ATRT-09. IDENTIFICATION OF POTENTIAL GENETIC DRIVERS OF METHOTREXATE (MTX) RESISTANCE IN ATYPICAL TERATOID RHABDOID TUMOURS (ATRT) THROUGH A GENOME-WIDE RNAI SCREEN

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ATRT of the CNS constitute a group of rare and aggressive early childhood tumors with poor prognosis. While there are differing chemotherapeutic regimens for ATRT, high-dose MTX is a crucial component of many therapeutic protocols. Currently, the biological mechanisms contributing to the generation of MTX resistance in ATRT are unknown. To identify genes involved in MTX resistance in ATRT, an unbiased genome-wide RNAi screen on ATRT cell lines was conducted using 24,000 distinct shRNAs covering 8,000 genes. ATRT cells were transfected with a retrovirus containing pRS-shRNA vectors and treated with puromycin for selection. The resulting cells were treated with MTX to identify resistant clones and resistant colonies were then isolated and amplified individually. Presence of shRNA inserts in each colony was determined by PCR using pRS forward and reverse primers. PCR products within each of the three resistant colonies were sequenced, leading to the identification of three distinct genes, TGIF1, HIF3A and PGAM2, as potential indicators of resistance. Western blotting verified depletion of these proteins in their respective colonies. Proliferation assays were then conducted on cells from each resistant colony alongside control cells to confirm that the identified drivers conferred resistance. Sensitivity to MTX was significantly lower in TGIF1-depleted (IC50=212±8.48nM, n=3), HIF3A-depleted (IC50=52±4.68nM, n=3) and PGAM2-depleted (IC50=41±4.13nM, n=3) cells compared to control cells (IC50=19±2.87nM, n=3), ($p<0.001$). In addition, more than 60% of TGIF1, HIF3A, and PGAM2-depleted cells survived the maximum MTX treatment (100nM), while less than 20% of control cells survived this treatment. Our study using an unbiased genome-wide RNAi screen approach has shown that depletion of TGIF1, HIF3A and PGAM2 are potential molecular markers of MTX resistance in ATRT. Screening for their occurrence may help to identify patients at high risk of MTX resistance and may also serve as targets for future novel therapeutics development.

BASIC BIOLOGY

BIOL-01. THE RELATIONSHIP OF BRAIN ENDOTHELIAL WNT SIGNAL INHIBITION ON BLOOD-TUMOR BARRIER INTEGRITY

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The blood-tumor barrier (BTB) is the primary site of nutrient and drug transport to tumor cells such as malignant gliomas. Yet, signaling pathways and factors influencing BTB permeability are poorly understood. Previous studies demonstrate the role of WNT/β-catenin signaling in establishing and fortifying blood-brain barrier integrity in a non-diseased state. Additionally, WNT proteins are highly expressed in gliomas and their surrounding vasculature. Thus, we propose inhibition of WNT/β-catenin signaling at the brain endothelium of malignant glioma can impair BTB integrity to enhance permeability for select cytotoxic agents. We *used* immortalized mouse brain endothelial cells (bEnd.3), akin to brain tumor endothelium, treated for 24 hours with WNT inhibitors (ICG-001, IWR-1, and LGK974). Inhibition of WNT/β-catenin signaling was confirmed by gene expression of transcription factors (*Tcf4* and *Birc5)*. Cell viability was confirmed by CellTiter Glo®. Brain endothelial cell-cell interaction was evaluated by cell impedance and resistance via the Agilent xCELLigence and ABP TEER24 systems. Using qPCR and flow cytometry, we observed changes in expression and function of Abcb1 and Abcg2 transporters. Using an *in vitro* BTB (bEnd.3 cells and mouse H3.3WT/K27 glioma cells) we evaluated the effect of WNT inhibition on permeability and glioma viability. We found that *all the* inhibitors downregulated *Tcf4* and *Birc5* in brain endothelium dose-dependently. Viability with inhibitors demonstrated an IC_{50} of 28μM for ICG-001, and 42μM for both IWR-1 and LGK974. Endothelial cell-cell interaction was transiently decreased by approximately 50% with all inhibitors at 30 minutes; increasing closer to baseline after 2-4hrs. All WNT inhibitors dose-dependently decreased Abcg2 transporter expression and function. While *In vitro* BTB studies are ongoing, preliminary findings demonstrate increasing permeability of BTB amongst H3.3K27 glioma cells. Our results demonstrate potential of WNT inhibitors to modulate BTB integrity and drug efflux function. More studies are warranted to explore WNT/β-catenin signaling inhibition on BTB *in vivo*.

BIOL-03. PROTEIN TRANSLATION FROM NON-CODING GENOMIC LOCI PRODUCE BIOLOGICALLY-ACTIVE PROTEINS IMPLICATED IN CANCER CELL SURVIVAL IN PEDIATRIC BRAIN TUMORS

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Protein translation is both a fundamental cellular process essential for life as well as an oncogenic mechanism employed by tumors to enact cancer cell biology. While protein translation is most readily manifest in the ~20,000 known human protein coding genes, there are, in fact, several thousand additional regions of the cancer genome that are translated and contribute the complexity of the molecular milieu of cancer. Here, we systematically addressed the question of whether such uncharacterized genomic regions encode truly biologically active proteins and applied these findings to pediatric brain tumors. We experimentally interrogated 553 candidates selected from non-canonical open reading frame (ORF) datasets. Of these, 57 induced viability defects when knocked out in a broad array of human cancer cell lines. Upon ectopic expression, 257 showed evidence of protein expression and 401 induced gene expression changes. CRISPR tiling and start codon mutagenesis indicated that their biological effects required translation as opposed to RNA-mediated effects. We characterized several of these in the context of pediatric brain tumors, where dense CRISPR tiling screens revealed unique functional relevance of dozens of non-canonical ORFs in pediatric brain cancer cell survival. We found that one of these ORFs, ASNSD1 uORF, encodes a well-folded protein whose translation is a selective genetic dependency distinct from the adjacent ASNSD1 annotated protein. *In vitro* molecular biology assays confirmed the MYC-amplified medulloblastoma cell lines had a heightened dependency on this protein, and that MYC binds to the promoter of this gene, with MYC expression correlating with ASNSD1 in patient tumors. Co-immunoprecipitation assays defined ASNSD1 uORF as a novel member of the prefoldin complex of cytoplasmic protein stability regulators. Overall, our experiments suggest that the abundant protein translation found in the "non-coding" genome may produce biologically active non-canonical ORFs that are potential therapeutic targets.

BIOL-04. CYTOPLASM PROTEIN GFAP MAGNETIC BEADS CONSTRUCTION AND APPLICATION AS CELL SEPARATION TARGET FOR BRAIN TUMORS

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Background: It is very important to develop a highly efficient cerebrospinal fluid (CSF) detection system with diagnosis and prediction function, for which the detection of circulating tumor cells (CTCs) in CSF is a good choice. In contrast to the past use of epithelial EpCAM as CTCs separation target, a cytoplasm protein of GFAP antibody was first selected to construct highly-sensitive immunomagnetic liposome beads (IMLs). The validation and efficiency of this system in capturing CTCs for brain tumors were measured both in vitro and in vivo. The associations between the numbers of CTCs in patients with their clinical characteristics were further analyzed. Results: Our data show that CTCs can be successfully isolated from CSF and blood samples from 32 children with brain tumors. The numbers of CTCs in CSF were significantly higher than those in blood. The level of CTCs in CSF was related to the type and location of the tumor rather than its stage. The higher the CTCs number is, the more possibly the patient will suffer from poor prognosis. Genetic testing in GFAP CTC-DNA by sanger sequencing, q-PCR and NGS methods indicated that the isolated CTCs (GFAP+/EGFR+) are the related tumor cell. For example, the high expression of NPR3 gene in CSF CTCs was consistent with that of tumor tissue. Conclusions: The results indicated that GFAP-IML CTCs isolation system, combined with an EGFR immunofluorescence assay of antitumor marker, can serve as a brand-new method for the identification of CTCs for brain tumors. Via lumbar puncture, a minimally invasive procedure, this technique may play a significant role in the clinical diagnosis and drug evaluation of brain tumors.

BIOL-05. MAPK PATHWAY INHIBITION SENSITIZES TO IMMUNOTHERAPY IN BRAF-MUTANT GLIOMAS

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Background: BRAF alterations frequently occur in pediatric low-grade gliomas. Previously, we showed that dabrafenib and trametinib (D+T) that target MAPK pathway can mediate the antitumor effect in a preclinical model of BRAF-mutant glioma (PMC5342782). Here, we further