

SP5

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Novel binary targeting molecule enhances radiation response in glioma model by induction of DNA damage and delay of DNA repair

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The most common and deadliest primary brain tumor derives from the cells that support neurons in the brain and is called glioblastoma multiforme (GBM). Although glioblastomas are known to be one of the most radioresistant types of tumor, development of new targeted drugs and their combination with radiotherapy have spawned renewed interest and enthusiasm for investigating new treatments possibilities. The aim of this study was to investigate the effect of ZRBA1, EGFR/DNA damage binary targeting molecule, in combination with radiation on proliferation of human glioma cells. The effect of ZRBA1 on the radiosensitivity of U87 and U373 cell lines was evaluated using clonogenic assays and spheroid migration assay whereas DNA damage and cell cycle progression were evaluated by FACS assays. Our data revealed that exposure of GBMs cells to ZRBA1 before irradiation resulted in an increase in radiosensitivity with dose enhancement factors at surviving fraction of 0.1 ranging from 1.3 to 1.7. Additionally, such combinational treatment caused strong cell cycle arrest in the G2/M phase (up to 72h post-treatment), which was accompanied by increased level of phosphorylated H2AX histone (gamma-H2AX). Importantly, in contrast to Temozolomide which enhances radiation response most effectively in MGMT-negative cells, radio-sensitizing properties of ZRBA1 does not depend on the MGMT methylation status. Overall, we demonstrated that ZRBA1 can enhance tumor cell radiosensitivity in vitro and suggest that this effect could be related to an inhibition of DNA repair. Therefore we postulate that ZRBA1 may be developed as a potent and innovative radio-sensitizing agent to treat malignant glioma tumors.

SP6

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Preclinical Evaluation of Oral Metronomic Topotecan and Pazopanib for the Treatment of Aggressive Extracranial Pediatric Solid Tumors

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Background: Metronomic chemotherapy with VEGF pathway inhibitors is a highly effective strategy to inhibit angiogenesis and

tumor growth. We tested efficacies of daily oral metronomic topotecan and pazopanib, as monotherapy and combination, in three pediatric extracranial tumors mouse models; and the effect of prolonged therapy with the combination on tumor behaviour in a neuroblastoma mouse xenograft model. Findings: In vivo, the combination demonstrated significant anti-tumor activity compared to respective single agents in all models. Reductions in viable Circulating Endothelial Progenitors and tumor microvessel density were correlated with tumor response and therefore confirmed the antiangiogenic activity of the regimens. However, combination also caused significantly higher myelotoxicity. For studying the tumor behaviour to our therapy, a time-response study (28, 56 and 80 days) was conducted in SK-N-BE(2) xenograft model. We found that only combination-treated animals survived till 80 days. However, tumors in these animals started growing gradually after 50 days. Unlike single agents, all three durations of combination significantly lowered microvessel densities, compared to control; with higher pericyte coverage after 56 and 80 days. The combination increased hypoxia, proliferation and glycolysis in the tumor. Conclusion: Combination of metronomic topotecan and pazopanib has superior efficacy than either single agents, which is attributed to superior antiangiogenic activity. However, prolonged treatment with combination can have additive myelotoxicity and may encounter adaptive resistance in neuroblastoma, associated with metabolic reprogramming and increased proliferation of the tumor cells.

SP7 Withdrawn

SP8

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Glioma stem cell specific microRNA-mRNA interaction network

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The role of cancer stem cells in tumor formation and tumor heterogeneity is currently one of the most researched topics in cancer biology. A better understanding of molecular mechanisms regulating the biology of cancer stem cells may ultimately help provide a better management of cancer patients. Various individual or families of microRNAs have been shown to have oncogenic or tumor suppressor function in glioblastoma (GBM). MicroRNAs have functional relevance in regulation of critical genes and pathways implicated in maintenance of glioma stem cell (GSC) properties. To avoid inclusion of inherent bias of miRNA-target prediction algorithms, we have applied biochemical methods to establish direct miRNA-mRNA interaction network relevant and specific to GSCs. We have generated an unbiased global miRNA mediated RNA-RNA interactome by performing RNA-sequencing all RNA species

(small and large RNAs) isolated from AGO2-miRISC (microRNA-induced silencing complex) of GSCs and normal human neural stem cells (hNSCs). Additionally, we have also established this interactome after exposure of GSCs and normal hNSCs to hypoxia, a key tumor micro-environmental factor that is known to be pivotal in generating GBM heterogeneity. The rank order list of miRNA-mRNA interaction nodes generated from RNA sequence reads reveals that enrichment of specific RNAs in functional AGO2-miRISC is not a direct function of their relative abundance in cells, thus this biochemically generated interactome is distinct from that generated by bioinformatics tools. We demonstrate that scope and influence of GSC specific miRNA-mRNA network and specific nodes of this interactome varies with hypoxia and tumor region in GBMs.

SP9

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Nilotinib inhibits pediatric high-grade glioma cell growth by blocking PDGFR

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Solid tumours arising from malignant transformation of glial cells are one of the leading causes of central nervous system tumor related death in children. Tumor recurrence in spite of rigorous surgical and chemoradiation therapies remains a major hurdle in management of these tumors. Here, we have investigated the efficacy of second-generation receptor tyrosine kinase (RTK) inhibitor nilotinib as therapeutic option for management of pediatric gliomas. We have utilized two independent pediatric high glioma cell lines with either high platelet-derived growth factor alpha (PDGFR α) or high PDGFR α expression in our in vitro assays to investigate the specific downstream effects of Nilotinib treatment of these cells. Using in vitro cell based assays we show that nilotinib inhibits PDGF-BB dependent activation of PDGFR. We further show that nilotinib is able to block cell proliferation and anchorage dependent growth via blockade of AKT and ERK1/2 signaling pathways. Our results suggest that nilotinib may be effective for management of PDGFR α dependent group of pediatric gliomas.

SP10

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Neurodevelopmental implications of DLX2 homeobox gene expression in human gangliogliomas

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Introduction: Gangliogliomas are low-grade, well differentiated neuroepithelial tumours of the central nervous system (CNS) comprised of neoplastic glial and neuronal cells. From microarray data, gangliogliomas overexpress the homeobox gene DLX2 required for differentiation and migration of inhibitory interneurons in the embryonic forebrain. We are interested in the role DLX2 plays in specifying neural progenitor fate. We hypothesize that in CNS progenitors, DLX2 promotes neural cell fate while simultaneously repressing glial fate. Methods: DLX2 expression was examined in a cohort of ganglioglioma FFPE sections using immunohistochemistry and immunofluorescence labelling. To examine co-localization of DLX2 with a glial specific marker, double immunofluorescence staining of DLX2 with glial fibrillary acidic protein (GFAP) was carried out. Results: Out of 30 patient samples examined, 10 samples expressed DLX2. Double immunofluorescence studies with GFAP determined that DLX2 co-localizes with GFAP expressing cells. Conclusions: Although DLX2 was not expected to co-localize with GFAP, as we hypothesized that DLX2 represses glial cell fate, GFAP may also be expressed in CNS progenitors specified to become neurons. To verify GFAP expressing cells are indeed from a neuronal lineage, co-expression studies with DLX2 and established markers for neurons, including synaptophysin and NeuN, will be carried out. In addition, co-expression of DLX2 with nestin and OLIG2, a marker for oligodendroglia, will be examined.

SP11

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Glia maturation factor (GMFb) promotes glial and neuronal tumor cell differentiation

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Introduction: GMFb was identified as a factor promoting glial cell process outgrowth in vitro and is predicted to be a member of the actin depolymerization factor (ADF) family. GMFb is highly expressed in the nervous system, with cytoplasmic expression in neurons and glia. We sought to understand the role of GMFb in CNS development and in gliomas. Methods: Anti-peptide antibodies to GMFb were generated. Co-immunoprecipitations (co-IP) were performed with actin antibodies. Glioma cells were treated with cytochalasin D to depolymerize actin or with colchicine to disrupt microtubules. Cis-retinoic acid (RA) was used to promote neurite outgrowth. Phosphorylation status of GMFb was ascertained using Western blots. Results: Co-IP experiments