ETMM-04. AURKA INHIBITION REPROGRAMS METABOLISM AND IS SYNTHETICALLY LETHAL WITH FATTY ACID OXIDATION INHIBITION IN GLIOBLASTOMA MODEL SYSTEMS

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Aurora kinase A (AURKA) has emerged as a viable drug target for glioblastoma (GBM), the most common malignant primary brain tumor in adults with a life expectancy of 12–15 months. However, resistance to therapy remains a critical issue, which partially may be driven by reprogramming of metabolism. By integration of transcriptome, chromatin immunoprecipitation with sequencing (CHIP-seq.), assay for transposase-accessible chromatin with sequencing (ATAC-seq.), proteomic and metabolite screening followed by carbon tracing (U-^{13}C-Glucose, U-^{13}C-Glutamine and U-^{13}C-Palmitic acid) and extracellular flux analysis we provided evidence that genetic (shRNA and CRISPR/Cas9) and pharmacological (Alisertib) AURKA inhibition elicited substantial metabolic reprogramming supported in part by inhibition of MYC targets and concomitant activation of PPARA signaling. While glycolysis was suppressed by AURKA inhibition, we noted a compensatory increase in oxygen consumption rate fueled by enhanced fatty acid oxidation (FAO). Whereas interference with AURKA elicited a suppression of c-Myc, we detected an upregulation of PGC1A, a master regulator of oxidative metabolism. Silencing of PGC1A reversed AURKAi mediated metabolic reprogramming and sensitized GBM cells to AURKAi driven reduction of cellular viability. Chromatin immunoprecipitation experiments showed binding of c-Myc to the promoter region of PGC1A, which is abrogated by AURKA inhibition and in turn unleashed PGC1A expression. Consistently, ATAC-seq. confirmed higher accessibility of a MYC binding region within the PGC1A promoter, suggesting that MYC acts as a repressor of PGC1A. Combining alisertib with inhibitors of FAO or the electron transport chain exerted substantial synergistic growth inhibition in PDX lines in vitro and extension of overall survival in orthotopic GBM PDX models without induction of toxicity in normal tissue. In summary, these findings support that simultaneous targeting of oxidative energy metabolism and AURKAi might be a potential novel therapy against GBM.

ETMM-05. LACTIC ACID FACILITATES GLIOBLASTOMA GROWTH THROUGH MODULATION OF THE EPIGENOME

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Glioblastoma (GBM) is the most common primary malignant brain tumor with an unfavorable prognosis. While GBMs utilize glucose, there are other carbon sources at their disposal. Lactate accumulates to a significant amount in the infiltrative margin of GBMs. In the current study, we demonstrated that lactate rescued patient-derived xenograft (PDX) GBM cells from nutrient deprivation mediated cell death and inhibition of growth. Transcriptome analysis, ATAC-seq and CHIP-seq. showed that lactic acid exposure entertained a signature of cell cycle progression and oxidative phosphorylation (OXPHOS) /tricarboxylic acid (TCA)-cycle. LC/MS analysis demonstrated that U-13C-Lactate elicited substantial labeling of TCA-cycle metabolites, acetyl-CoA and histone protein acetyl-residues in PDX derived GBM cells. Given that acetyl-CoA is pivotal for histone acetylation we observed a dose-dependent elevation of histone marks (e.g. H3K27ac), which was rescued by genetic and pharmacological inhibition of lactic acid-uptake, ATP-citrate lyase, p300 histone-acetyl-transferase and OXPHOS, resulting in reversal of lactate mediated protection from cell death. CHIP-seq. analysis demonstrated that lactic acid facilitated enhanced binding of H3K27ac to gene promoters and cis-regulatory elements. Consistently, ATAC-seq. analysis highlighted enhanced accessibility of the chromatin by lactic acid. In a combined tracer experiment (U-13C-glucose and 3-C13-lactate), we made the fundamental observation that lactic acid carbons were predominantly labeling the TCA cycle metabolites over glucose, implying a critical role of lactic acid in GBMs. Finally, pharmacological blockage of the TCA-cycle, using a clinically validated drug, extended overall survival in an orthotopic PDX model in mice without induction of toxicity, implying a critical role of lactic acid in GBMs and establishing lactic acid metabolism as a novel drug target for GBM.

ETMM-06. ELEVATED MITOCHONDRIAL TOM20 EXPRESSION SUPPRESSES GLIOMA MALIGNANCY BY ENHANCING OXIDATIVE PHOSPHORYLATION

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BACKGROUND: Malignant glioma display a metabolic shift towards aerobic glycolysis with reprogramming of mitochondrial oxidative phosphorylation (OXPHOs). However, the underlying mechanism for this metabolic switch in glioma is not well elucidated. Mitochondrial translocases of the outer/inner membrane (TOMs/TIMs) import proteins into mitochondria, and could thereby regulate OXPHOs. The objective of this study is to investigate the expression of TOM/TIM members in glioma, as well as their functional and therapeutic implications. **METHODS:** Transcriptome sequencing (RNA-seq), real-time PCR, Western blot, and immunohistochemistry were used to identify Tom20 as a significantly downregulated TOM/TIM protein in 20 paired glioma/Peritumoral tissues. To study the biological function of Tom20 in glioma, we interrogated metabolic alterations in Tom20 overexpressed glioma cells by GC-MS metabolomics, acetyl-CoA assay, and Seahorse assay. We compared the cell proliferation and viability profiles between Tom20 overexpressed and control cells in vitro and in vivo. To investigate the therapeutic implication of Tom20 expression, we tested OXPHOs inhibitor metformin in Tom20 overexpressed cells and xenograft mouse models. **RESULTS:** We find that Tom20, a critical component of the mitochondrial outer membrane translocases, is downregulated in malignant gliomas. Using an integrative approach spanning bioinformatic analysis, metabolomics, and functional approaches, we reveal that Tom20 elevation activates mitochondrial OXPHOs in glioma cells and reduces tumor malignancy. We also find that Tom20 upregulation sensitizes glioma cells to metformin in vitro, and improves the therapeutic efficacy of metformin in glioma in vivo. **CONCLUSION:** Our work defines Tom20 as a glioma suppressor and an indicator of metformin treatment in glioma.

ETMM-07. HYPOXIC REGULATION OF METABOLIC AND STRUCTURAL GENES IN T98 GLIOBLASTOMA MULTIFORME CELLS BY RNA SEQUENCING

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Glioblastoma multiforme (GBM) is the most common primary brain cancer and carries a very poor prognosis. The GBM tumor microenvironment is characterized by regions of profound hypoxia, which are associated with a variety of alterations in gene expression that confer survival, proliferation, and resistance to therapy. Multiple mechanisms have been implicated in hypoxia-associated GBM behavior including upregulation of pathways involved in angiogenesis, immunosuppression, and glucose metabolism. Our study aimed to identify changes in gene expression induced by hypoxia among T98G cells via total RNA sequencing. Human T98 GBM cell lines were cultured in a humidified incubator at 37° C and 5% CO_2 and were grown in normoxia (21% O_2) or hypoxia (95% N_2, 5% $C0_2$) for 72 hours. Total RNA was harvested, and global gene expression was evaluated via total RNA sequencing. Standard bioinformatics analysis was performed to identify changes in expression associated with hypoxia. Hypoxia in T98 cells led to significant upregulation of genes implicated in canonical glycolysis, focal adhesion, extracellular matrix reorganization, and endoplasmic reticulum-associated protein processing. We document 690 genes and 11 associated KEGG pathways that demonstrated significant enrichment ($p \leq 0.01$ with Bonferroni, Benjamini, and False Discovery Rate corrections) induced by hypoxia. Notably, upregulation of the IRE1-mediated unfolded protein response was observed. DrugBank database analysis identified four molecules targeting genes upregulated in hypoxic T98G cells: tenecteplase ($p = 0.013$, 5 gene targets), succinic acid ($p = 0.02$, 7 targets), artenimol ($p = 0.013$, 13 targets), and copper ($p = 0.0015$, 22 targets). We document 733 genes and 6 associated KEGG pathways significantly downregulated ($p \leq 0.01$) in hypoxia, including genes associated with DNA replication and repair, mitotic processes, and spliceosome function. Total RNA sequencing showed hypoxic upregulation of genes involved in various pathways associated with neoplastic GBM behavior and identified multiple candidate molecules which may hold therapeutic potential.

ETMM-08 METABOLIC REGULATION OF THE EPIGENOME DRIVES LETHAL INFANTILE EPENDYMOMA

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Ependymomas are malignant glial tumours that occur throughout the central nervous system. Of the nine distinct molecular subgroups of

ependymoma, Posterior Fossa A (PFA), is the most prevalent, occurring in the hindbrain of infants and young children. Lacking highly recurrent somatic mutations, PFAs are thought to be a largely epigenetically driven entity, defined by hypomethylation at the histone 3 lysine 27 residue. Previous transcriptional analysis of PFAs revealed an enrichment of hypoxia signaling genes. Thus, we hypothesized that hypoxic signaling, in combination with a unique metabolic milieu, drive PFA oncogenesis through epigenetic regulation. In this study, we identified that PFA cells control the availability of specific metabolites under hypoxic conditions, resulting in diminished H3K27 trimethylation and increased H3K27 acetylation *in vitro* and *in vivo*. Unique to PFA cells, transient exposure to ambient oxygen results in irreversible cellular toxicity. Furthermore, perturbation of key metabolic pathways is sufficient to inhibit growth of PFA primary cultures *in vitro*. PFA cells sequester s-adenosylmethionine while upregulating *EZHIP,* a polycomb repressive complex 2 (PRC2) inhibitor, resulting in decreased H3K27 trimethylation. Furthermore, hypoxia fine-tunes the abundance of alpha-ketoglutarate and acetyl-CoA to fuel demethylase and acetyltransferase activity. Paradoxically, a genome-wide CRISPR knockout screen identified the core components of PRC2 as uniquely essential in PFAs. Our findings suggest that PFAs thrive in a narrow "Goldilocks" zone, whereby they must maintain a unique epigenome and deviation to increased or decreased H3K27 trimethylation results in diminished cellular fitness. Previously, we showed that PFAs have a putative cell of origin arising in the first trimester of development. Using single-cell RNAseq and metabolomics, we demonstrate that PFAs resemble the natural metabolic-hypoxic milieu of normal development. Therefore, targeting metabolism and/or the epigenome presents a unique opportunity for rational therapy for infants with PFA ependymoma.

ETMM-09 TARGETING GLIOBLASTOMA MULTIFORME METABOLISM AT THE INVASIVE TUMOR FRONT

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Glioblastoma (GBM) is a primary malignant brain tumor with a median survival under two years. The poor prognosis GBM caries is largely due to cellular invasion, which enables escape from resection and drives inevitable recurrence. Numerous factors have been proposed as the primary driving forces behind GBM's ability to invade adjacent tissues rapidly, including alterations in its cellular metabolism. Though studies have investigated links between GBM's metabolic profile and its invasive capabilities, these studies have had two notable limitations. First, while infiltrating GBM cells utilize adaptive cellular machinery to overcome stressors in their microenvironment, the cells at the invasive tumor front have rarely been sampled in previous studies, which have primarily used banked tissue taken from the readily accessible tumor core. Second, studies of invasion have primarily used two-dimensional (2D) culture systems, which fail to capture the dimensionality, mechanics, and heterogeneity of GBM invasion. To address these limitations, our team developed two complementary approaches: acquisition of site-directed biopsies from patient GBMs to define regional heterogeneity in invasiveness, and engineering of three dimensional (3D) platforms to study invasion in culture. Through utilization of these platforms, and by taking advantage of the system-wide, unbiased screens of metabolite profile and gene expression available, our team looked to accomplish the goal of identifying targetable metabolic factors which drive cellular invasion in GBM. Pilot RNA-Sequencing data revealed 87 of the top 250 (35%) genes preferentially expressed in the tumor invasive edge, and 30 of the top 250 (12%) genes preferentially expressed in the tumor core were involved in cellular metabolism. KEGG pathways analysis demonstrated enrichment of glycolytic, pentose phosphate, and response to amino acid starvation pathways at the tumor invasive edge. These preliminary studies demonstrate a distinct metabolic phenotype in invasive GBM cells which will be further explored with system wide screens.

METABOLIC FLUXES AND SIGNALING OF METABOLIC PATHWAYS

FSMP-01. ID1 MEDIATES ONE-CARBON MEDIATED PURINE SYNTHESIS IN GLIOBLASTOMA

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Inhibitor of DNA-binding-1 (ID1) is a transcriptional regulatory protein involved in maintenance of self-renewal and inhibition of differentiation, and acts as a key regulator of tumorigenesis in glioblastoma. Studies suggest that de novo purine synthesis is essential for the maintenance of rapid proliferation rates in glioma initiating cells. We hypothesise that ID1 plays a role in reprogramming one-carbon mediated de novo purine synthesis, thereby metabolically contributing to the tumorigenic advantage seen in ID1-high glioblastoma cells. The effect of ID1 regulation on metabolic reprogramming of glioblastoma was studied using ID1-knockout U251 glioblastoma cell lines. Protein expression analysis and liquid chromatography mass-spectrometry were respectively used to assess expression and concentration of metabolic enzymes and intermediates of one-carbon and de novo purine synthesis pathways. CD44 expression was analyzed as a marker of cancer stem cells. The expression of DHFR and MTHFD2 was significantly decreased after ID1 knockout. Furthermore, PAICS expression, and overall concentration of IMP, AMP, GMP, and ATP were reduced after ID1 knockout. ID1 expression in glioblastoma tumor xenografts was associated with positive expression of one-carbon metabolism and purine synthesis enzymes, while $ID1^{-/-}$ cells within the same xenograft had significantly reduced expression of these enzymes. The expression of CD44 was reduced after ID1 knockout. This data suggests that ID1 mediates an increase in one-carbon mediated *de novo* purine synthesis, thereby regulating metabolic reprogramming in glioblastoma cells. The correlation between CD44 and ID1 expression provides further support that ID1 maintains a less differentiated phenotype in a subset of glioblastoma cells, and metabolic reprogramming is one of the mechanisms through which this phenotype, and the capacity for self-renewal are maintained. Further elucidation of the mechanisms through which ID1 mediates metabolic reprograming of glioblastoma cells can lead to developing effective combination therapies coupling chemotherapeutic strategies with targeting of metabolic programs used by cancer initiating cells.

FSMP-02. CHANGES IN GLUTAMINE METABOLISM INDUCED BY OXALOACETATE IN GLIOBLASTOMA

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Anhydrous Enol-Oxaloacetate (AEO) has been shown to significantly increase survival and decrease tumor growth rates in animal models of glioblastoma and hepatocellular carcinoma. In the body, AEO is metabolized to "oxaloacetate" (OAA). Earlier, we demonstrated that AEO drastically reduced Warburg glycolysis in glioblastoma cells which was determined by the increase in pyruvate to lactate ratio and a 48.8% decrease in lactate production in ^{13}C-labeled glucose metabolism studies. We have expanded this previous work to examine ^{13}C-labeled glutamine metabolism. Cultured solid tumor cancer cells strongly rely on both glucose and glutamine to synthesize carbon intermediates for anaplerotic reactions. With treatment of OAA, we hypothesize that glutamine-derived OAA may be reduced which can be tracked through the use GC-MS based ^{13}C-labeled glutamine isotopomer experiments. Patient-derived glioblastoma cells were grown in 15 mM glucose and 2 mM glutamine containing DMEM medium supplemented with 2 mM OAA for 10 days. 24 hours prior to harvesting the cells, 4 mM of [U-^{13}C]glutamine was introduced to the medium. OAA treated cells showed significant decrease in the protein levels of lactate dehydrogenase A and C which indicates the switching off of glycolysis to support the utilization of elevated OAA levels during glutamine metabolism in the TCA cycle. ^{13}C mass distribution analysis showed significant decrease in malate, aspartate and citrate pools (malate: 8.8%, $p = 0.0098$; aspartate: 9.2%, $p = 0.0064$; citrate: 9.5%, $p = 0.0036$) in their M+4 isotopomer labeling in OAA treated group compared to the control group. Decrease in lactate generation may reduce cancer proliferation, migration and invasion. Together, these data provide alternative way to modulate energy metabolism in glioblastoma using AEO treatment. Similarly, AEO treatment in other solid tumors (e.g. pancreatic ductal adenocarcinoma) may produce altered glutamine metabolism which may be of therapeutic value.

FSMP-03. INVESTIGATING CO-OPTED ASTROCYTIC METABOLISM IN MELANOMA BRAIN METASTASIS

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Melanoma, an aggressive form of skin cancer, frequently metastasizes to the brain. While peripheral melanoma is largely treatable, MBM fail to respond to current therapeutics and is a clear unmet clinical need. Initial