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