MEDU-44. TARGETING SHH SIGNALING VIA PI3K/MTOR INHIBITION IN MEDULLOBLASTOMA AND EWING SARCOMA

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BACKGROUND: The phosphoinositide 3-kinase (PI3K) and sonic hedgehog (SHH) pathways play important roles in medulloblastoma (MB) and other pediatric cancers. Aberrant activation of the PI3K pathway has been shown to be an important regulator of cancer cell proliferation, metabolism, protein synthesis and apoptosis. Additionally, mTOR activation contributes to therapy resistance likely in part through SMO-independent activation of the transcription factor GLI. Thus we sought to evaluate the effects of combined PI3K/mTOR inhibition in medulloblastoma and Ewing sarcoma. METHODS: Medulloblastoma (DAOY and D556) and Ewing Sarcoma cell lines (TC71, RDES) were grown in 2-D culture to investigate the effects of pharmacologic inhibition of PI3K and mTOR (using BYL-719, a p110α isoform specific PI3K inhibitor and OSI-027, a catalytic inhibitor of mTOR1/2, respectively) on protein kinase signaling, cell proliferation, colony formation, intracellular localization of GLI, and GLI target gene expression. RESULTS: Of all four class I PI3Ks only the p110α isoform is required for MB cell proliferation and colony formation. Pharmacologic targeting of the p110α isoform of PI3K with BYL-719 synergized with the catalytic mTORC1/2 inhibitor OSI-027, resulting in inhibition of effector pathways, and blocked cell proliferation and colony formation in both medulloblastoma and Ewing Sarcoma. Moreover, dual PI3K/mTOR inhibition resulted in decreased nuclear localization of GLI and reduced GLI target gene expression in both pediatric tumor cell lines. CONCLUSIONS: Inhibition of p110α and mTOR exerts potent antineoplastic effects on cancer cell proliferation and transformation. This effect may be due in part to inhibition of GLI nuclear localization. Thus, besides established roles in inhibition of cancer cell proliferation and protein synthesis, dual inhibition of p110α and mTOR is particularly promising for targeting SHH-driven cancers such as medulloblastoma and Ewing sarcoma.

MEDU-46. IDENTIFICATION AND CHARACTERIZATION OF TUMOR-INITIATING CELLS IN RECURRENT MEDULLOBLASTOMA

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Medulloblastoma represents the most common childhood brain tumor. Despite aggressive multimodal therapy, a significant proportion of surviving patients suffer from severe treatment-related side effects, including tumor relapse. Thus, there is an urgent need for novel therapeutic modalities that can improve patient survival while minimizing tumor recurrence. Our recent study identified GNAS as a novel tumor-suppressor gene in Sonic hedgehog-driven medulloblastomas (Ref.1). Low expression or mutation of GNAS was tightly correlated with poor prognosis of Sonic hedgehog-group tumors and their resistance to the treatment of Sonic hedgehog signaling inhibitors alone. Ablation of the single GNAS gene in anatomically-distinct progenitors in the ventricular zone of the cerebellum and dorsal brainstem is sufficient to induce medulloblastoma formation, recapitulating their human counterparts. Gsα activation suppresses Sonic hedgehog signaling by regulating both the cAMP-dependent PKA pathway and ciliary trafficking of Hedgehog pathway components. Elevation of a Gsα effector, cAMP, effectively inhibits tumor growth in GNAS mutants. Strikingly, we find that in the drug-resistant tumor lesion and recurrent tumors after radiation therapy, there is an increase of Olig2 expression and Olig2+ progenitor cells. High levels of Olig2 are correlated with poor prognosis of medulloblastomas in human patients. We show that elimination of the mitotic Olig2-expressing cells blocks tumor progression in Olig2-TK transgenic mice. In addition, cell type-specific deletion of Olig2 halts tumor progression. Moreover, we find that Olig2 activates gene regulatory networks inferred for tumor cell stemness and regulates chromatin accessibility of oncogenic factors by altering epigenome landscapes. Furthermore, our single cell analyses reveal that Olig2-expressing progenitors are the distinct tumor-initiating cells during the onset of primary and recurrent medulloblastoma, highlighting potential therapeutic avenues for treating recurrent medulloblastomas by targeting Olig2-expressing progenitors. Ref.1: He, X. et al., "The G protein-alpha subunit Ga is a tumor suppressor in Sonic hedgehog-driven medulloblastoma". Nat Med 20: 1035–1042.

MEDU-47. PEROXIREDOXIN1 IS A THERAPEUTIC TARGET IN GROUP-3 MEDULLOBLASTOMAS

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Group-3 Medulloblastomas (MBL) has the worst prognosis due to its resistance to radiation and chemotherapy with a 5-year survival of 30%. Thus, there is an urgent need to elucidate targets that can sensitize Group-3 tumors to conventional treatments. We identified PRDX1 as a candidate therapeutic target for therapy sensitization in group-3 tumors. PRDX1 catalyzes the conversion of hydrogen peroxide to water and oxygen. We hypothesized that inhibiting PRDX1 would lead to oxidative stress and increase susceptibility to ionizing radiation via extensive DNA damage. Accordingly, when PRDX1 was targeted using Adenanthin (specific chemical inhibitor) or RNAi, Group-3 MBL (D425-MED) cells were rendered hypersensitive to radiation. Mechanistically, targeting PRDX1 resulted in an increase in reactive oxygen species, extensive oxidative DNA damage and an induction of the apoptotic pathway. Similarly, overexpression of PRDX1 in MBL cells susceptible to radiation (DAOY, UW-228) resulted in radiation resistance. However targeting PRDX1 in normal astrocytes did not have any sensitization effects. The in-vitro results were validated in-vivo using flank tumors (Adenanthin) and an orthotopic murine model (both RNAi and Adenanthin) using Group-3 MBL cells (D425-MED) and patient derived xenografts (MB3W1). Briefly, mice bearing Group-3 MBL tumors (D425-MED / MB3W1) when subjected to treatment with Adenanthin combined with radiation achieved a synergistic increase in survival. To fully evaluate the therapeutic potential of PRDX1 across all groups of MBL, we determined the expression of PRDX1 in a validated MBL tumor micro-array (TMA) by immunohistochemistry and correlated it with patient characteristics, therapeutic response and clinical outcomes. We also evaluated the role of PRDX1 in Group-3 MBL stem cells with respect to radiation resistance, invasion and migration. The results from these experiments will be presented in the meeting. Our data suggest that PRDX1 is a therapeutic target in Group-3 MBL and Adenanthin as a small molecule inhibitor of PRDX1.

MEDU-48. MRI TEXTURAL FEATURES CAN DIFFERENTIATE PEDIATRIC POSTERIOR FOSSA TUMORS

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BACKGROUND: Medulloblastoma (MB), Ependymomas (EP), & Gliomas are the most common posterior fossa (PF) tumors in children. Reliable, non-invasive biomarker that can distinguish these tumor types would significantly aid clinical management. We recently developed a new computerized descriptor, CoLlAGe, that distinguishes subtly-different pathologies in adult brain tumors. CoLlAGe measures the orientation of local intensity gradients as an estimate of lesion heterogeneity. In this work, we hypothesize that CoLlAGe can identify subtle micro-architectural differences between MB, EP and Glioma on routine MRI scans. METHODS: A retrospective cohort of 59 studies (MB = 22, EP = 12, Glioma = 25) was acquired. After registration, scans were age normalized, skull-stripped, and corrected for intensity inhomogeneities. Every lesion was then expert delineated to obtain a region of interest (ROI). 52 CoLlAGe features were extracted from every ROI for every MRI sequence (T1w, T2w, FLAIR). Wilcoxon rank sum test was used to identify top 10 CoLlAGe features within a training set of 30 patients (MB = 11, EP = 6, G = 13), for differentiating MB from (EP+Glioma), & Glioma from (MB+EP). Using top features, random forest classifiers were built for every sequence, and validated on an independent set of 29 studies (MB = 11, EP = 6, G = 12) by using area under receiver operating characteristic curve (AUROC). RESULTS: On the independent set, we found that sum variance and entropy of CoLlAGe ($p < 0.002$) on T2w protocol could reliably distinguish MB from (EP+Gliomas) (AUROC = 0.88), and entropy and sum average of CoLlAGe on T2w ($p < 0.0003$) could differentiate Gliomas from (MB+EP) (AUROC = 0.72). CONCLUSION: Our results suggest that entropy feature of CoLlAGe, a measure of tumor heterogeneity, enabled discrimination of MB from (EP+Glioma). Prospective validation of these features in a larger cohort may improve personalized treatment management for PF tumors.