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BACKGROUND: Understanding how aberrant transcription factors (TFs) hijack normal development to induce oncogenesis is a critical question in oncology. Forkhead box (FOX) proteins are a superfamily of transcriptional regulators characterized by a forkhead DNA-binding domain. Within this family, *Forkhead Box R2* (*FOXR2*) has been identified as a candidate structural variant (SV) driver in a subset of pediatric cancers including CNS embryonal tumors and peripheral neuroblastoma. While FOXR2 has been shown to stabilize MYC isoforms, the mechanistic details through which it enhances tumor formation, other non-SV mechanisms of activating aberrant expression, and the true extent of its role as an oncogene across all cancers have not been systematically evaluated. METHODS: We applied an integrative approach using transcriptomics, epigenetics, in vitro cancer models, and in vivo mouse models to systematically evaluate the mechanisms by which *FOXR2* is activated across human cancers. RESULTS: We performed a pan-cancer analysis of *FOXR2* activation across over 10,000 adult and pediatric cancer samples, and surprisingly found *FOXR2* to be aberrantly upregulated in 70% of all cancer types (including diffuse midline gliomas), and 8% of all individual tumors. *FOXR2* expression occurred predominantly in the absence of rearrangement/fusions, single nucleotide variants, or copy number aberrations at the DNA level. Transcriptomic and epigenomic analyses show the vast majority of tumors (78%) aberrantly express *FOXR2* through a previously undescribed epigenetic mechanism via hypomethylation of a novel promoter. Using both in vitro and in vivo models, we demonstrate that *FOXR2* expression is both sufficient and necessary for transformation across multiple lineages, including DMGs. **CONCLUSION**: Taken together, this study demonstrates that *FOXR2* is a novel and potent oncogene across pediatric and adult cancers, and highlights a new epigenetic mechanism by which its expression is activated.

DIPG-20. COPPER CHELATION THERAPY TARGETS S-ADENOSYLMETHIONINE (SAM) METABOLISM AND EPIGENETIC REGULATORS IN DIFFUSE INTRINSIC PONTINE GLIOMA (DIPG)

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DIPG is an incurable pediatric brain cancer of the ventral pons characterized by its complex epigenetic profile. Up to 81% of patients present with mutation H3K27M, resulting in the global reduction of H3K27 trimethylation and increased H3K27 acetylation, deregulating gene expression through an aberrant pattern of epigenetic modification. These alterations affect many cellular and metabolic mechanisms, complicating the search for effective targeted therapeutic strategies. Copper is highly abundant in the pons and is essential for normal brain function and development. However, excess copper accumulation is implicated in several neurological diseases and cancers. Incidentally, recent investigations have indicated the H3-H4 dimer can interact with copper acting as a reductase enzyme. Copper chelation therapy is clinically approved for pediatric patients with Wilson's Disease, improving their neurological symptoms, and is being trialed in several cancers. We therefore hypothesized copper chelation may represent an effective therapeutic strategy for DIPG. Copper chelator tetraethylenepentamine (TEPA) decreased cell growth and induced apoptosis in DIPG cell lines. To understand these results, unbiased RNA-seq and metabolomics analyses were performed, revealing downregulation of EZH2, DNMT1 and DNMT3B, upregulation of KDM6B and the disruption of key enzymes in the S-adenosylmethionine (SAM)-cycle. Importantly, TEPA downregulated SAM, which donates methyl groups for methylation, S-adenosylhomocysteine, its post-methylation product and α-ketoglutarate, a co-factor for KDM6B. Western blots confirmed the reduced expression of EZH2, DNMT1 and DNMT3B, with further blots examining the chromatin cell fraction revealing modulation of H3K27 trimethylation through copper or TEPA stimulation, and reduction of H3K27 acetylation by TEPA. Importantly, in vitro combinations with Panobinostat were synergistic, while in vivo investigations demonstrated TEPA improved survival in an orthotopic patient derived xenograft (PDX) model, showing complete tumor regression in 25% of treated mice. This study indicates a novel use for copper chelators as epigenetic drugs, and their potential as therapeutics for DIPG.

DIPG-21. DIPG CELLS ALTER THE PERMEABILITY OF THE BLOOD-BRAIN BARRIER IN THE BRAINSTEM LEADING TO TREATMENT FAILURE.

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Diffuse Intrinsic Pontine Glioma (DIPG) is an aggressive paediatric high-grade glioma with no effective therapies. The blood-brain barrier (BBB) presents a significant obstacle for delivery of therapeutics into the brain, especially in the brainstem. This study aims to investigate the effect of DIPG cells on the BBB in the brainstem. We hypothesized that the location of DIPG may result in a less permeable BBB than other brain regions. We compared two independent orthotopic models with the same DIPG cells injected in the cortical region or brainstem. We found that treatment with mTOR inhibitor, temsirolimus, significantly extended survival for cortical tumors compared with the same tumor in the brainstem (p=0.0097). Immunohistochemical analysis showed significant reduction of mTOR target, P-p70S6K, in the cortical region compared to brainstem in treated animals, with pharmacokinetic analysis confirming significantly higher temsirolimus levels in the cortical region. These findings suggest that cortical tumors respond better than brainstem tumors, and that a less permeable BBB in brainstem tumors contributes to treatment failure. To understand whether DIPG cells affect the BBB in the brainstem, single-cell RNA sequencing experiments were conducted on vasculature isolated from DIPG and matrigel-injected mice. Gene-ontology overrepresentation analyses identified downregulation in the P38MAPK pathway in endothelial cells from DIPG-injected mice, suggesting the potential for therapeutic manipulation with cytokines. Treatment with SNGR-TNFα, a derivative of an agent successfully used in improving drug penetration in CNS lymphoma patients, in an in vitro BBB/DIPG model significantly reduced transendothelial resistance, and further exploration into the effects on the BBB in vivo is currently being undertaken. Our studies indicate that the intact BBB in the brainstem in DIPG is a major reason for treatment failure, and DIPG cells directly influence the vasculature and response to treatment. This may lead to a novel DIPG treatment strategy and for other brain tumors.

DIPG-22. MODIFYING THE TUMOR MICROENVIRONMENT WITH A TIM-3 MONOCLONAL ANTIBODY AS A THERAPEUTIC STRATEGY FOR DIPGS

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Diffuse Midline Glioma, encompassing Diffuse Intrinsic Pontine Gliomas (DIPGs), are the most aggressive pediatric brain tumors. Their meager survival has not changed despite the combination of radiotherapy with targeted therapies emphasizing the urgent need for effective treatments. TIM-3 (HAVCR2) is a member of the T-cell im-