TMET-34. RADIATION METABOLOMICS IN PRIMARY HUMAN MENINGIOMA AND SCHWANNOMA: EARLY EXPERIENCE AND INITIAL RESULTS

Mark Dougherty, Eric Taylor, Marlan Hansen

only 20 μL of microdialysate, representing a short and feasible 10 minutes of intraoperative collection time. Enrichment analysis of each patient's tumor vs. brain ranked extracellular metabolome highlighted marked metabolic convergence within the most aggressive regions of molecular diverse tumors (FDR = 0). Pathway analysis revealed significant enrichment for large neutral amino acid pathways, including valine, leucine, and isoleucine biosynthesis (p=1.6E-9) and degradation (p=0.001) and glycine, serine, and threonine metabolism (p=4.7E-5). Notably, this amino acid signature was not as abundantly present in nonenhancing tumor when compared to enhancing tumor (Average tumor/brain: 1.9x vs. 4.3x, respectively), suggesting upregulation of neutral amino acid transporters in enhancing tumor or delivery from plasma into the CNS via a disrupted BBB. Interestingly, guanidinoacetate (GAA) was our most highly conserved and upregulated metabolite (128.9x in tumor vs. brain). Given its co-production with ornithine, the precursor to protumorigenic polyamines, we posit that GAA may serve as a biomarker of increased ODC activity in live human gliomas. In conclusion, intraoperative HMW microdialysis feasibly offers improved opportunities to perform glioma metabolic biomarker and therapeutic discovery.

TMET-33. ELONGATION CONTROL OF MRNA TRANSLATION SUPPORTS GROUP 3 MEDULLOBLASTOMA ADAPTATION TO NUTRIENT DEPRIVATION

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Group 3 affiliation and *MYC* genetic amplification are associated with poor life expectancy and substantial morbidity in children suffering from medulloblastoma (MB). However, the high metabolic demand induced by MYC-driven transformation sensitizes MYC-overexpressing MB to cell death under conditions of nutrient deprivation (ND). Additionally, MYC-driven transformation is known to promote mitochondrial oxidative phosphorylation (OXPHOS). We previously reported that eukaryotic Elongation Factor Kinase 2 (eEF2K), the master regulator of mRNA translation elongation, promotes survival of MYC-overexpressing tumors under ND. Interestingly, eEF2K is overexpressed in MYC-driven MB and our preliminary proteomics data highlight large-scale alterations in OXPHOS components affecting eEF2K deficient MB cells. We therefore hypothesized that eEF2K activity is required for the selective translation of mRNAs needed for efficient OXPHOS, and for the progression of MYC-driven MB. We performed Multiplexed enhanced Protein Dynamic Mass Spectrometry in eEF2K knockdown MYC-overexpressing D425 MB cells to identify mRNAs selectively translated upon eEF2K activation. Messenger RNAs encoding multiple (9 out of 10 detected) components of the mitochondrial OXPHOS pathway are selectively translated upon eEF2K activation. Inactivation of eEF2K by genetic KO leads to the disassembly of electron transport chain (ETC) complexes I-IV without affecting mRNA levels of their respective components. Consistently, *eEF2K* KO MB cells display decreased mitochondrial membrane potential and 20% increased proton leak thorough the mitochondrial membrane. In addition, eEF2K inactivation results in increased Group 3 MB cell death under ND and doubles survival of MB bearing mice fed with calorie restricted diets ($p < 0.05$). Control of mRNA translation elongation by eEF2K is critical for mitochondrial ETC complex assembly and efficient OXPHOS in MYC-overexpressing MB, likely representing an adaptive response by which MYC-driven MB cells cope with acute metabolic stress. Future therapeutic studies will aim to combine eEF2K inhibition with caloric restriction mimetic drugs as eEF2K activity appears critical under metabolic stress conditions.

TMET-34. RADIATION METABOLOMICS IN PRIMARY HUMAN MENINGIOMA AND SCHWANNOMA: EARLY EXPERIENCE AND INITIAL RESULTS

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INTRODUCTION: Meningiomas and schwannomas account for 45% of primary CNS tumors. Yet when surgery and radiation fail, no further treatments exist. Metabolomics has been used to discover new cancer therapies; however, to date few have used metabolomics to study meningiomas and schwannomas. Here we present initial results and lessons learned from this novel endeavor. METHODS: Primary tumors were obtained from patients during surgery and immediately taken for culturing or xenograft implantation. Upon reaching >90% confluence, cultures were treated with 0gy, 3gy, 10gy, or 20gy gamma radiation, then flash frozen 6 or 72 hours post-treatment. Xenograft tumors were implanted in nude mice. MRI 4 weeks post-implantation confirmed tumor viability. Mice were then given 10gy, 20gy, or sham radiation treatment. Xenografts were harvested 72 hours post-treatment. Metabolites were measured with a ThermoISQ gas chromatography-mass spectrometer. RESULTS: Eleven meningiomas and nine schwannomas were successfully cultured. Unsupervised hierarchical clustering of cultures demonstrated greater influence from tumor of origin than from radiation. Univariate analysis of schwannoma xenografts demonstrated elevated ornithine following radiation (fold change 1.62; $P = 0.008$). However, principal component analysis did not show significant between-group differentiation. Orthotopic meningioma xenografts did not produce sufficient tissue for metabolomics; however, subsequent subcutaneous implants have been successful (data forthcoming). CONCLUSION: Standard cell cultures did not reveal significant metabolic changes following radiation; it is unclear whether this was due to culture technique or inter-tumor heterogeneity. In radiated schwannoma xenografts, elevated ornithine may implicate related pathways such as ornithine decarboxylase-mediated polyamide synthesis for DNA double-strand break repair. Compared to other '-omics' studies, metabolomics requires more tissue per sample (>10mg) and is more sensitive to environmental conditions. Thus, large sample sizes are needed to detect significant changes, and xenografts are likely superior to cell culture. Future plans include increased xenograft sample size and stable isotope tracing for pathway analysis.

TMET-35. ONE CARBON AND NUCLEOTIDE METABOLISM ARE NOVEL TARGETS IN PEDIATRIC BRAINSTEM TUMORS

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Diffuse intrinsic pontine glioma (DIPG) are inoperable tumors of the brainstem with no cure. The median survival of children with DIPG is less than 2 years and 5-year overall survival is only 1%. Genomic studies have identified recurrent mutations in histone H3, ACVR and TP53 genes in DIPG. However, there are still no breakthrough drug targets for DIPG. To better understand the disease biology and find new drug targets for DIPG, we recently performed joint pathway analysis of DIPG metabolites (*untargeted metabolomics*) and differentially expressed genes (*RNAseq*) relative to normal human neural stem cells. We discovered that specific enzymes of the de novo purine biosynthesis pathway (DNPB), serine synthesis and the associated and one-carbon (1C) metabolism are highly active and the most upregulated in DIPG tumors and cell lines. Gain and loss of function experiments as well as pharmacological studies indicated a mechanistic link between DIPG mutations and DNPB pathway and the dependence of DIPG cells on specific DNPB and 1C metabolism enzymes for in vitro and in vivo growth. The 1C pathway is crucial for rapidly proliferating malignant cells as it senses and regulates cellular nutrient status by allocating and cycling 1C-groups between different acceptor compounds. It controls synthesis of nucleotides, amino acids, glycogen and phosphoglyceride precursors, S-adenosylmethionine (SAM), glutathione and NADPH. Thus, besides dispensing carbon atoms it also regulates cellular epigenetic and redox status. An important carbon donor is serine which is synthesized from glucose at 3-phosphoglycerate step in glycolysis. Untargeted as well as carbon and nitrogen tracing experiments indicated large folate pools in DIPG cells that can serve as a buffer to potentially blunt antifolate therapy. Experiments are underway to understand the importance of the folate cycle in DIPG and design novel and translatable combination therapies for DIPG.

TMET-36. ACID CERAMIDASE INHIBITION EXPLOITS SPHINGOLIPID VULNERABILITIES IN IDH MUTANT GLIOMAS

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The presence of the IDH mutation in gliomas is a major classifier of brain tumor subtypes and has several important implications for cancer growth. Our recent work uncovered that IDH-mutant tumors are susceptible to increased apoptosis via alterations of the sphingolipid pathway due to their excess production of pro-apoptotic ceramides over pro-proliferative sphingosine 1-phosphate (S1P). To that end, we proposed that this rheostat can be modulated to induce cell death in IDH^{mut} tumors by targeting acid ceramidase, a critical sphingolipid enzyme in gliomas. We hypothesize that pharmacological inhibition of acid ceramidase will increase ceramide levels and therefore induce apoptosis in IDH^{mut}gliomas. Using a preliminary drug screen, we have identified a group of haloacetate C2-ceramide derivatives known as SOBRACs that potently inhibit acid ceramidase. We selected five candidate compounds from this family and assessed the effectiveness of each drug in 3 I IDH^{mut} (BT142, TS603, & U251mut) and 3 IDH^{mut}