients presumed cured after primary tumor removal and therapy, can carry non-proliferating 'dormant' disseminated cancer cells (DCCs) for years before reactivating to form incurable metastasis. In addition, DCCs show resistance to standard treatments by reprogramming themselves in a niche-dependent manner. Our lab specifically focused on the dormant nature of minimal residual disease in cancer by understanding the biology of dormant DCCs and their reactivation, to target them and prevent relapse. We focus on identifying the mechanisms of microenvironmental cues and cross-talk with DCCs, epigenetic and immune regulation of DCCs, and developing therapeutic approaches to target DCCs clinically.

1. Early dissemination and early DCC dormancy. Our lab has discovered that dormant breast cancer DCCs and metastasis can originate very early during cancer evolution, disseminating during pre-malignant stages and aided by innate immune cells. We identified a mechanism for early dissemination whereby Her2 aberrantly activates a program similar to mammary ductal branching that spawns early DCCs (eDCCs) capable of forming metastasis after a dormancy phase. We also revealed how the HER2 oncogene activates through CCL2 signaling the recruitment of tissue resident macrophages that help eDCCs to enter circulation. Targeting these macrophages early in cancer evolution reduced metastasis late in cancer progression. Together, we try to understand how eDCCs orchestrate signals intrinsic to cancer cells and those from the microenvironment to remain dormant and persistent and how alterations of these signals lead to reactivation into outgrowing metastases. We also aim to identify markers that might pinpoint early DCCs vs. late DCCs and may allow selectively targeting these cells.

To this end we recently completed a full single cell sequencing study of early and late primary lesions as well as DCCs from matched early and late lungs identifying the transcriptional programs that drive early DCC dormancy. Understanding mechanisms of disseminated cancer cell (DCC) plasticity, represented by the transitions between epithelial and mesenchymal cell fates (EMT and MET), has been shown to closely associate with tumor cell proliferation, cancer dormancy, and metastasis. Our early DCC studies have identified the pluripotency regulator ZNF281 (aka Zfp281, ZBP99, GZP1) in controlling anterior-posterior axis formation and epiblast maturation through direct transcriptional and epigenetic regulation of Nodal/TGFb signaling pathway, which is well known for its roles in EMT and cancer metastasis as well as in BCa stemness. ZNF281 induces a permissive state for heterogeneous mesenchymal-like (M-like) transcriptional programs. These programs carry a dormancy signature and are absent in proliferative primary tumors and metastasis.

- Harper, K, Sosa MS., et al., (2016) *Nature*. PMID 27974798
- Hosseini et al., (2016) *Nature*. PMID 27974799
- Linde and Casanova-Acebes et al., (2018) *Nat Commun*. PMID 29295986
- Nobre et al., (2022) *Nat Cancer*. PMID: 36050483

2. Stem cell and niche biology in cancer dormancy. We have explored why in many patients DCCs persist and remain dormant in the bone marrow and other organs. We found that the bone marrow contains high levels of TGFβ2, which induced dormancy via TGFβ-RI/RII/RIII complexes and p38 signaling. We used mouse genetics to show that a key source of TGFβ2 is the NG2+/Nestin+ mesenchymal stem cells (MSCs) that induce hematopoietic stem cell dormancy and self-renewal and also proved that these MSCs induce and maintain dormancy of breast cancer DCCs in the bone marrow. We also determined that the retinoic acid signaling via the nuclear receptor NR2F1 is required for survival of HNSCC DCCs in the bone marrow but it controls dormancy of these same DCCs in the spleen and lungs. Interestingly the expression of NR2F1 can be initiated in the primary site by a combination of hypoxic signals and the presence of specialized macrophages that coordinate the induction of NR2F1 and SOX9 and prime intravasating DCCs to enter dormancy post extravasation. We have also discovered that dormant tumor cells assemble their own pro-dormancy niche by coordinating the above signals with the upregulation if collagen-III and the assembly of curly collagen matrices that sustain dormancy via DDR1 signaling STAT1 signaling. We have also explored how in lung cancer niches orchestrated by tissue resident macrophages (TRMs) and how these are altered by early lung cancer cells and later recruited bone marrow derived macrophages regulate lung cancer growth initiation. This work revealed that TRMs orchestrate a tissue regenerative and immune evasive program that enables lung cancer initial growth. Together these studies have provided a molecular description of how a reciprocal interaction between DCCs and their niches are essential for survival and dormancy of DCCs across different cancers.

- Bragado, P., et al.,(2013). *Nat. Cell Bio.* PMID 24161934
- Sosa MS et al. (2015) *Nat Commun*. PMID 25636082
- Nobre AR et al., (2021) *Nat Cancer*. PMID: 34812843
- Di Martino Jet al., (2021) *Nat Cancer*. PMID: 35121989
- Casanova-Acebes, M., et al., (2021) *Nature*. PMID: 34135508
- Borriello L. et al., (2022) *Nat Commun*. PMID: 35110548

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3. Mechanism of uveal melanoma dormancy and therapy resistance. The primary concern in uveal melanoma (UM) patient is the development of liver metastasis after primary tumor resection and the lack of effective treatment to treat the same. UM DCCs can leave the primary tumor at very early stages of progression as observed in other types of cancers, seed target organs and remain dormant for years, until some change “awakens” DCCs causing metastasis after 10-15 years. We hypothesize that dormant DCCs enter quiescence and cannot be eradicated by anti-proliferative therapies. We further hypothesize that these mechanisms could be targeted to prevent awakening and metastasis, as well as to identify biomarkers that could be used to predict dormant disease in patients and therapy response. This approach has not been attempted in uveal melanoma. The primary aim of this effort in the lab is to provide the preclinical basis for new treatment options for early as well as late-stage UM patients. TGFβ2 signaling was found to be implicated in the dormancy of HNSCC DCCs in the bone marrow, demonstrated as being a ‘restrictive soil’ compared to the lungs for example. Interestingly, liver cells also express TGFβ2. Thus, we hypothesize that TGFβ2 could be maintaining UM DCCs dormancy in the liver for years until age-related changes in the liver ME induces their awakening. The lab is also currently exploring this aspect of dormancy-maintaining signaling as well as optimizing targeted therapies as a possible therapy option for UM patients.

- Lapadula et al., 2023 *Mol Cancer Ther.* PMID: 36223548

- Kadamb and Lopez-Anton et al., (2023) In Preparation
- The et al., (2020) *Mol Cancer Ther* PMID: 32430489
- Lapadula et al., (2019) *Mol Cancer Res* PMID: 30567972

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4. Tissue homeostasis, aging, and dormancy: We are dissecting out the interaction between tissue resident host cell populations in the metastatic niche and DCCs, and how this may regulate dormancy and re-awakening. Once DCCs arrive at distal sites they are exposed to niche-specific homeostatic processes. These processes are largely driven by stromal and immune cell populations. Given that dormancy is a long-lived process that can span many years, we hypothesize that these homeostatic processes likely induce dormancy, and that the loss of tissue homeostasis results in DCC re-awakening. Over the span of an organism's life, many age-related changes result in a loss of homeostasis which have been shown to affect DCC re-awakening. Age-induced modifications in pulmonary fibroblast WNT-signaling induces a dormancy-to-reactivation switch in melanoma DCCs resulting in efficient metastatic outgrowth in aged lungs. We are also beginning to investigate how age-related changes in the immune and stromal compartment may influence dormancy and re-awakening at distal sites and how specific factors that reverse aging phenotypes may affect the fate of DCCs and their switching from dormancy to reactivation in melanoma and breast cancer.

- Fane et al., (2022) *Nature*. PMID: 35650435

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5. Epigenetic programs of dormancy and therapeutic targeting. Targeting dormancy mechanisms for metastasis suppression: Analysis of the transcriptional and epigenetic mechanisms active in dormant DCCs pinpointed retinoic acid as a micro-environmental pro-dormancy cue. We found that the orphan nuclear receptor NR2F1, which regulates lineage commitment and is silenced in human tumors, is spontaneously upregulated in solitary dormant tumor cells. Retinoic acid induces TGFβ2, NR2F1 expression, and dormancy of DCCs. We found that a transient treatment with a low dose of 5-azacytidine (AZA), followed by all-trans retinoic acid (atRA), restored the NR2F1-driven program and long-term in vivo quiescence of previously malignant cells. We showed that NR2F1-independent pathways induced in parallel upon reprogramming also play an important role in dormancy induction and they depend on TGFβ-SMAD4 signaling. This mechanism is due to specific remodeling of enhancers linked to TGFb-SMAD4 signaling. The AZA+atRA strategy is currently being used in a clinical trial in prostate cancer. Our epigenetics work has also revealed that in melanoma mutations in the chromatin regulator ARID2 acquire a mesenchymal slow cycling buyt highly disseminating phenotype. Further analysis of epigenetic pathways focused on the histone variants macroH2A, especially the isoform macroH2A2, and we found this histone variant enforces a stable dormant phenotype in DCCs by activating dormancy and senescence genes that limit metastasis initiation. We showed that TGFβ2 and p38a/β also upregulate macroH2A and thus induce DCC dormancy.

Recently, we capitalized on this knowledge and discovered an agonist of the NR2F1 that specifically activates dormancy programs or neural crest stem cell commitment that drives stably malignant cells into a persistent growth arrest in target organs. In addition, we discovered long ago that dormant cells depend on the unfolded protein response for survival and specifically on the PERK pathways. We have now identified an inhibitor the PERK pathway, which is required for the survival of dormant DCCs and when used in vivo blocks metastasis by eradicating dormant DCCs during quiescence. This inhibitor is currently in clinical trials.

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- Khalil B.D. et al, (2022). *J. Exp. Med*. PMID 34812843
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Key Words: *intestinal homeostasis and cancer, diet, mouse models, inflammation, stem cells*

Our laboratory is focused on cellular and molecular mechanisms of intestinal homeostasis and perturbations that cause intestinal inflammation and tumor development. This encompasses development of mouse genetic models and the impact of environmental alterations linked to elevated risk for human inflammatory bowel disease and colon cancer.

We demonstrated an orchestrated reprogramming of intestinal epithelial cells as they migrate from the stem and progenitor cell compartment in the crypt and undergo maturation, normally 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.

Diet and Aging Impact on intestinal stem cells and mucosal lineages:

Colon cancer incidence in humans is strongly linked to long term dietary patterns. We model this in the mouse using a purified rodent diet (NWD1) that adjusts multiple key nutrients to mimic exposure to each at its level present in human populations with much higher incidence of colorectal cancer.

Feeding NWD1 accelerates and increases tumor development in mouse genetic models of intestinal cancer, regardless of etiology, mechanism, or altered genetic drivers, and can also influence site of tumor development in the small and large intestine. However, >70% of human colon tumors are sporadic, developing in the absence of known inherited genetic factors, and arising after 5-6 decades of life, with incidence largely determined by long term dietary patterns. Importantly, wild-type mice rarely, if ever, develop sporadic tumors when fed standard diets, but feeding wild-type mice NWD1 for 1-2 years causes 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 develop, the mucosa, though seemingly normal, is altered at the cellular and molecular levels: there is increased immune cell infiltration, elevated serum cytokine levels, altered lineage specific markers, and elevated Wnt signaling and ectopic Paneth cell marker expression throughout the small intestinal and colonic mucosa.

Our recent work has focused on the profound role of nutritional exposures in determining which and how stem cells function in eventually giving rise to tumors, making extensive use of single cell technology and novel bioinformatic data analysis. NWD1 initiates mucosal remodeling by the epigenetic down-regulation of a key gene in the most common stem cells which alters their mitochondrial structure and function. This represses their stem cell functions, recruiting alternate stem cells to maintain the tissue, but this also reprograms mucosal cells to stimulate chronic inflammation, a key driver of tumor development. Strikingly, many of these nutritionally induced changes mimic those that cause human inflammatory bowel disease that also raises risk for tumor development. A major finding is that there is failure of cells to develop properly as they move through what we have termed a developmental restriction point in the tissue, triggering the alternate stem cell recruitment. We also discovered a similar compromise of stem cell function and failure of cell developmental maturation in the intestine of aging mice. In both systems this was dynamic, reversed by either nutritional shifts or drugs that target important pathways of metabolism. The finding that nutritional exposure has such a profound effect in determining which and how stem cells function raises an intriguing question: rapid evolutionary expansion of species and higher taxa to occupy new niches – termed "*adaptive radiation*" - depends on the ability of organisms to adapt to and utilize new food sources. Therefore, the plasticity of intestinal epithelial cells to adapt, including alterations determining how and which stem cells function as stem cells, may have arisen to provide this flexibility to organisms to function in new ecosystems.

Collaborations: The gut also harbors billions of bacteria, creating a complex ecosystem interacting with stem and progenitor cells and differentiated lineages of the mucosa. A collaboration with Libusha Kelly (Computational Biology) has pursued her discovery of gut microbe mediated synthesis of sulfide that is released into the gut lumen. The sulfide then can chemically reduce susceptible chemical bonds (e.g. azo bonds) in a wide range of compounds, altering effects of these compounds on the intestinal mucosa and also having systemic effects. The Kelly and Augenlicht labs established that this can be regulated by diet, both by major alterations of the intestinal microbe populations, and by altering level of the amino acid cysteine, a principal substrate for bacterial synthesis of sulfide. These reactions can be important in reprogramming intestinal stem cells and lineages in development of inflammation and tumor risk, in damaging the mucosa, and in potentially modifying drug efficacy. We also have important collaborations with Winfried Edelmann (Cell Biology) on mechanisms of nutritional-genetic interactions that drive tumor development, and with Vern Schramm (Biochemistry) in his pioneering work on development of novel transition state analogues as therapies for disease. This collaboration among Schramm, Augenlicht and Edelmann is investigating the efficacy and mechanism of one such drug for colon cancer, targeting a unique synthetic lethality of genes in methyl group metabolism that expands the range of colon and other tumors that can be effectively treated.

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Key Words: Pluripotent Stem Cells, Hematopoietic Stem Cells Red Blood Cells, Sickle Cell Disease, Gene Therapy, Genome Modification.

CULTURED RED BLOOD CELLS:

We have developed a method 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 hemophilia A.

GENE THERAPY:

We are studying the biology of hematopoietic stem cells in the context of sickle cell disease and have developed a CRSPR-based methods to perform knock-in of mini-circles in human hematopoietic stem cells for gene therapy of hemoglobinopathies and other genetic disorders affecting erythroid cells.

FOR MORE DETAILS: https://www.einstein.yu.edu/faculty/4981/eric-bouhassira/

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Single Molecule Imaging of Transcription Factors Involved in Cancer, Hematopoiesis and Ageing

Human tumorigenesis is a complicated process marked by a loss of the cell's ability to regulate critical cellular processes, such as transcription, RNA processing and translation, leading to uncontrollable cell growth. As our understanding of tumorigenesis becomes more sophisticated, a combinatorial approach is necessary to better understand the dynamic coordinated action of very large multi-subunit enzymes and protein complexes controlling these key cellular processes. Recent advances in single-molecule imaging provide an unprecedented spatiotemporal window into probing dynamic functional interactions between these heterogeneous large multi-subunit transcription assemblies and chromatin in real-time. In addition, high-resolution cryo-electron microscopy and genome-wide binding assays allow us to rapidly survey detailed functional interactions on physiologically relevant substrates with limited amounts of samples. Armed with these advanced *in vitro* and *in vivo* approaches, our lab seeks to gain a mechanistic understanding of how transcription complexes dynamically regulate expression of tumor suppression pathways and how these processes are altered in cancer.

Specifically we apply these tools to mechanistically dissect how the p53 tumor suppressor protein communicates with multiple transcription assemblies, such as chromatin remodeling factors (PBAF), core promoter recognition factors (TFIID), and RNA Polymerase II, to circumnavigate the repressive effects of chromatin on transcription. We are also applying these same strategies to study how oncogenic mutants of p53 and chromatin-remodeling complexes mechanistically function to promote tumor formation. Our long-term goal is to determine the molecular origin of cancer and additional diseases related to hematopoiesic dysfunction, eye development and ageing using our multi-disciplinary approach centered around advanced single molecule imaging, genome-wide studies, and structural biology.

Development of single molecule imaging systems to study how transcription factors alter chromatin structure and regulate transcriptional bursting

To understand how proteins engage chromatin at high temporal and spatial resolution, our group has established numerous in vitro and in vivo systems utilizing high-resolution co-localization, single molecule FRET, and dynamic live cell imaging. Strikingly, our live cell imaging studies find that transcription factors and chromatin remodelers, including p53, RNA Polymerase II and PBAF, dynamically cycle on and off the genome in spatial hubs of activity on the timescale of seconds to minutes. The chromatin structure of underlying target sequences and enzymatic activities associated with the transcription factors/chromatin remodelers dictate their dynamic cycling and binding kinetics. Oncogenic mutations in transcription factors can also affect the binding kinetics of associated proteins that are being co-loaded onto the genome. In addition, we find that single molecule kinetic binding profiles of transcription factors are associated with different cellular states during development. Furthermore, we are also adapting our imaging systems to directly assess the enzymatic activity of chromatin remodelers and enzymes that translocate along the genome.

In collaboration with Rob Singer's lab at Einstein, we have also developed a live-cell multicolor single molecule imaging system to examine how our transcription factors dynamically regulate transcriptional bursting of tumor suppression genes. Using these imaging systems, we find that patterns of transcription factor binding and transcriptional bursting display memory effects to fine-tune expression of genes. Future work will be to develop this high resolution imaging in live mice and live-cell image based drug screening platforms that rapidly determine combinatorial effects of epigenetic inhibitors in different cell types and diseased cells.

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Tumor MicroEnvironment of Metastasis

Areas of Research:

Cell biology and biophysics, cancer biology, and the integration and validation of clinical imaging platforms with high resolution intravital multiphoton microscopy. Development of multiphoton imaging technology to identify tumor cell dissemination mechanisms in both primary and secondary tumor sites during metastatic progression. Development of new optical microscope technology in the Gruss Lipper Biophotonics Center.

Professional Interests:

John Condeelis' research interests are in optical physics, cell biology and biophysics, cancer biology and mouse models of cancer. He and his collaborators developed the multiphoton imaging technology and animal models used to identify invasion and intravasation micro-environments in mammary tumors. Integration of intravital multiphoton imaging with computational /systems analysis of living breast tumors identified the dominant tumor cell phenotypes contributing to invasion and dissemination during metastasis. This led to the discovery and verification of the paracrine interaction between tumor cells and macrophages in vivo, the role of macrophages in the migration of tumor cells during HGF- dependent tumor cell streaming to blood vessels and the mechanism of tumor cell dissemination from primary tumors via TMEM (Tumor MicroEnvironment of Metastasis) to distant metastatic sites. Based on these results, cell collection techniques, including the in vivo invasion assay were developed for the collection of migrating and disseminating macrophages and tumor cells. This led to the discovery of the mouse and human invasion signatures, and the TMEM, MenaCalc and MenaINV markers for assessing risk of metastasis and prediction of response in breast cancer patients to both chemotherapy, and receptor tyrosine kinase and tyrosine kinase inhibitors used to suppress metastasis.

John Condeelis has devised optical microscopes for uncaging, biosensor detection and multiphoton imaging for these studies and has used novel caged- enzymes and biosensors to test, in vivo, the predictions of the invasion signatures regarding the mechanisms of tumor cell dissemination and metastasis. He is one of the founding co-directors of the Integrated Imaging Program (IIP) dedicated to the integration and validation of clinical imaging platforms, including digital pathology, with high resolution optical imaging in the Gruss Lipper Biophotonics Center. He is now the Director of the Integrated Imaging Progam (IIPCR). The IIPCR is dedicated to curing metastatic cancer. He has authored more than 360 scientific papers on various aspects of his research.

For a complete list of publications please visit https://www.einsteinmed.edu/labs/john-condeelis/publications/

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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. DNA mismatch repair (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 mutations 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 novel anticancer treatments including immune therapeutic approaches.

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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 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 their contributions to chromatin assembly. We also dissect their function *in vivo* by methods of *Drosophila* genetics. More globally, we are trying to elucidate the roles nuclear enzymes in the hierarchical organization of the chromosome.

1. Nucleosome assembly mechanisms and biological functions of chromatin assembly factors

In vivo, canonical nucleosomes are assembled by two families of proteins, core histone chaperones, such as NAP-1, and ATP-dependent motor factors, such as ACF (ATP-utilizing Chromatin assembly Factor). ACF consists of a SNF2-like ATPase ISWI and a polypeptide termed Acf1. We study ATP-dependent chromatin assembly *in vitro* to elucidate molecular events that take place during the formation of nucleosomes.

In addition to ACF, two other ISWI-containing complexes, ToRC (comprising Tou, ISWI and CtBP) and RSF (Rsf1 and ISWI), mediate similar reactions and 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. It 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.

2. Mass spectrometry tools for the analyses of chromatin structure and stable protein complexes

The factors of nuclear DNA metabolism rarely act as individual polypeptides but, rather, within stable multi-subunit complexes. The existing methods to characterize native complexes suffer from several drawbacks. Tag-affinity purification of ectopically expressed subunits is prone to an unacceptably high rate of false-positive IDs. On the other hand, conventional chromatography to achieve apparent homogeneity is time-consuming and can only be applied to abundant proteins. We have devised, implemented and validated a highly innovative, streamlined method termed MS-Enabled Rapid protein Complex Identification (MERCI) to rapidly identify stable native complexes. It relies on partial enrichment of a target protein in sequential FPLC steps, quantitative shotgun proteomics of chromatographic fractions and correlation analyses. MERCI allows rapid identification of scarce complexes without a loss of the precision emblematic of the classical FPLC approach. It makes characterization of native complexes a routine laboratory technique and allows characterization of trace amounts of protein complexes with a virtually assured result.

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.

We examined phenotypes associated with the depletion of H1 by RNAi in flies. 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 in *Drosophila* larval cells. H1 negatively regulates DNA replication in conjunction with a SNF2 ATPase SUUR that forms a native complex (SUMM4) with an insulator protein Mod(Mdg4).

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.

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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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Kinase signaling pathways in adult blood development and blood cancers

My lab studies the signal transduction pathways that affect the early fate decisions of adult hematopoietic stem cells (HSCs) as they psrogress 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. We use a combination of genetically modified mice, cell lines, and patient samples to explore the mechanisms by which signaling pathways maintain both normal and leukemic hematopoiesis.

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, protein synthesis, and autophagy. 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 have found that deletion of multiple PI3K isoforms impacts normal HSC function, including self-renewal, proliferation after infection or chemotherapy treatment, and differentiation along different blood lineages. Strikingly, we found that deletion of all 3 PI3K isoforms that are needed to transduce cytokine and growth factor signals results in a phenotype resembling human myelodysplastic syndrome (MDS). We are now using this PI3K triple knockout mouse model to study the mechanisms of MDS initiation and progression to acute myeloid leukemia (AML).

Roles of kinase of signaling pathways in blood malignancies

AML is a genetically diverse disease, but activation of the PI3K pathway has been reported in up to 80% of cases. We are using several different mouse models of AML to examine the roles of individual PI3K isoforms in leukemic stem cells (LSCs), which is the cell population in AML patients that has self-renewal activity, and has been implicated in relapse. In particular, we are exploring how PI3K cooperates with epigenetic regulators in LSCs, and how PI3K in LSCs can promote evasion of the immune system. We are also studying the roles of PI3K and other signaling molecules in the normal and leukemic bone marrow microenvironment.

In addition, we recently discovered that RON kinase can be a new potential therapeutic target in a different blood cancer called myeloproliferative neoplasms (MPN). Future work will focus on understanding the mechanisms of RON signaling and regulation in MPN cells, and on strategies for therapeutic targeting of RON in this disease.

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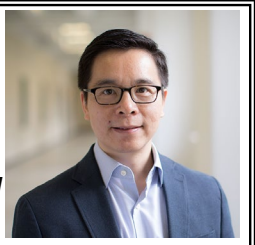

Key Words: ***mammary stem/progenitor cells, breast cancer stem cells, drug resistance, metastasis***

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 stem cell plasticity in tumorigenesis

Using in vitro organoid culture and in vivo lineage tracing strategies, we have identified novel unipotent stem/progenitor cells that are responsible for the development of distinct mammary epithelial cell lineages and may serve as origins of distinct breast cancer subtypes. We have also uncovered a crucial role of de-differentiation of these unipotent cells to multipotent stem-like cells in breast cancer malignant progression. Our ongoing work focuses on determining the mechanisms of de-differentiation to multipotency, dissecting the interaction of de-differentiated stem-like cells with the host immune system, developing strategies for targeting these pre-cancer stem cells.

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 regulators in controlling metastatic colonization, a rate-limiting step of the metastatic cascade that involves cancer stem cells. We recently identified a key role of epigenetic regulator MLL3 mutation in enhancing stem cell activity and promoting the formation of aggressive cancer cells with hybrid epithelial-mesenchymal characteristics. In addition, we are interested in how cancer stem cells are regulated by and interact with the tumor immune microenvironment.

Selected Publications

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Key Words: Hematopoietic stem cell, Mitochondria, Leukemia, Myelodysplastic syndrome, Sickle cell disease

The central research goal of the Ito Lab is the expansion of our understanding of the regulatory pathways **controlling the equilibrium of healthy and malignant hematopoietic stem cells**. At the core of our work is the process of stem cell division, and the resulting balance between **self-renewal and differentiation**, which directly impacts tissue homeostasis. We are also devoting increased attention to targeting **cellular metabolism** and/or **mitochondrial physiology** as a therapeutic strategy and are cutting 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 **single cell approaches** and **live imaging** to **track stem cell fate** *in vivo* and **leukocyte behavior** in **sickle cell disease** animal models will 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**.

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Molecular Mechanisms of Virus Entry and Exit.

For more information, please see our lab homepage:

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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 an important 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.

Complete List of Published Work:
https://www.ncbi.nlm.nih.gov/sites/myncbi/margaret.kielian.1/bibliography/45469240/public/?sort=date&direction=descending

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Cell Death and Mitochondrial Biology: Fundamental Mechanisms and Roles in Human Disease

The most basic decision that any cell makes is to proliferate/hypertrophy, differentiate, or die. Our laboratory studies fundamental mechanisms of cell death and the roles of cell death in normal biology and human disease. At least a dozen cell death programs have been recognized to date (Cell Death Differ, 2018; Physiol Rev, 2019). Little is understood about how they interconnect mechanistically or functionally to bring about cell death. *The overarching goal of our work is to create a unified model of cell death that integrates seemingly "individual" cell death programs*. Our hypothesis is that "individual" cell death programs, which often undergo activation in the same cell, are components of an integrated cell death response that has arisen over evolution. Thus far, we have elucidated basic aspects of cell death biology including mechanisms that mediate apoptosis and regulated forms of necrosis, molecular interconnections among these programs, and connections with other aspects of mitochondrial biology (Mol Cell, 2004; PNAS, 2007; JBC, 2007; PNAS, 2012; Cell Death Differ, 2014; Nature, 2016; Science, 2018; Nat Commun, 2022).

Although our primary focus is basic mechanisms, we are also interested in translational implications. While some of our work has involved models of cancer (Cell Death Differ, 2005; JBC, 2010; Cancer Res, 2011, PLOS One, 2015) and diabetes (Diabetes, 2013; Sci Rep, 2017; Dev Cell, 2021), our primary translational focus is heart disease, the major cause of mortality worldwide. A number of years ago, our lab provided the first evidence that regulated forms of cell death play critical roles in the pathogenesis of myocardial infarction ("heart attack") (Circulation, 2000; JMCC, 2000; AJP, 2003) and heart failure (JCI, 2003). More recently, the lab has contributed to the development of small molecule and peptide drug prototypes that modulate cell death and may provide the basis for new therapies for heart diseases and other conditions (Nat Chem Biol, 2019; Nat Cancer, 2020). We continue to use cell and *in vivo* myocardial infarction models in our current work aimed at unifying cell death mechanisms because at least 7 cell death programs are activated in this syndrome.

Current projects

1. A novel platform for caspase-9 activation to bring about necrosis – not apoptosis
   We have identified a new molecular platform – distinct from the apoptosome – for caspase-9 activation, which directs caspase-9 to induce necrosis rather than apoptosis. This pathway appears to operate in a variety of cell types as well as during myocardial infarction *in vivo*. We are currently characterizing the activating complex structurally (manuscript in preparation). In addition, much future works remains including identification of what we believe are endogenous danger-associated molecular patterns that trigger the assembly of the complex and delineation of the downstream signaling pathway.
2. Role of necroptosis in myocardial infarction
   Necroptosis, a regulated form of necrosis defined by RIPK3 → MLKL signaling, is important in cardiac damage during myocardial infarction. In many paradigms, RIPK3 is activated by RIPK1. However, we believe that, during myocardial infarction, RIPK3 activation is triggered by the presence of double stranded nucleic acids in the Z-conformation in the cytoplasm. We are currently delineating this pathway, which we suspect may be a prototype for roles of Z-nucleic acids in other aspects of cell signaling. We are also developing a novel small molecule RIPK3 inhibitor as a potential therapeutic in myocardial infarction.
3. Function of the mitochondrial ATP synthase *in vivo*
   The mitochondrial ATP synthase catalyzes the generation of >90% of ATP in eukaryotic cells, but its necessity for maintaining cellular energetics has not been defined in an intact organism. We devised an *in vivo* strategy to progressively deplete the entire 28-subunit mitochondrial ATP synthase monomer, and dimers thereof, in heart muscle cells, a cell type chosen because of its high energetic demands. Unexpectedly, mice whose heart muscle cells contain 10-20% of the monomeric complex and 2% of dimers remain clinically indistinguishable from controls for weeks, retain normal cardiac function, and

exhibit only moderate exercise deficits. Cardiac mitochondrial ATP synthesis rates and concentrations are reduced ~50%, roughly equivalent to those in end-stage human heart failure. Following this period of compensation, all mice transition somewhat precipitously to heart failure and die, but remarkably this occurs without further decreases in ATP synthesis rates and concentrations. Experiments are currently underway to understand the compensatory changes that allow mice to cope with such marked depletion of the mitochondrial ATP synthase in an energetically demanding cell type and to delineate the mechanisms that mediate the transition to heart failure.

A second part of this work has been to test a hypothesis that has been advanced by others that the mitochondrial ATP synthase serves a second function as a mitochondrial permeability transition pore, a channel in the inner mitochondrial membrane that mediates necrotic cell death. Our data in isolated mitochondria, cells, and intact mice demonstrate that, rather than serving as the mitochondrial permeability transition pore, the mitochondrial ATP synthase is in fact a negative regulator of this pore (manuscript in revision at PNAS).

4. Interrelationships between cellular senescence and cell death in aging
   We have performed a genome-wide CRISPR screen to identify proteins that direct cells under stress toward a senescent versus cell death fate. We are currently studying these molecules in models of senescence relevant to aging, including in the heart.
5. ER-mitochondrial relationships in regulating necrotic cell death and metabolism
   We have previously created novel peptides and small molecules that conformationally active or inhibit mitofusins (MFNs) (*Nature*, 2016; *Science*, 2018), which are large GTPases involved in mitochondrial fusion and ER-mitochondrial tethering. We are now using these reagents to perturb the physical distance and extent of overlap between ER and mitochondria. We have used MFN inhibitors to limit ER-mitochondrial interactions resulting in decreases in infarct size during myocardial infarction, effects we hypothesize are mediated by decreases in ER to mitochondria Ca^{2+} transfer (manuscript in preparation). We are also attempting to do the opposite: augment ER-mitochondrial interactions and Ca^{2+} transfer to boost myocardial metabolism during heart failure.
6. Regulation of ferroptosis by OPA1
   We have discovered that OPA1 markedly sensitizes cells to ferroptosis, an iron-dependent form of necrotic cell death mediated by hydroperoxidation of phospholipids in cellular membranes. Unexpectedly, this sensitization to ferroptosis is independent of the ability of OPA1 to promote mitochondrial fusion. Rather, by maintaining mitochondrial homeostasis and function, OPA1 stimulates the generation of mitochondrial lipid ROS and suppresses an ATF4-mediated integrated stress response that would otherwise upregulate mechanisms that inhibit ferroptosis (manuscript in review at Mol Cell). This axis has potential implications for several diseases.

Link to publications:
https://pubmed.ncbi.nlm.nih.gov/?term=kitsis+rn&sort=date

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Gene regulatory mechanisms that mediate lung cancer evolution

Lung adenocarcinoma (LUAD) is the most common subtype of non-small cell lung cancer and remains a leading cause of cancer-related death. Next-generation sequencing studies have identified a number of recurrent genetic drivers (such as mutations in *KRAS* and *TP53*); however, this somatic variation does not fully explain key features of cancer evolution including progression toward metastasis and intratumoral heterogeneity. Our group aims to understand how non-genetic mechanisms contribute to lung cancer initiation and progression through the lens of chromatin biology.

In genetically engineered mouse modeling studies, cell-type specific transformation of alveolar type II (AT2) cells with $Kras^{G12D}$ and loss of *Tp53* lead to a natural cancer evolution process that recapitulates phenotypic hallmarks of human disease. Using this model, our recent work employed single-cell epigenomics to study dysregulation of chromatin state across lung cancer evolution. We identified a continuum of heterogeneous chromatin states with reproducible epigenomic features across individual lung tumors, suggesting conserved routes of cancer progression. Using both functional and computational approaches, we annotated transcription factor (TF) regulators and downstream gene programs associated with these diverse chromatin profiles. These discernable gene regulatory modules were linked to certain aspects of cancer progression including loss of cellular identity, inflammation, and metastasis. Our work also identified the emergence of a pre-metastatic gene regulatory program that arises in a rare cell population in primary tumors and primes cells for metastatic seeding. We identified RUNX2 as an important regulator of this cell state and are developing new models to study the role of RUNX TF biology in lung cancer progression.

Cellular identity is dictated by chromatin structure, which is maintained by a complex network of *cis*- (e.g., enhancer, promoters, and insulators), and *trans*- (e.g., chromatin modifiers, TFs, and adaptors) factors. Our previous work found that early stages of LUAD progression are associated with the loss of AT2 identity and the acquisition of epigenomic states similar to developmentally related cell types. We seek to further understand how lung cancer initiation ultimately leads to decoupling from an AT2 cell identity and increased epigenomic plasticity. In addition, we are interested in investigating how disruption of the tumor microenvironment influences LUAD cell state. To address these questions, we pair lung cancer modeling with epigenomic technologies (including single-cell ATAC-sequencing, single-cell multiomic analysis, and spatial approaches) to dissect mechanisms important for the maintenance and persistence of dysregulated cell states in lung cancer. We flexibly utilize genetically engineered mouse models, alveolar human and murine organoids, and cell-based assays to perform functional studies. We continue to expand our technological and modeling toolkit to study early and late stages of lung cancer progression with the overarching goal of better understanding and targeting lung cancer progression.

Research directions in the lab:

1. Perturbation of candidate chromatin regulators associated with aberrant cell states in lung cancer
2. Interrogation of mechanisms that drive epigenomic plasticity and cell identity shifts in LUAD
3. Investigation of the role of the tumor microenvironment and loss of tissue homeostasis in shaping cell state in lung cancer cells
4. Utilization of varied single-cell epigenomic technologies to decipher gene regulatory networks in normal lung and lung cancer cells

Selected Publications:

*Co-first; ^Co-corresponding authors

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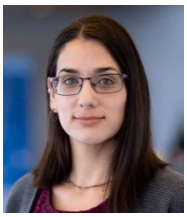

Mechanisms that regulate maintenance of niches for hematopoietic stem cells in aging and cancer.

Life-long production of blood depends on the ability of hematopoietic stem cells (HSCs) to self-renew, differentiate, and form all blood cell lineages. HSCs rely upon a tight interaction with their microenvironment in the bone marrow (also termed the "niche") to preserve quiescence and maintain normal blood output. These interactions are based upon membrane-bound, locally secreted, and/or long-range signals produced by a complex network of blood vessels, sympathetic nerve fibers, mesenchymal stem cells (MSCs), stromal cells, and hematopoietic cells. Aging of the hematopoietic system is associated with an age-dependent decline in HSC function leading to myeloid cell expansion and a reduction in lymphoid output that contribute to the development of myeloid malignancies. Many advances have been made toward deciphering intrinsic mechanisms that control HSC aging. However, the dependence of HSC dysfunction on the aging microenvironment remains underexplored.

Our interest is to understand mechanisms that control niche aging and contribute to the remodeling of niches that support myeloid malignancy and leukemic stem cells (LSCs). We use rigorous genetic models that target different niche constituents combined with innovative 3D imaging technology to detect endogenous HSC distribution in their native microenvironment. These approaches will provide novel insights into the molecular mechanisms underlying age-dependent stem cell dysfunction and identify factors that can be targeted to develop novel therapies to rejuvenate stem cell niches.

Primary interests of the lab:

1. Sympathetic nerves and adrenergic signals as regulators of niche homeostasis. Our previous work established that signals from the sympathetic nervous system (SNS) control hematopoietic aging and highlight niche-derived factors as critical regulators of HSC longevity, rejuvenation of which can directly benefit aged stem cells (*Maryanovich et al., Nature Medicine 2018*). Future efforts are oriented toward identifying targets downstream of the SNS that control niche and HSC aging.

2. Reactive oxygen species (ROS) role in remodeling niches to support myeloid malignancies and leukemic stem cells (LSCs). Myeloid malignancies elicit alterations to the HSC niche that promote oncogenic expansion and eradication of healthy stem cells. The SNS was shown to be protective against myeloproliferation, as sympathetic denervation or deletion of β- adrenoreceptors accelerated the development of myeloproliferative disease (*Hanoun et al., Cell Stem Cell 2014*). Our work has shown that SNS signals control homeostasis of niche-derived ROS and that leukemic progression favors high oxidative stress, suggesting that SNS signals may mitigate ROS levels in the BM microenvironment to protect HSC niches during leukemogenesis.

3. HSC niche metabolism and its role during aging and leukemogenesis. Our goal is to determine how aging and leukemogenesis affect the metabolic activity of niche constituents and perturb their ability to support hematopoiesis. We will investigate the role of the SNS in controlling

niche cell metabolism and whether disruption of metabolic activity in the niche can deteriorate both its structure and function to facilitate hematopoietic aging and precondition niches to support leukemic infiltration. We will focus on glucose metabolism, fatty acid metabolism, and mitochondrial oxidative phosphorylation, pathways shown to be critical for stem cell maintenance.

Selected Publications:

Pinho S, Wei Q, **Maryanovich M**, Zhang D, Pierce H, Nakahara F, Di Staulo A, Bartholdy B, Xu J, Borger D, Verma A and Frenette PS. VCAM1 confers innate immune tolerance on haematopoietic and leukaemic stem cells. **Nature Cell Biology**. 2022

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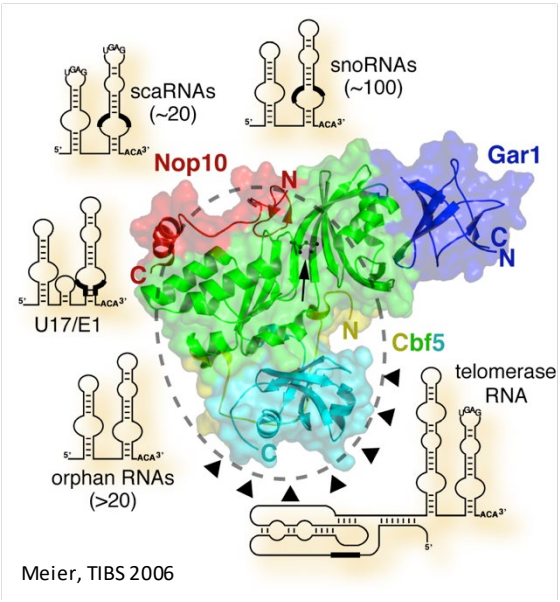
RIBONUCLEOPROTEINS IN CANCER AND HUMAN REPRODUCTION

Our group is studying the mechanism and regulation of nucleolar ribonucleoprotein biogenesis in relation to genetic disease, cancer, and human reproduction. Presently we are pursuing two main areas of research:



First, we are analyzing the biogenesis and function of small nucleolar ribonucleoproteins (snoRNPs) of the H/ACA class and how minor deviations from their natural assembly pathway can lead to cancer and bone marrow failure. Human H/ACA ribonucleoproteins are important for many basic cellular processes including protein synthesis, pre-mRNA splicing, and genome integrity. The different functional classes of H/ACA RNPs isomerize some 130 uridines to pseudouridines in ribosomal (r) and spliceosomal small nuclear (sn) RNAs, process rRNA, stabilize telomerase RNA, yield microRNAs, and harbor yet to be determined roles. Each of these functions is specified by one of over 500 H/ACA RNAs, each of which associates with the same four core proteins to form an H/ACA RNP. The central core protein, NAP57 (aka dyskerin or in yeast Cbf5p), is mutated in the predominant X-linked form of the inherited bone marrow failure syndrome dyskeratosis congenita (DC). NAP57 is an oncogenic protein, whereas the H/ACA RNP-specific assembly factor SHQ1 is a tumor suppressor of prostate and other cancers. We identify how disease causing and oncogenic point mutations in these proteins perturb H/ACA RNP assembly and how it can lead to an imbalance in cellular protein expression and genome instability. Among other cell biological approaches, we are employing CRISPR/Cas9 and RNAseq technology to get to the bottom of this. We are further collaborating with structural biologists and clinical scientists.

Second, we investigate the function of nucleolar channel systems (NCSs) in the cell and in human reproduction. During the height of receptivity of each menstrual cycle, NCSs transiently develop in the nuclei of endometrial epithelial cells (EECs). They are implicated in the preparation of the endometrium for uterine attachment of the fertilized egg. Although the molecular mechanisms of embryo implantation in humans are poorly understood, NCSs remain unexplored as candidate markers or potential prerequisites for implantation. This can be attributed to the fact that, despite their discovery over 50 years ago, until recently identification of NCSs was limited to electron microscopy. We identified molecular markers of NCSs, which finally affords simple and quantitative detection of these organelles at the light microscopic level. We are now exploiting our discovery to understand the cellular biology of NCSs and their regulation and function in uterine biology. To boost success rates of embryo transfer during in vitro fertilization cycles, we apply our technology for timing endometrial receptivity before embryo transfer by NCS detection in uterine secretions.

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Genetics of hematopoietic stem cell (HSC) clonal dynamics and leukemia predisposition

Every day, each one of us produce hundreds of billions of specialized blood cells through a process of hematopoietic differentiation originating from a pool of long-lived hematopoietic stem cells (HSCs). As we age, some HSCs acquire mutations that causes them and their progeny to expand resulting in the formation of their own distinct "clones". This condition known as clonal hematopoiesis is ubiquitous with advancing age affecting 10%-20% of population by the age of 70 years. Individuals with clonal hematopoiesis have an increased risk hematological malignancies such as leukemia and myelodysplastic syndromes. If we could identify and prevent such clones from expanding this can dramatically reduce risk for blood cancers. Despite its importance, we still don't understand why these clones emerge only in some individuals and why only a small subset of such carriers' eventually progresses to cancer?

Our laboratory leverages human genetic studies such as genome-wide association studies (GWAS) and studies on rare families to address these questions. Our goal is to identify and experimentally study germline risk variants that predispose individuals to clonal hematopoiesis and myeloid malignancies. Our recent work has uncovered a previously unappreciated mechanism by which inherited genetic variants influence clonal blood disorders by modulation of HSC function and self-renewal (Bao et al., *Nature* 2020; Bick et al., *Nature* 2020).

We routinely model genetic risk variants into primary human hematopoietic stem and progenitor cells using CRISPR/Cas9 approaches and study their impact on stem cell function and clonal expansion of mutant HSCs. We are developing engineered mouse models to study mechanisms of blood cancer predisposition. Since the majority of these genetic variants are in noncoding genomic regions, we are employing high throughput approaches that we have developed to systematically test their effects on transcriptional activity (Ulirsch et al., *Cell* 2016). Our studies will provide critical insights into how germline variants influence the emergence of clonal hematopoiesis and its progression to hematological malignancies.

Research Directions

1) Mechanisms of germline predisposition to clonal hematopoiesis and leukemia.
2) Investigation of the role of interactions between germline and somatic variants in shaping hematopoietic stem cell clonal fitness.
3) Genome engineering of human normal, preleukemic and leukemic hematopoietic stem and progenitor cells using CRISPR/Cas9.
4) Application of multiplexed functional assays and genetic screens to prioritize germline genetic variants underlying clonal blood disorders.

Selected Publications

Bao EL*, **Nandakumar SK***, Liao X*, Bick A, Karjalainen J, Tabaka M, Gan OI, Havulinna A, Kiiskinen TT, Lareau CA, de Lapuente Portilla AL, Li B, Emdin C, Codd V, Nelson CP, Walker CJ, Churchhouse C, de la Chapelle A,

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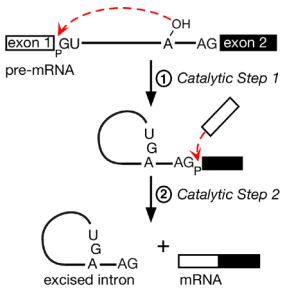
RNA Processing – Mechanisms and Disease

Intron removal, a defining feature of eukaryotic cell biology, is catalyzed in two chemical steps by the spliceosome, a 60S complex composed of 5 snRNAs and >100 proteins. We study spliceosome function and spliceosomal mutations.

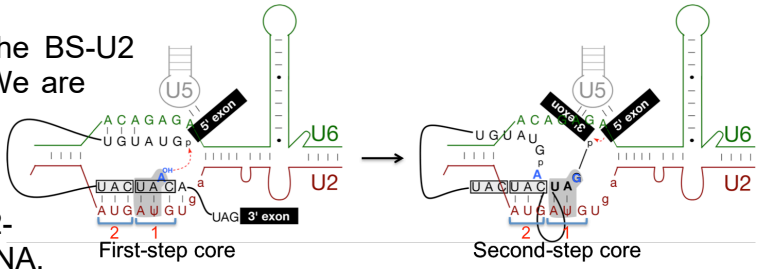


Do variant snRNAs make variant spliceosomes? One 'black hole' in RNA biology is the identification of 50-300nt RNAs, missing in modern-day sequencing datasets. Vertebrate genomes reveal hundreds of snRNA gene loci; with few exceptions, only the most abundant canonical snRNAs have been investigated. We found that expression of variant-snRNAs changes greatly during development. We are investigating how this impacts spliceosome function.

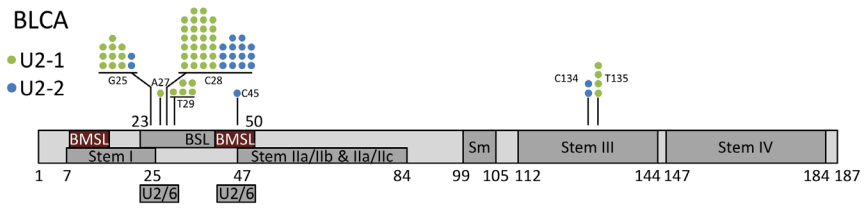
snRNA	Mature Length	Annotated Loci
U1	163	143
U2	190	70
U4	143	91
U5	116	32
U6	106	1333
U4atac	127	18
U6atac	126	41

Catalytic center interactions. RNA–RNA base pairing of U2 snRNA to the intron branch site (BS) is essential for spliceosome assembly and first-step catalysis. We developed an orthogonal system, wherein a second-copy U2 with grossly substituted BS-U2 base-pairs mediates splicing of a cognate reporter gene. This produces a non-essential second spliceosome that can be characterized in vivo. These properties allowed us to test whether the BS-U2 duplex exists at the time of first-step catalysis. (It does.)

What is the second-step catalytic core? The BS-U2 duplex does not remain for the second step. We are using our orthogonal systems to elucidate a 3'SS binding site within the second-step core, demonstrating that the first-step branch structure becomes partially unpaired from U2-GUAGUA, allowing the 3'SS to bind on U2 snRNA.



Splicing and disease. U2 snRNP protein SF3b1 and other U2 proteins are highly mutated in myelodysplastic syndromes, AML, and other cancers. However, there has been no information regarding U2 snRNA mutations. Our analysis of U2 variant loci and novel bioinformatic pipelines allowed us to interrogate U2 RNA mutations in >1000 whole genome sequences (WGS) from the Cancer Genome Atlas (TCGA). We identified mutations in both U2-1 and U2-2 snRNAs, within the branch-recognition stem-loop (BSL), recurrent in bladder cancer (BLCA); we identified the same mutations in bladder cancer organoid lines (Lee et al., Cell 2018). The BSL contributes to recognition of the branch site as well as 3'SS. Genetic screens in yeast for improved splicing of non-consensus 3'SS yielded the same BSL mutations. Using CRISPR-Cas9 edited human cell lines, we are investigating molecular consequences of these U2 mutations and the mechanism of resultant splicing changes.



BSL = branch recognition stem-loop

BMSL = branch helix-mimicking stem-loop: Wojtek Galej, Science 2022

Recent Publications (selected)

Maron, M.I., Casill, ,A.D., Gupta, V., Roth, J.S., Sidoli, S., Query, C.C., Gamble, M.J., and Shechter, D. (2022). Type I and II PRMTs inversely regulate post-transcriptional intron detention through Sm and CHTOP methylation. ***Elife***: e72867. doi: 10.7554/eLife.72867. PMID34984976 PMC8765754

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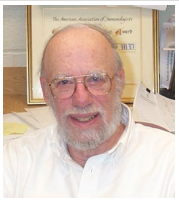

Our laboratory is studying how antibody-forming cells respond to antigen by undergoing somatic hypermutation and class switch recombination so that they can produce higher affinity antibodies with more useful effector functions. The molecular and biochemical mechanisms of antibody variable region hypermutation 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 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 how changes in the DNA sequence of antibody genes, chromatin structure and transcriptional elongation lead to the targeting and regulation of AID. In collaboration with Dr. Thomas MacCarthy at Stony Brook, the role and evolution of the antibody V region sequence is also analyzed computationally using data bases of the human antibody response. The highly mutagenic processes required to generate antibody diversity also leads to B cell lymphomas and we are trying to understand how AID contributes to human Chronic Lymphocytic Leukemia in collaboration with Dr. Nicholas Choirazzi from the Feinstein Institute at the Northwell Health Center.

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

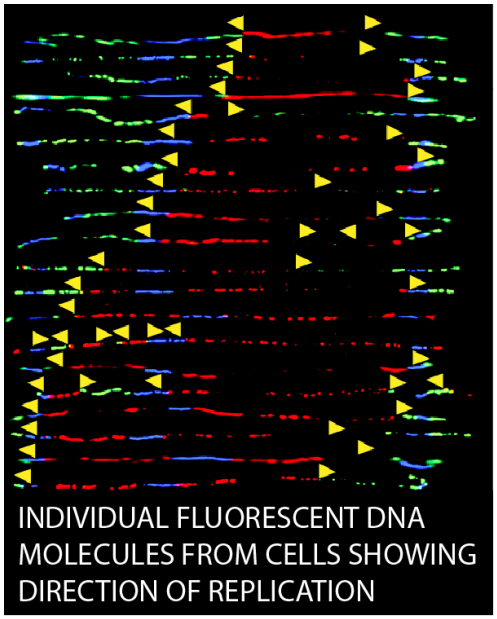
A major interest of our lab is the organization and regulation of the DNA replication program in mammalian cells and its effect on gene expression. One area we have been concentrating on the role of DNA replication in neuronal diseases such as Alzheimer's. Alzheimer's disease (AD) is a neuro-degenerative disease that has been studied for more than three decades. Surprisingly after all this effort, there is still no unequivocal treatment, and the cause is not known.

We are also focused on understanding the role of genomic instability at human chromosomal fragile sites in cancer and aging.

Genome instability occurs in the early development of many cancers and other diseases and is thought to be caused by replication fork stalling. This is clinically relevant since most current cancer therapies are based on radiation or chemotherapeutic agents that stall or block forks. Manipulating cellular responses to fork stalling may therefore prove crucial in improving effectiveness of current chemotherapies.

Long term interests:

• Difficult to replicate regions accumulate secondary structures which, if not properly resolved, may block DNA replication fork movement and lead to genome instability. We are studying mechanisms to resolve these barriers for efficient and faithful DNA replication.• The role of DNA replication of genes involved in neurological diseases such as Alzheimer's. We will examine critical genes in the brain that undergo mutations with aging.
• Genome protection by telomeres.
• Aneuploidy leading to infertility and pregnancy loss.
• Mechanisms leading to breaks at sites that result in chromosomal rearrangements frequently detected in cancer cells.



INDIVIDUAL FLUORESCENT DNA MOLECULES FROM CELLS SHOWING DIRECTION OF REPLICATION

Current projects include a wide range of interests:

• The replication of two repeated sequence DNAs that are involved in Alzheimer's disease: alpha satellite DNA and the ribosomal DNA genes.
• The DNA2 nuclease/helicase can cleave G-quadruplex structures which are one of the most common non-B structures present in DNA. We study the effect on the deletion of DNA2 on DNA replication.
• Replication of the alpha satellite DNA at centromeres of specific human chromosomes. These include chromosome 21 and the Y chromosome. The Y chromosome is lost with aging, and we are studying the

replication of this chromosome to understand more about this loss. Chr 21 undergoes trisomy in Down syndrome and people with Down syndrome have a higher risk of getting Alzheimer's symptoms.

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FOLLOWING THE TRAVELS OF RNA

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.

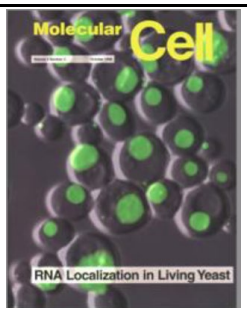
Our new technology, based on in situ hybridization allows us to visualize specific nucleic acid sequences within individual cells. Synthetic nucleic acid probes are labeled with fluorochromes. Subsequently these molecules are hybridized to the cell and detected using high resolution digital imaging microscopy.

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

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.



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

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. We have labelled a number of genes in mice that are important in neuronal function and can image their mRNAs in cultured neurons and in brain tissue. The imaging reveals a complicated pathway for the mRNAs to arrive at sites of synaptic contact and translate in order to consolidate proteins into local “hotspots”. This represents the molecular basis of memory formation.

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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.

Selected Publications:

Willcockson, M. A., S. E. Healton, C. N. Weiss, B. A. Bartholdy, Y. Botbol, L. N. Mishra, D. S. Sidhwani, T. J. Wilson, H. B. Pinto, M. I. Maron, K. A. Skalina, L. N. Toro, J. Zhao, C. H. Lee, H. Hou, N. Yusufova, C. Meydan, A. Osunsade, Y. David, E. Cesarman, A. M. Melnick, S. Sidoli, B. A. Garcia, W. Edelmann, F. Macian and A. I. Skoultchi (2021). "H1 histones control the epigenetic landscape by local chromatin compaction." Nature 589(7841): 293-298.

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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 the cell surface 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, 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 a neighboring 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. Using a panel of different CHO glycosylation mutants developed in this lab, we showed that inhibition of Notch signaling by the Fringe glycosyltransferase requires the addition of a Gal residue to O-fucose glycans on Notch. 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 now exploring its functions in the regulation of Notch signaling in mammals.

We are also investigating roles for glycans in reproduction. We have found that MGAT1 and complex N-glycans are essential for spermatogenesis and male fertility, and we are testing the hypothesis that they play an important role in spermatid/Sertoli cell interactions. Small molecule inhibitors are being sought using an in silico docking screen of ~1 million compounds. Candidates will be tested in cell-based assays and ultimately in mice for contraceptive effects. We have shown that deletion of the glycoprotein basigin (CD147) in spermatogonia gives rise to a similar block in spermatogenesis and are investigating whether the N-glycans on basigin are responsible for that phenotype. We are also examining the consequences of deleting MGAT2 in spermatogonia to allow hybrid but not complex N-glycan synthesis.

Finally, Chinese hamster ovary (CHO) cell glycosylation mutants developed in this laboratory are used by us and many academic and biotech laboratories for a variety of purposes including to develop new methods such as a novel approach to tracking glycan epitopes on the cell surface, a method to analyse glycans expressed using scRNA-seq termed sgRNA-seq, assays for Notch and growth factor signaling, and assays to characterize glycosyation mutations that cause rare congenital disorders of glycosylation (CDGs).

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

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Molecular Regulation and Therapeutic Targeting 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 HSC and committed progenitors. Cell-intrinsic as well as cell-extrinsic aberrations give rise to a highly diverse pool of 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, heterogeneity, and regulatory mechanisms governing pre-LSC and their subclonal diversity, dynamics, and 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, progression, and therapeutic resistance of pre-LSC and LSC. To identify and functionally study implicated pathways we are utilizing stem and progenitor cell subsets isolated by means of multi-parameter high-speed fluorescence-activated cell sorting (FACS). We are using experimental tools including at the single-molecule and single-cell level, as well as molecular biological methods for forced expression or inactivation of molecular targets, 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 mechanisms in normal and leukemic stem cells. We are studying murine genetic models as well as primary human samples from patients with pre-leukemic conditions and 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 governing normal and malignant hematopoiesis, including stem cell subclonal heterogeneity, dynamics, and competition (focusing on transcription and signaling, incl. single-molecule and single-cell studies)
- Development and preclinical testing of novel therapeutics targeting aberrant stem cells
- Translational computational biology (e.g. integrated analysis of WGS, scRNA-seq, ChIP-seq, CUT&TAG data etc.; including from longitudinally sampled, sorted / single stem cells from patients and mouse genetic models)

Selected publications:

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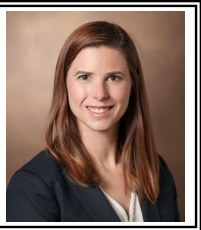
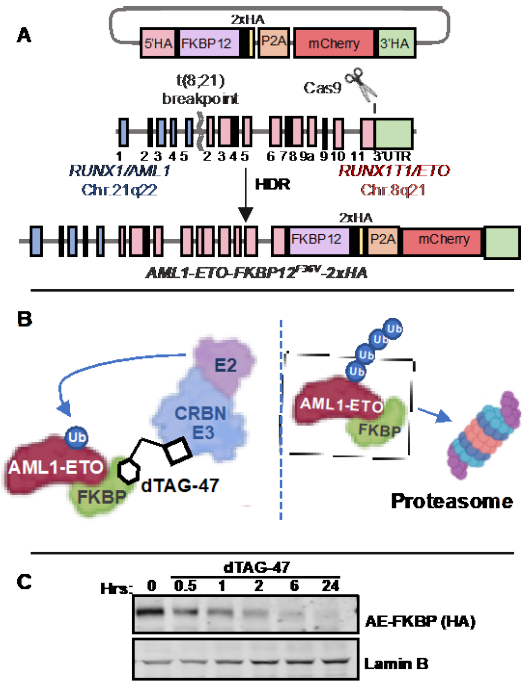


Using CRISPR-mediated Chemical Genetics to Define Oncogenic Transcription Networks



Degron tags promote rapid degradation of endogenous proteins. (A) The strategy for editing the AML1-ETO locus utilizes Cas9, gRNA targeting the last exon, and an HDR plasmid that will integrate an FKBP-based degron tag, 2xHA, and an mCherry selectable marker. (B) The resulting AML1-ETO-FKBP12^{F36V} is then sensitive to the bifunctional small molecule, dTAG-47. One half of the molecule binds the the FKBP tag and the second half binds and recruits the CRBN E3 ubiquitin ligase, leading to AML1-ETO degradation. (C) dTAG-47 treatment results in the rapid degradation of the endogenous AML1-ETO protein.

The disruption of normal gene expression programs is a hallmark of all cancers. One common way in which this occurs is through chromosomal translocation that results in the generation of gain-of-function transcription factor fusion proteins. For example, PAX3-FOXO1, arises from the t(2;13) translocation, and is the defining feature of a subset of highly aggressive, pediatric rhabdomyosarcoma (RMS), while AML1-ETO arises from the t(8;21) translocation in acute myeloid leukemia (AML). Both of these translocations target transcription factors that regulate critical cell fate decisions, and both are thought to be the initiating event and an ideal therapeutic target in the cancer in which they arise. Thus, it is essential to understand how these oncogenic transcription factors alter transcriptional programs to drive cancer development.

Historically, our ability to understand how sequence-specific transcription factors rapidly and specifically alter transcriptional programs has been limited by a toolbox of very slow genetic and knockdown strategies that take days to weeks before transcription factor activity can be assayed. Therefore, while direct transcriptional effects occur within minutes to hours, these models take days to establish, which results in the detection of secondary and/or compensatory transcriptional changes that often mask the direct/immediate effects of transcription factor disruption. In order to overcome these technical limitations, we use CRISPR-mediated genome editing to introduce degron tags into endogenous transcription factor loci. This chemical-genetic approach allows rapid transcription factor degradation (minutes to hours) following PROTAC (e.g., dTAG-47, see figure above) treatment, and effectively collapses the timeframe for assaying transcriptional changes, chromatin states, and genome-wide transcription factor occupancy from days to hours. We also incorporate proteomics-based methods to identify associated protein complexes and cooperating transcription factors. Combined, these approaches are allowing us to define the mechanism of action of specific oncogenic transcription factors (e.g., AML1-ETO and PAX3-FOXO1). Moreover, we aim to address fundamental questions in the transcription field including how oncogenic transcription factors are influenced by and exert influence over the chromatin landscape, how multiple sequence-specific transcription factors cooperate to fine-tune gene expression, and how enhancer activity functions to control gene transcription.

Selected Publications:

1. Layden HM, Eleuteri NA, Hiebert SW, **Stengel KR**. (2021) A protocol for rapid degradation of endogenous transcription factors in mammalian cells and identification of direct regulatory targets. *STAR Protoc.* 2(2): 100530. PMID: 34041503.

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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. 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 HSC, 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.

Identification of novel molecular safeguards of adult and cancer stem cells

Teamed-up with Dr. Ana Maria Cuervo (Dept. of Developmental & Molecular Biology), leader in highly precise protein degradation and aging biology, we have recently discovered an important role for chaperone-mediated autophagy in the maintenance of functional HSC (*Dong et al., Nature 2021*). Current efforts investigate the role of this and other stress-related molecular defense mechanisms in leukemic stem cell evolution and maintenance.

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 have been investigating the precise molecular mechanism of action, particularly focusing on metabolic and non-enzymatic molecular pathways relying on iron (*Kao et al. bioRxiv 2021*) – 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) how PU.1 gene expression networks functionally cooperate with age-associated inactivation of epigenetic regulators, e.g. TET2 (*Aivalioti et al., Blood Cancer Disc 2022*) and DNMT3A.

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 (*Will et al., Blood 2012*; *Kao et al., STM 2018; Shastri et al., JCI 2018, Okoye-Okafor et al., JEM 2022*).

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, and cancer genetics, and involves all aspects of tumor-host interactions. Thus, we constantly draw upon the most recent advances in these fields to address mechanism questions that are related to lymphoma initiation, development, and therapeutic response. As each lymphoma entity often relates to a specific B/T cell activation/differentiation state that is phenotypically "frozen" by the malignant transformation process, our research also provides valuable insights to the regulatory processes that govern the normal immune system. **Our studies have three major goals**: to better understand 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 particularly important stage in B cell development that is specifically evolved to support the production of high affinity antibodies and B cell memory. Dysregulated GC responses underlie the development of many types of B cell lymphomas and autoimmune diseases. 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. Mutated B cells undergo positive and negative selections through interactions with 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 a coordinated response to various extracellular and intracellular stimuli; yet the precise sequence and nature of these stimuli are incompletely understood.

Our previous studies have revealed important mechanisms that govern the expression and activity of BCL6, the master regulator of GC response, and demonstrated functional interactions between BCL6 and several cell signaling pathways including RhoA, NF-κB, and Jak/STAT3. In the context of diffuse large B-cell lymphomas (**DLBCL**), another focus of our investigation was on the IL-6/Jak/STAT3 signaling pathway. We have characterized expression regulation of STAT3, cause and consequences of its aberrant activity in DLBCL development and therapeutic response. The functional contribution of this pathway to normal plasma cell maturation was also investigated.

Since 2016, the major focus of our research program has switched to adult T-cell leukemia/lymphoma (**ATLL**), a disease of malignant CD4+ T cells that develops in 4-5% of individuals infected with the human T-cell lymphotropic virus 1 (**HTLV-1**). The medium survival of ATLL patients diagnosed in North America is only about 7 months, pointing to an urgent unmet need in the therapeutic space. Mainly because of the Bronx's immigrant makeup, the Montefiore Medical Center treats a sizable fraction of all ATLL cases in the U.S., and thus we have unique access to ATLL patient samples and unmatched opportunities to perform preclinical and clinical studies. We recently reported that ATLL patients diagnosed in North American (**NA-ATLL**) have a distinct genomic landscape compared to the Japanese cohort (**J-ATLL**). Specifically, NA-ATLL is

characterized by a much higher frequency of prognostic epigenetic mutations and is targetable preclinically with DNA de-methylation drugs. Ongoing studies in our laboratory support the concept that NA- and J-ATLL share similarities in clinical behavior and pathogenesis but also have clear distinctions in mutation pattern, cell cycle regulation, immunophenotype, and response to experimental therapeutics. Since host anti-HTLV-1/anti-ATLL immune response plays a key role in ATLL development, we have also begun to examine how host immunity, in particular $CD8^+$ cytotoxic response, is impacted during ATLL development. Our preliminary findings support the concept that for an ATLL treatment to be most effective, ATLL-induced immunosuppression must be overcome along with recruitment restoration of anti-ATLL $CD8^+$ T cell effector and memory responses. Looking forward, insights uncovered from our ongoing projects are expected to pave the way for novel pathway-targeted and immune-modulatory therapies for ATLL patients and possibly other types of T-cell malignancies.

We are currently pursuing the following research questions:

1. How does BCL6 contribute to the pathogenesis and cell identity of NA-ATLL?
2. What is the genetic and clonal evolution basis that may contribute to the differences between NA- and J-ATLL?
3. How do the ATLL cells suppress the function of normal T cells to achieve immune escape?

Selected Publications:

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