



## **Stromal Mutations: Cause and Consequence in Cancer Biology**

### ***Virtual Workshop***

#### Talk Abstracts

##### **Sheila Stewart, Washington University School of Medicine in St. Louis**

###### **Age-related stromal changes drive breast cancer**

Age is the single largest risk factor for the development of cancer, but how age impacts the molecular mechanisms that drive cancer remain poorly understood. While it is clear that age-related accumulation of cell autonomous mutations contributes to tumorigenesis, the central role age-related changes in the tumor microenvironment play in the transformation process is becoming more fully appreciated. Underscoring the importance of an aged microenvironment in cancer development are findings that senescent fibroblasts, which accumulate with age, directly stimulate preneoplastic and neoplastic cell growth and tumor progression. Investigations into how senescent fibroblasts promote tumorigenesis revealed that they express a plethora of growth factors, extracellular matrix remodeling enzymes, chemokines, and cytokines collectively referred to as the senescence associated secretory phenotype (SASP). To identify a role for senescent stromal cells in a spontaneous model of breast cancer, we used single cell RNA-Sequencing (scRNA-Seq) to interrogate the cell types and their functional status in the MMTV-PyMT breast tumor model. This analysis revealed the presence of senescent cancer associated fibroblasts (senCAFs) that we also identified in a human triple negative breast cancer scRNA-Seq database. To establish a role for senCAFs in breast cancer, we mated the PyMT model to the INKATTAC (INK) mouse that allowed us to inducibly eliminate senescent cells. We found that the elimination of senCAFs reduced tumor growth and unleashed the killing power of NK cells. We will discuss the mechanism by which senCAFs impact NK cell killing.

##### **Peter Adams, Sanford Burnham Prebys**

###### **The role of aged stroma/microenvironment in cancer initiation and progression**

Aging exerts a notable influence on incidence of liver cancer. Liver cancer incidence is extremely low until the age of 49 years, after which incidence rates begin to increase with the steepest increase between ages 60-69 years of age. The mechanistic underpinnings as to how aging affects liver cancer development remain unclear, necessitating focused research for better early detection and prevention for this aged population. Our hypothesis posits that age-driven changes render aged liver more susceptible to oncogenic stress and tumorigenesis. To investigate how the liver changes with age, we profiled the transcriptome and epigenome of healthy livers from both young and aged mice, revealing pronounced disruptions to these networks with aging. In aged hepatocytes, we identified heightened tumor suppressor, oncogene, and pro-inflammatory signaling, marked by elevated cytokine and IFN-stimulated gene (ISG) expression, at the bulk and single cell level. Employing adeno-associated virus (AAV) mediated transgene expression, we determined Stat1 is both necessary and sufficient for the elevated ISG expression in old wild type mice. To explore the influence of aging on the response to

oncogene activation, we used AAV-mediated delivery of activated Myc oncogene to young and aged mouse livers. Analysis of old liver expressing Myc unveiled altered transcriptomic profiles associated with loss of liver identity, an elevated tumorigenic index, and activation of specific signaling pathways, notably IFN-stimulated genes. We determined that this ISG upregulation is evident in numerous models of oncogenic stress and transformation in older mice and is also associated with MAFLD progression in human patients. Remarkably, inhibiting JAK/STAT signaling alongside Myc expression in aged mice led to high-grade hepatocyte dysplasia and tumor formation, suggesting an aged liver in a state of “precarious balance”, due to concurrent oncogenic and tumor suppressor pathways, but protected against neoplastic progression by IFN-signaling. Our findings highlight an age-driven vulnerability to cancer and suggest potential interventions to modulate the precarious balance of oncogenic and tumor suppressor pathways and cancer susceptibility in liver aging and MAFLD.

**Fuchou Tang, BIOPIC, Peking University, China**

### **Single-cell multiomics sequencing reveals prevalent genomic alterations in tumor microenvironment cells of human colorectal cancer**

To what extent stromal cells in the tumor microenvironment are transformed by colorectal cancer (CRC) cells is unexplored. To dissect alterations in these non-malignant cells, we performed single-cell multiomics sequencing of patients with microsatellite-stable CRCs and cancer-free, elderly individuals. We found that somatic copy number alterations (SCNAs) are prevalent in immune cells, fibroblasts, and endothelial cells in both the tumor microenvironment and the normal tissues of each individual. Moreover, the proportions of fibroblasts with SCNAs in tumors are much higher than those in adjacent normal tissues, with gain of chromosome 7 strongly enriched in the tumor microenvironment, clearly indicating clonal expansion. Furthermore, five genes (BGN, RCN3, TAGLN, MYL9, and TPM2) are identified as fibroblast-specific biomarkers of poorer prognosis of CRC. We also systematically analyzed point mutations in tumor microenvironment cells for CRC patients. Our study provides strong evidence and functional relevance of pervasive genomic alterations in the tumor microenvironment cells in CRC.

**Kornelia Polyak, Dana-Farber Cancer Institute, Harvard Medical School**

### **Stromal drivers of immune escape during breast tumor progression**

The tumor microenvironment shapes tumor evolution. The ductal carcinoma in situ (DCIS) to invasive breast carcinoma (IBC) transition is a key step of tumor progression, but the underlying mechanisms are still poorly defined. We have analyzed genetic, gene expression, epigenetic profiles of cells composing normal breast tissue, DCIS, and IBC and identified cell type-specific changes during tumor progression. Importantly, while we could not detect common progression stage-specific alterations in tumor epithelial cells, myoepithelial cells and cells composing the microenvironment showed consistent differences between DCIS and IDC. Specifically, we identified differences in T cells as the main distinguishing feature between DCIS and IBC and discovered a novel cycling regulatory T cell population (cycTreg) as the primary orchestrator of the immune suppressive environment during invasive progression. Our single cell transcriptomic profiles also revealed a Treg and cancer-associated fibroblast (CAF) crosstalk critical for Treg expansion and tumor growth. Multimodal analysis of patient cohorts demonstrated that cycling Treg predicts recurrence in low-grade DCIS and poor patient outcomes in IBC, and that increased CAF-immune-DCIS proximity is prognostic of progression to IBC. Our data highlight the role of microenvironmental, especially immune-related changes in driving breast tumor progression.

**Ken Lau, Vanderbilt University**

### **Tracking Clonality in tumors and their microenvironments**

Clonal expansion serves as a key indicator of functional significance within specific cell populations exhibiting genetic alterations. Clonal expansion is typically identified in tumor cells that present a significant level of mutations and is more difficult to detect in non-tumor cells. Using NSC-seq, a newly designed and validated multi-purpose single-cell CRISPR platform, we developed a synthetic barcode mutation approach to assess clonality *in vivo*, even in non-tumor cells, while incorporating assigned cell state information. We applied NSC-seq to demonstrate polyancestral initiation in colonic precancers, revealing their origins from multiple normal founders. We coupled this analysis to single-cell multi-omic

profiling data as part of the Human Tumor Atlas Network (HTAN) to demonstrate a 15-30% incidence of polyancestral colonic precancers. We also use NSC-seq to preliminarily assess clonal expansion of non-tumor cell populations to demonstrate the feasibility of this approach.

In addition, I will discuss a mechanistic story on how external stressors induce mutations in non-tumor cells. Inflammation is a major predisposing factor to tumorigenesis, and heat is a cardinal feature of inflammation. We show that heat-exposed Th1 cells selectively developed mitochondrial stress and DNA damage that activated Tp53 and STING pathways. While many died, surviving Th1 cells had increased mitochondrial mass and showed improved activity. Mechanistically, electron transport chain complex 1 (ETC1) was rapidly impaired under fever-range temperatures and this was specifically detrimental to Th1 cells. Furthermore, cells with elevated ETC1 signatures exhibited a higher mutation burden than those without. Thus, we demonstrate one external mechanism by which microenvironmental cells can selectively accumulate mutations in an inflammatory setting.

### **Jan van Deursen, Unity Biotechnology**

#### **Non-cell autonomous TME alterations from stromal cell mutations**

Cancer treatment options that cause genotoxicity have been known to activate the DNA damage response pathway, which induces p53-p21. This leads to cell-cycle arrest followed by cell death or senescence, both of which are key determinants of positive outcomes in cancer treatment. However, this response is not exclusive to cancer cells and can affect normal cells in the tumor environment and the patient's body as a whole. Such widespread activation of the p53-p21 axis in healthy tissues and organs raises questions about the potential impact on short- and long-term treatment outcomes. Recent findings indicate that elevated p21 causes Rb-hypophosphorylation, triggering significant changes in the transcriptional landscape. Of these, repression of E2F-regulated genes by hypophosphorylated RB is long-known to drive cell-cycle arrest, whereas its widespread activation of gene expression is a more recent discovery. The Rb-regulated genes activated in response to p21 induction are functionally highly diverse and include key drivers of immunoclearance and functions relevant to cell proliferation, survival, migration and adhesion, energy metabolism, angiogenesis, and the extracellular matrix. These functions are all implicated in tumor etiology and the development of age-related diseases. To gain a deeper understanding of the impact of genotoxic cancer treatments on cancer treatment outcomes, it will be important to manipulate the p53-p21 axis in innovative animal models. The upcoming workshop will explore this opportunity and delve into the underlying concepts to enable a more comprehensive understanding of cancer treatment options.

### **Alex Maslove, Albert Einstein College of Medicine**

#### **Single-molecule mutation analysis as a genome integrity measure**

In the intricate landscape of genomic research, especially within the contexts of cancer, aging, and age-related diseases, accurately identifying DNA mutations is of paramount importance. Single Molecule Mutation Sequencing (SMM-seq) stands out as a refined methodology that enhances our capability to detect somatic mutations and structural variants, significantly impacting the assessment of genome integrity. Being both cost-effective and adaptable, SMM-seq is ideal for large-scale studies aimed at assessing mutagenic exposures and the burden of somatic mutations across populations. Its significance is particularly pronounced in oncology, where a deep understanding of the mutation landscape is crucial for accurate cancer diagnosis and effective therapy. Additionally, as a biomarker for aging, SMM-seq illuminates the molecular underpinnings of the aging process—a factor closely linked with cancer progression. This technique contributes substantially to precision medicine, subtly yet significantly advancing our ability to develop preventive strategies and enhance our understanding of molecular dynamics. Through meticulous application and study, SMM-seq is poised to enrich our insights into cancer genetics and shape the future of healthcare and disease management.

**Cancer-associated but not cancer-specific: fibroblast reprogramming by mechanical environment**

Following tissue injury, various precursor cells – collectively called ‘fibroblasts’ are activated into so-called myofibroblasts to produce extracellular matrix and acquire smooth muscle features. Hallmark of this transition is the formation of contractile stress fibers which generate higher force upon incorporation of  $\alpha$ -smooth muscle actin ( $\alpha$ -SMA). Myofibroblast activation is part of our body’s normal response to injury and aims at rapidly restoring mechanical stability and tissue integrity. Rapid repair comes at the cost of tissue contracture due to the inability of the myofibroblast to regenerate tissue. When contracture and extracellular matrix remodeling become progressive and manifest as organ fibrosis, stiff scar tissue obstructs and ultimately destroys organ function. This includes fibrosis affecting vital organs, such as heart, liver, kidney, and lung.

Likewise, myofibroblasts are activated at the interface between cancers and their surrounding connective tissue as part of the stromal reaction to tumours. It is still unclear whether and when activation of such cancer-associated fibroblasts is meant to contain the cancer by forming an encapsulating scar tissue or unintentionally promotes tumor progression and metastasis by generating tumor-permissive conditions. In any event, the myofibroblast scar represents a mechanical obstacle for immune cells and/or therapeutics to access and kill the tumor cells.

Because cancer-associated fibroblasts are not special fibroblasts but fibroblasts in a special microenvironment, they are subject to the same activation pathways as fibroblasts during wound healing and fibrosis. Pivotal for the formation and persistence of myofibroblasts are mechanical stimuli arising during tissue repair and chronic presence of inflammatory cells. I will provide an overview on our current projects aiming at investigating of how mechanical factors not only promote acute myofibroblast activation but drive their persistence through epigenetic reprogramming. By understanding and manipulating the fundamental mechanisms of myofibroblast mechanoperception, we will be able to devise better therapies to reduce organ scarring, support normal wound healing, and to manipulate myofibroblast activation in the tumor stroma.