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Tumor heterogeneity is one of the hallmarks of glioblastoma multiforme (GBM). Morphology within a given GBM tumor can be extremely variable where some regions of the tumor have a soft, gel-like structure while other areas are dense and fibrous. Abnormal mechanical stress and tissue stiffening caused by cancer proliferation are believed to affect vascularity by compressing structurally weak blood vessels and restricting the supply of nutrients and oxygen to the tissue. These effects contribute to a hypoxic microenvironment that promotes disease progression and chemoresistance. The genetic and molecular mechanisms that govern tissue stiffness within GBM tumors, however, are largely unknown. Magnetic Resonance Elastography (MRE) is an emerging technique for quantifying tissue stiffness non-invasively. We have evaluated 10 GBM patients by MRE imaging obtained prior to surgical resection. During surgery, 2–7 stereotactically navigated biopsies were collected from locations within the tumor with varying degrees of measured stiffness. Biopsies were processed to extract RNA, proteins, polar metabolites and lipids. Biomolecules were analyzed on relevant -omics platforms (RNA sequencing, MS-proteomics and lipidomics, NMR of polar metabolites). Differential expression and gene set enrichment analysis of patient paired biopsies indicate an overall increase in macrophage infiltration and extracellular matrix re-organization associated with increased tumor stiffness. Among the most highly upregulated genes in stiff tumor tissue were lymphatic endothelial hyaluronic acid receptor 1 (LYVE-1) and macrophage receptor with collagenous structure (MARCO), both of which have been associated with immune cell infiltration and tissue stiffness. Our preliminary findings offer novel insights into tumor morphology in GBM that can be inferred from imaging prior to surgery. This can be used to identify tumor regions with high risk of progression and infiltration, thereby informing and guiding surgical strategy and may ultimately lead to novel treatment strategies.

OTEH-8. PATHWAY-BASED APPROACH REVEALS SENSITIVITY TO RADIATION WHEN TARGETING E2F1 IN GLIOBLASTOMA

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The great phenotypic heterogeneity of glioblastoma (GBM) – both inter and intratumorally – has hindered therapeutic efforts. While genome-based molecular subtyping has revealed that GBMs may be parsed into several molecularly distinct categories, this insight has not translated to a significant extension of patient survival. We hypothesize that, rather than gene expression as a whole, analysis of targetable pathways could yield important insights into the development of novel classification schemes and, most importantly, to targeted therapeutics. Here, we interrogated tumor samples using a pathway-based approach to resolve tumoral heterogeneity. The Cancer Genome Atlas samples were clustered using gene set enrichment analysis and the resulting 3 clusters were informative of patient survival and only modestly overlapped with prior molecular classification. We validated our approach by generating gene lists from common elements found in the top contributing genesets for a particular cluster and testing the top targets in appropriate gliomasphere patient-derived lines. Samples enriched for cell cycle related genesets showed a decrease in sphere formation capacity, proliferation and in vivo tumor growth when E2F1, our top target, was silenced. Consistent with our theory, E2F1 knockdown had little or no effect on the growth of the non-enriched lines, despite their ability to proliferate in vitro and in vivo. We similarly analyzed single cell RNAseq datasets and correlated cell cycle and stemness signatures with the gene lists we generated, concluding that cells with stem cell signatures were depleted of E2F1 and its downstream targets. Finally, we confirmed a connection between E2F1 and cellular inhibitor of PP2A (CIP2A) in a cluster of samples. Loss of function studies reveal a diminished capacity for DNA damage regulation in E2F1 activated samples. Our studies relate inter- and intratumoral heterogeneity to critical cellular pathways dysregulated in GBM, with the ultimate goal of establishing a pipeline for patient- and tumor-specific precision medicine.

OTEH-9. SCRNA SEQUENCING OF PRONEURAL GBM AVATAR MODEL REVEALS ACQUISITION OF ONCOGENIC TRANSCRIPTIONAL PROGRAMMING AND INFERS A DEVELOPMENTAL PATH TOWARDS A GENOMICALLY UNSTABLE STATE

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Glioblastomas (GBMs) are the most common malignant primary brain tumors, and the paucity of novel treatments warrants an investigation of its origins and development into aggressive, lethal tumors. Koga & Chaim *et al.* have recently shown that human pluripotent stem cells (hiPSCs) with different combinations of driver mutations can be differentiated into neural progenitor cells (NPCs) and engrafted into mice to form high grade gliomas (iHGGs). In this work, scRNA seq analysis was used to investigate the development of *TP53−/−;PDGFRAΔ8–9* iHGGs, an avatar model that has been shown to recapitulate the proneural subtype of GBM. After re-engrafting the primary avatar cultures (secondary tumor stage), the *TP53−/−;PDGFRAΔ8–9* iHGGs developed diverse transcriptional programming and acquired a subpopulation of cells with high expression of known GBM oncogenes, such as MYC, CDK4, and PDGFRA. Notably, when all datasets were aggregated, this oncogene amplifying transcriptional program became the largest source of variation between all stages and replicates of the *TP53−/−;PDGFRAΔ8–9* iHGGs. Indicated by a larger total copy number variation (CNV), this oncogene-amplifying program was associated with a genomically unstable developmental state. Trajectory inference could track the development of this population from the initial primary culture of *TP53−/−;PDGFRAΔ8–9* iHGG. Differential gene expression analysis identified distinct divergences in clonal evolution—e.g., high expression of the S100 protein family in one cluster—following the acquisition of this genomically unstable state. Lastly, genomic PCR was used to ascertain whether these changes in transcriptional programming were reflected in changes in DNA copy number and identified DNA amplifications of MYC and CDK4. Our scRNA seq analysis of the GBM avatar model platform provides novel insight into how oncogenic states in GBM develop from a small number of driver mutations.

OTEH-10. EVOLUTIONARY TRAJECTORY OF EPIGENOMIC OF GLIOMAS

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Gliomas are the most common malignant brain tumor, have an aggressive behavior, and invariably relapse and progress. Despite the recent advancements, little is known about the role of the epigenome in glioma disease progression and recurrence. To investigate the molecular dynamics over time and in response to therapeutic pressures, the Glioma Longitudinal AnalySiS (GLASS) Consortium, a multinational collaboration, is investigating epigenome-wide molecular data from primary and recurrent matched pairs, including IDH mutant (IDHmut) and IDH wildtype (IDHwt) gliomas. We have compiled a total of 357 samples comprising 143 primary-recurrent pairs profiled by DNA methylation, of which 157 samples have genomic data (WXS/WGS) and 120 have transcriptomic data (RNAseq). IDHwt gliomas have a distinct epigenetic evolution compared to IDHmut after treatment. IDHwt gliomas are more epigenetically stable over time, while IDHmut gliomas display a loss of DNA methylation throughout disease progression. Next, we investigated the molecular drivers of longitudinal gliomas by integration of DNA methylation and gene expression data. We identified epigenetic activation of cell cycle pathways in recurrent IDHmut compared to initial tumors. Transcription factors musculin, ZNF367, and ZNF682 are enriched among recurrent IDHmut gliomas and potentially regulate IDHmut recurrence and/or progression. We next used a DNA methylation-based deconvolution approach to estimate the tumor microenvironment (TME) composition. We found that the TME among IDHmut subtypes (Codel, GCIMP-high, and GCIMP-low) presented less immune infiltration than IDHwt (Classic-like, Mesenchymal-like, and PA-like). Post-treatment, we found a decrease of CD4+T and an increase of CD8+T cells in IDHmut. In conclusion, IDHmut gliomas present a more unstable epigenome, while the epigenome of IDHwt gliomas seems relatively preserved after treatment. We identified potential master regulators of cell cycle deregulation of IDHmut recurrence. Finally, the TME differs across IDHmut and IDHwt gliomas and the cell composition changes over time.

OTEH-11. SINGLE CELL RNA SEQUENCING TO IDENTIFY CELLULAR HETEROGENEITY WITH IN PITUITARY ADENOMAS

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Pituitary adenomas (PA) are one of the most common primary brain tumors and comprise approximately 15% of brain neoplasms. Most PA are