Invited Speaker Abstracts

IA01 Cohesin-mediated chromosome folding in healthy and cancer cells. Job Dekker[1]. [1]Howard Hughes Medical Institute, University of Massachusetts Chan Medical School, Worcester, MA.

The genome folds in a series of domains, referred to as Topologically Associating Domains (TADs). TADs are involved in long-range gene regulation by constraining enhancer-promoter interactions. In cancer, genetic and epigenetic alterations can lead to TAD disruption and this in turn can lead to inappropriate enhancer-promoter interactions. There are now several important examples where TAD disruption leads to oncogene activation. Therefore, understanding the molecular mechanisms of TAD formation is critical for understanding gene regulation in healthy and in cancer cells. TADs are formed by the activity of the cohesin complex. Cohesin extrudes loops along chromatids leading to TAD formation The ring-like cohesin complex also mediates sister chromatid cohesion by encircling pairs of sister chromatids. Whether the two activities involve similar mechanisms of DNA engagement is not known. We implemented an experimental approach based on isolated nuclei carrying engineered cleavable cohesin complexes to precisely control cohesin ring opening so that its role in chromatin looping could be studied under defined experimental conditions. This approach allowed us to identify cohesin complexes with distinct biochemical, and possibly structural properties, that mediate different sets of chromatin loops. When the cohesin ring is opened, cohesin complexes at CTCF sites are released from DNA and loops at these elements are lost. In contrast, cohesin-dependent loops within chromatin domains and that are not anchored at CTCF sites are more resistant to RAD21 cleavage. The results show that the cohesin complex mediates loops in different ways depending on genomic context and suggests that it undergoes structural changes as it dynamically extrudes and then encounters CTCF sites.

IA02 Single cell epigenomics. Bing Ren[1]. [1]Ludwig Institute for Cancer Research, La Jolla, CA, Center for Epigenomics and Institute for Genomic Medicine, University of California San Diego, La Jolla, CA, Department of Cellular and Molecular Medicine, University of California San Diego School of Medicine, La Jolla, CA.

Covalent modifications to DNA and histone proteins along the chromatin fiber, play a critical role in gene regulation and tumorigenesis. Together with chromatin accessibility and 3D architecture of the genome, they are collectively referred to as the epigenome. Delineating the epigenome in normal and tumor cells is a necessary step towards a molecular understanding of malignant transformation. The high inter- and intra-tumoral heterogeneity presents a significant hurdle to conventional epigenome assays. Recent advances in single cell epigenome assays have overcome this bottleneck. We have used single-cell chromatin accessibility assays to analyze 30 adult human tissue types from multiple donors and defined cell-type-specificity of potential gene regulatory elements in 111 adult cell types. We further integrated these datasets with single-cell chromatin accessibility data from 15 fetal tissue types to reveal the status of open chromatin for approximately 1.2 million candidate *cis*-regulatory elements (cCREs) in 222 distinct cell types comprised. We used these chromatin accessibility maps to systematically interpret the noncoding variants associated with complex human traits and diseases. We further examined chromatin accessibility landscapes in clinical glioma specimens at single cell resolution and dissected intra-

tumoral genetic heterogeneity. We observed sub-populations of tumor cells characterized by focal DNA amplifications including extrachromosomal circular DNA (ecDNA) that harbor highly transcribed oncogenes. The maps of open chromatin helped define cellular states of distinct tumor cell populations characterized by unique regulatory landscapes.

IA03 RNA modification in human cancer. Chuan He[1]. [1]The University of Chicago / Howard Hughes Medical Institute, Chicago, IL.

Over 150 types of post-transcriptional RNA modifications have been identified in all kingdoms of life. We have discovered two RNA demethylases, FTO and ALKBH5, which catalyze oxidative demethylation of the most prevalent modifications of mammalian messenger RNA (mRNA) and other nuclear RNA, N^6-methyladenosine (m^6A). These findings indicate that reversible RNA modification could impact biological regulation analogous to the well-known reversible DNA and histone chemical modifications. We have also characterized proteins that selectively recognize m^6A-modified mRNA and affect the translation status and lifetime of the target mRNA, as well as molecular machines that deposit the m^6A methylation on mRNA. Functional studies reveal m^6A methylation as a critical mechanism to group transcripts for coordinated metabolism, translation, and decay, allowing timely and coordinated protein synthesis and transcriptome switching during cell differentiation and development. Misregulations of these processes lead to human diseases such as cancer. I introduce critical roles of mRNA m^6A methylation in tumor immunology and anti-cancer immunotherapy.

IA04 Deconvolution of chromatin programs of stemness at single-cell resolution. Marco Gallo[1]. [1]University of Calgary, Calgary, AB, Canada.

Genetic, epigenetic and functional intratumoral heterogeneity have been well documented in glioblastoma. However, how these three layers of complexity interface with each other is still incompletely understood. Here we describe how we have adopted orthogonal approaches to test the hypothesis that genetic and epigenetic factors act hierarchically to define functional properties of glioblastoma cells. Hi-C and single-cell epigenomic assays demonstrated how chromatin proteins can shape 3D genome organization and transcriptional profiles associated with stem-like or differentiated states. These data showcase how epigenetics and 3D genome architecture influence the functional status of glioblastoma cells. Further, we created a computational tool – Copy-scAT – that uses single-cell epigenomic data to infer copy number variants in individual cells. Copy-scAT enabled the investigation of the interface between genetics and epigenetics. We found that genetic subclones from the same tumor can have different chromatin accessibility profiles. Interestingly, we also found that subclones could differ in functional output, including proliferative potential. Overall, our results suggest that genetic factors may influence epigenetic profiles and ultimately functional properties of glioblastoma cells.

IA05 Dissecting glioblastoma by single cell RNA-seq. Itay Tirosh[1]. [1]Weizmann Institute of Science, Rehovot, Israel.

Cellular heterogeneity is a fundamental property of glioblastoma that represents a central barrier for effective therapies. I will present our studies of glioblastoma by single cell RNA-seq, in

which we focus on the malignant cells, define their diversity of cellular states and explore the mechanisms that generate heterogeneity and its functional significance. We find that glioblastoma tumors consistently contain cells in four cellular state and that all four are proliferative and have previously been proposed to reflect glioma stem cells. These four states recapitulate expression programs from neural development as well as a mesenchymal-like state. We demonstrate a high degree of cellular plasticity, in which cells rapidly transition between those states, and we further examine the role of macrophages in driving transitions towards the mesenchymal state through secretion of Oncostatin M. These results revise our understanding of glioblastoma, explain the basis for previously defined subtypes, and suggest that future therapies will have to co-target multiple cellular states in order to improve patient survival.

IA06 Immunocytokines to treat glioblastoma. Michael Weller[1]. [1]Department of Neurology, University Hospital and University of Zurich, Zurich, Switzerland.

Glioblastoma is a poorly immunogenic tumor. Successes with recent immunotherapies approaches, notably with immune checkpoint inhibition, in extracranial malignancies have not been translated to glioblastoma so far. There is therefore an urgent need for new strategies to convert the immunologically “cold” tumor microenvironment in glioblastoma into a “hot” one to enable more effective antitumor immunity. Using the L19 antibody, which is specific to a tumor-associated epitope of extracellular fibronectin, we developed antibody cytokine fusion molecules, referred to as immunocytokines, with interleukin-2 (IL-2), IL-12, or tumor necrosis factor (TNF). We showed that L19 accumulated in the tumor microenvironment in two orthotopic immunocompetent mouse glioma models. Furthermore, intravenous administration of L19-mIL12 or L19-mTNF cured a proportion of tumor-bearing mice whereas L19-IL2 had no such effect. This therapeutic activity was not observed in RAG−/− mice or upon depletion of CD4 or CD8 T cells, suggesting that adaptive immunity mediates the antitumor immunity in these models. Mechanistically, both immunocytokines promoted the accumulation of tumor-infiltrating lymphocytes and increased the amounts of proinflammatory cytokines within the tumor microenvironment. In addition, L19-mTNF induced tumor necrosis. Systemic administration of the fully human L19-TNF fusion protein to patients with glioblastoma (NCT03779230) is safe, decreases regional blood perfusion within the tumor, is associated with increasing tumor necrosis and an increase in tumor-infiltrating CD4 and CD8 T cells. Clinical trials in recurrent and newly diagnosed glioblastoma are ongoing.

IA07 Integrated development of a brain-penetrant EGFR inhibitor and non-invasive predictive biomarker of response for glioblastoma. David A. Nathanson[1], Timothy Cloughesy[1], Michael Jung[1]. [1]University of California Los Angeles, Los Angeles, CA.

The epidermal growth factor receptor (EGFR) is mutated and/or amplified is nearly 60% of patients with glioblastoma (GBM) and has been validated as a key oncogenic driver of malignancy. However, clinical trials using EGFR inhibitors designed for primary malignancies located outside of the brain (e.g., EGFR mutant lung cancer) have failed to improve on the clinical outcomes of patients with GBM. This failure can be attributed to the fact that existing EGFR inhibitors have low brain penetration and, in certain instances, poor activity against the oncogenic EGFR alterations uniquely present in GBM (e.g., amplified wild-type EGFR and mutant EGFRvIII). To address this critical unmet need, we have developed ERAS-801 – a novel

EGFR tyrosine kinase inhibitor that was specifically designed for EGFR altered GBM. Relative to numerous FDA approved EGFR TKIs (e.g., erlotinib, lapatinib, osimertinib (Tagrisso), ERAS-801 shows extremely high unbound brain exposures (~120% relative to plasma), potency against both amplified wild-type EGFR and mutant EGFRvIII, and superior activity in patient-derived EGFR mutant orthotopic GBM xenografts. The high activity of ERAS-801 was corroborated in a preclinical trial consisting of 30 unique patient derived GBM mouse models, where ERAS-801 was efficacious in over 90% of the EGFR-altered GBM models tested. Finally, given the functional relationship between aberrant EGFR signaling and glucose metabolism, we provide strong evidence that fluorodeoxyglucose (FDG) positron emission tomography (PET) imaging can serve as a robust, rapid, and non-invasive predictive biomarker of ERAS-801 activity. Together, our results present a novel, highly brain-penetrant EGFR TKI optimized specifically for GBM coupled with an imaging biomarker that is on track for an IND submission in Q1 2022.

IA09 Consistent metabolic adaptations in stressful tumor microenvironments. M. Celeste Simon[1]. [1]University of Pennsylvania, Philadelphia, PA.

Solid tumors reside in harsh tumor microenvironments (TMEs) together with various stromal cell types. During tumor progression and metastasis, both tumor and stromal cells undergo rapid metabolic adaptations. Tumor cells metabolically coordinate or compete with their “neighbors” to maintain biosynthetic and bioenergetic demands while escaping immunosurveillance or therapeutic interventions. Here, I will provide an update on metabolic communication between tumor cells and heterogeneous stromal components in primary and metastatic TMEs, and discuss emerging strategies to target metabolic communications for improved cancer treatments.

IA11 Targeting the PI 3-Kinase pathway in brain cancers. Lewis C. Cantley[1]. [1]Weill Cornell Medicine, New York, NY.

Phosphoinositide 3-Kinase-alpha (PI3Kalpha), encoded by PIK3CA, is activated by insulin and other growth factors to mediate nutrient uptake and cell growth. The PIK3CA gene is one of the most frequently mutated oncogenes in brain cancers. In addition, mutations and deletions in the PTEN gene, whose protein product degrades the phosphoinositides generated by PI3Kalpha, also occur frequently in brain cancers. Activation of the PI3Kalpha pathway is required for insulin to stimulate glucose uptake and cell growth. The genetic aberrations in PIK3CA and PTEN allows brain tumors to grow more rapidly when serum insulin levels are high. Although more than 20 PI3Kalpha inhibitors have entered clinical trials for treating PIK3CA mutant cancers, most have failed, in part because of associated toxicities, including hyperglycemia and rashes, but also due to limited effectiveness in suppressing tumor growth. Data will be presented showing that dietary and pharmaceutical interventions that limit elevation of serum glucose and serum insulin dramatically improve responses to PI3Kalpha inhibitors in mouse models of cancer by preventing insulin-dependent activation of PI3K in tumors. As a consequence of these preclinical studies, several human clinical trials that use dietary intervention and/or drugs to lower serum insulin in patients taking PI3Kalpha inhibitors are opening in our cancer center, including a glioblastoma trial.

IA12 Aberrant DNA repair as a therapeutic target in glioblastoma. Petra Hamerlik[1].
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Faithful completion of DNA replication is essential for genome integrity. The integrity of mammalian genomes is continuously challenged by DNA double-strand breaks (DSBs), cytotoxic lesions that can be generated by cell-intrinsic processes, such as DNA replication, transcription, and metabolism, as well as cell-extrinsic events such as DNA damaging therapies. Thus, a functional DNA damage response (DDR) promoting DNA repair and cell cycle checkpoints is crucial for cellular survival. The DDR encompasses a network of proteins that sense and respond to DSBs formation. The failure or error-prone repair of DSBs can lead to cell death or accumulation of deleterious gross chromosomal aberrations thereby promoting genomic instability and tumorigenesis. Several studies including ours showed that malignant gliomas exhibit constitutive activation of the DDR, a network whose various facets have been implicated in early-stage protection against tumor progression. The understanding of the role the DDR plays in cancer is critical to successfully target difficult to treat or aggressive cancers such as glioblastoma.

IA13 Advances in the biology and treatment of pediatric CNS tumors. Mark W. Kieran[1].
[1]Day One Biopharmaceuticals, South San Francisco, CA.

There have been considerable advances in the biologic characterization of pediatric brain and spinal tumors. These have spanned multiple areas including technical advances in imaging, neurosurgical navigation, improved preclinical models and perhaps of greatest impact, the ongoing “omics” revolution. This later advance has led to the recent modification of the WHO classification schema for both adult and pediatric tumors of the central nervous system. Most importantly, the identification of specific molecular targets in tumors of the central nervous system has opened the door to the translation of many novel pathway inhibitors into the clinic. Multiple examples of the advances in the technical approach to brain tumors, as well as examples of successful targeted therapies will be discussed. Unfortunately, many identified mutations have not responded to the corresponding targeted agent and the reasons for this this will also be explored.