synapses, reduces glioma proliferation in patient-derived DIPG xenografts. This emerging understanding of brain cancer neurophysiology reveals new therapeutic targets and highlights commonly used drugs about which more study is required in this disease context.

HGG-05. NEURONAL ACTIVITY PROMOTES PEDIATRIC HIGH-GRADE GLIOMA GROWTH THROUGH A NLGN3-CSPG4 SIGNALING AXIS

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High-grade gliomas, including diffuse intrinsic pontine glioma (DIPG), are a lethal group of cancers whose progression is strongly regulated by neuronal activity {Venkatesh 2015}{Venkatesh 2017}{Venkatesh 2019}. One way in which glioma cells sense neuronal activity is via interaction with the ectodomain of post-synaptic adhesion protein neuroligin-3 (NLGN3), which is cleaved and released into the tumor microenvironment (TME) by the sheddase ADAM10. This interaction drives glioma growth, but the relevant binding partner of shed NLGN3 (sNLGN3) on glioma cells is currently unknown. Here, we report that sNLGN3 binds to chondroitin sulfate proteoglycan 4 (CSPG4), in turn inducing regulated intramembrane proteolysis (RIP) of CSPG4, and initiating a signaling cascade within DIPG cells to promote tumor growth. CSPG4 RIP involves activity-regulated ectodomain shedding by ADAM10 and subsequent gamma secretase-mediated release of the intracellular domain in healthy oligodendroglial precursor cells (OPCs), putative cells of origin for several forms of high-grade glioma {Sakry 2014}{Nayak 2018}. Incubation of high-grade glioma cells or healthy OPCs with recombinant NLGN3 is sufficient to augment ADAM10-mediated ectodomain release of CSPG4 and subsequent gamma secretase-mediated cleavage of the CSPG4 intracellular domain (ICD). Pre-treatment of glioma cells or OPCs with an ADAM10 inhibitor entirely blocks NLGN3-induced CSPG4 shedding. Acute depletion of CSPG4 via CRISPR gene editing renders glioma cells insensitive to the growth-promoting effects of NLGN3 application in vitro. We are now performing experiments to better discern how the CSPG4 ICD regulates signaling consequences downstream of sNLGN3 binding. In addition, we are using surface plasmon resonance to investigate whether the shed ectodomains of NLGN3 and CSPG4 remain in complex or only transiently interact. Altogether, our data form a critical missing link in understanding how glioma cells sense, translate and respond to neuronal activity in the TME and identify a new therapeutic target to disrupt neuron-glioma interactions.

HGG-06. EARLY GABAERGIC NEURONAL LINEAGE DEFINES DEPENDENCIES IN HISTONE H3 G34R/V GLIOMA

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High-grade gliomas harboring H3 G34R/V mutations exclusively occur in the cerebral hemispheres of adolescents and young adults, suggesting a distinct neurodevelopmental origin. Combining multimodal bulk and single-cell genomics with unbiased genome-scale CRISPR/Cas9 approaches, we here describe a GABAergic interneuron progenitor lineage as the most likely context from which these H3 G34R/V mutations drive gliomagenesis, conferring unique and tumor-selective gene targets essential for glioma cell survival, as validated genetically and pharmacologically. Phenotypically, we demonstrate that while H3 G34R/V glioma cells harbor the neurotransmitter GABA, they are developmentally stalled, and do not induce the neuronal hyperexcitability described in other glioma subtypes. These findings offer a striking counter-example to the prevailing view of glioma origins in glial precursor cells, resulting in distinct cellular, microenvironmental, and therapeutic consequences.

HGG-07. RADIATION INDUCED SENESCENCE IN DIFFUSE INTRINSIC PONTINE GLIOMA CELLS REVEALS SELECTIVE VULNERABILITY TO BCL-XL INHIBITION

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Diffuse intrinsic pontine glioma remains a devastating condition with a dismal five year survival rate less than 5%. New approaches for treating this aggressive disease are critical to driving progress. Conventional radiotherapy remains the cornerstone of treatment, with no chemotherapeutic agent found to improve survival. However, radiotherapy is often delivered as a palliative treatment, and disease often recurs 3–6 months after. Radiation causes DNA damage and oxidative stress yielding a senescent state of replicative arrest in susceptible cells. However, increasing evidence demonstrates malignant cells can escape senescence leading to tumour recurrence. Targeted ablation of non-replicating senescent tumour cells following radiation could negate tumour recurrence. It remains unknown whether DIPG undergoes senescence following radiation, and furthermore, whether senolytics can be utilised to target senescent DIPG cells. We employed radiation to induce a senescent state in primary human DIPG cell lines. Senescence was confirmed using SA-β-gal staining, lack of EdU incorporation and qRT-PCR to characterise the SASP in three primary human DIPG cell lines. RNA-sequencing on DIPG cells following radiation revealed senescence and SASP signatures. Viable cells that survive radiation were then utilised to screen candidate senolytic drugs, only Bcl-XL inhibitors demonstrated reproducible senolytic activity in radiation treated DIPG cells. Conversely, Bcl-2 inhibitors failed to show any consistent senolytic activity. These results demonstrate future possibilities of targeting radiation induced senescence in DIPG, using novel senolytic therapies and highlight Bcl-XL dependency as a potential vulnerability of surviving DIPG cells following exposure to radiation.

HGG-08. CREATION OF AN IN VITRO AND IN VIVO MODEL SYSTEM FOR THE STUDY OF H3.1K27M DIPG

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Diffuse Intrinsic Pontine Glioma (DIPG) is a devastating pediatric high-grade glioma that occurs in the brainstem with a median survival of less than 1 year. A greater understanding of the early tumorigenic events is essential for the development of effective therapeutics. DIPG is characterized by founder mutations in histone H3, either H3.1K27M or H3.3K27M. These mutations cause global hypomethylation, resulting in aberrant gene expression. Little is known about how this mechanism contributes to tumorigenesis. Interestingly, H3.1K27M DIPG show an increased incidence in females, whereas H3.3K27M DIPG shows no sex difference. This illustrates that the tumorigenic potential of H3.1K27M may be different between the sexes. Few models of DIPG incorporate the study of H3.1K27M despite the fact that it represents a unique opportunity to obtain valuable information on the tumorigenesis of DIPG through the study of the sex difference. Thus, we have created an *in vitro* and *in vivo* model system for H3.1K27M DIPG utilizing the RCAS mouse model system. This system utilizes RCAS vectors and a RCAS-ntva transgenic mouse line to deliver specific mutations to nestin expressing cells in the brainstem, including oligodendrocyte progenitor cells (OPCs), the predicted cell of origin. Delivering H3.1K27M, ACVR1 R206H, and PDGFaa at postnatal day 7 produces DIPG-like tumors *in vivo*, confirmed by H and E staining, between 60–110 days post injection. Additionally, confirmed through immunofluorescence staining, we can isolate a pure population of OPCs via immunopanning and infect them with RCAS vectors *in vitro* to produce stable expression of H3.1K27M. Introduction of H3.1K27M alone into male and female OPC cultures provides an opportunity to compare the early tumorigenic effects of H3.1K27M between the sexes *in vitro*. These results demonstrate that we have created an *in vitro* and *in vivo* H3.1K27M DIPG model system for the study of sex differences and tumorigenesis in DIPG.

HGG-09. TARGETING FACILITATES CHROMATIN TRANSCRIPTION (FACT) AS A NOVEL STRATEGY THAT ENHANCES RESPONSE TO HISTONE DEACETYLASE (HDAC) INHIBITION IN DIPG

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DIPG is an aggressive and incurable childhood brain tumour for which new treatments are needed. A high throughput drug screen of 3500 pharmaceutical compounds identified anti-malarials, including quinacrine as having potent activity against DIPG neurospheres. CBL0137, a compound modelled on quinacrine, is a novel anti-cancer compound which targets Facilitates Chromatin Transcription (FACT), a chromatin remodelling complex involved in transcription, replication, and DNA repair. CBL0137 effectively crosses the blood-brain barrier and has recently completed Phase I testing in adult patients. CBL0137 induced apoptosis in DIPG neurospheres and had profound cytotoxic activity against a panel of DIPG cultures. In a DIPG orthotopic model, treatment with CBL0137 significantly improved survival. We found that treatment with CBL0137 up-regulated TP53 and increased histone H3.3 acetylation and tri-methylation in DIPG cells. We therefore examined the interaction between CBL0137 and the histone deacetylase (HDAC) inhibitor panobinostat. *In vitro* experiments showed that the two agents had profound synergistic activity against DIPG neurospheres in clonogenic assays and enhanced caspase activation and apoptosis. The FACT subunit SSRP1 was found to directly interact with H3.3K27M and treatment with CBL0137 targeted this epigenetic defect, restoring histone H3.3 trimethylation and leading to tumor cell death. Transcriptomic analysis and immunoblotting indicated that combination treatment activated signalling pathways controlled by Retinoblastoma (RB)/E2F1 and subsequently increased phosphorylation and enzymatic activity of enhancer of zeste homolog 2 (EZH2). Consistent with the *in vitro* results, the combination of CBL0137 and panobinostat significantly prolonged survival in two independent orthotopic models of DIPG, while histological analysis showed restoration of H3K27me3 and decreased Ki67 positive cells. In addition to panobinostat, CBL0137 has been found to combine synergistically *in vitro* and *in vivo* with PARP and BET inhibitors. Given these promising results, a paediatric trial of CBL0137 will open through the Children's Oncology Group with an expansion cohort for DIPG patients.

HGG-10. THE BLOOD-BRAIN BARRIER IN DIPG: INVESTIGATING REGION-SPECIFIC DIFFERENCES IN PERMEABILITY

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Diffuse Intrinsic Pontine Glioma (DIPG) is the most aggressive pediatric high-grade glioma with median survival of only 12 months from diagnosis. Current therapies are essentially palliative. The blood-brain barrier (BBB) is a major obstacle, limiting delivery of effective chemotherapeutics into the brain. We hypothesized that tumors in the brainstem region have a BBB less permeable than tumors in other brain regions. We have confirmed the presence of an intact BBB in three orthotopic models of DIPG by Evans Blue extravasation assay. Immunohistochemical staining of CD13+ pericytes and CD34+ endothelial cells in healthy mouse brain compared to orthotopic DIPG model showed higher levels of both components in brainstem compared to cortical region. Single-cell RNA sequencing experiments are currently being undertaken to investigate region-specific differences in BBB cell populations and the impact of DIPG on signaling pathways that govern permeability. To determine if tumor location impacts therapeutic outcome, we performed *in vivo* efficacy studies with DIPG orthotopically injected into cortical region or brainstem region and treated with SAHA, HDAC inhibitor, or temsirolimus, mTOR inhibitor. Temsirolimus or SAHA was ineffective at extending survival in mice injected with DIPG in the brainstem compared to control. However, temsirolimus led to a significant improvement in survival in mice injected with DIPG cells in cortical region (median survival 85 days) compared to control (median survival 69 days ($P \leq 0.01$)). This suggests that the same tumor in cortical region may respond to systemic therapy that is ineffective in the brainstem and that the intact BBB in the brainstem is a major reason for treatment failure in DIPG. In conclusion, the BBB in the brainstem and in the presence of DIPG may be altered, changing signaling pathways that affect permeability. Understanding the brainstem cerebrovasculature may potentially lead to a novel strategy to treat DIPG as well as other brain tumors.

HGG-11. LEPTOMENINGEAL DISEASE AND TUMOR DISSEMINATION ALONG CSF PATHWAYS IN A MURINE DIPG MODEL: IMPLICATIONS FOR STUDY OF THE TUMOR-CSF-EPENDYMAL MICROENVIRONMENT

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Background: Leptomeningeal disease and hydrocephalus are present in up to 30% of patients with diffuse intrinsic pontine glioma (DIPG), however there are no animal models of cerebrospinal fluid (CSF) dissemination. As the tumor-CSF-ependymal microenvironment may play an important role in tumor pathogenesis, we identified characteristics of the Nestin-tumor virus A (Nestin-Tva) genetically engineered mouse model (GEMM) that make it ideal to study the interaction of tumor cells with the CSF and its associated pathways with implications for the development of treatment approaches to address CSF dissemination in DIPG. Methods: A Nestin-TVa model of DIPG utilizing the three most common DIPG genetic alterations (H3.3K27M, PDGF-B, p53) was used for this study. All animals underwent MR imaging and a subset underwent histopathologic analysis with H&E and beta-IV tubulin. Results: Tumor dissemination within the CSF pathways (ventricles, leptomeninges) was present in 76% (25/33) of animals, with invasion of the choroid plexus, disruption of the ciliated ependyma and regional subependymal fluid accumulation. Ventricular enlargement consistent with hydrocephalus was present in 94% (31/33). Ventricle volume correlated with region specific transependymal CSF flow (periventricular T2 signal), localized anterior to the lateral ventricles. Subependymal tumor cells were also present subjacent to the 4th ventricle in a post-mortem human specimen. Conclusions: This is the first study to report CSF pathway tumor dissemination an animal model of DIPG and is representative of CSF dissemination seen clinically. Understanding the CSF-tumor-ependymal microenvironment has significant implications for treatment of DIPG through targeting mechanisms of tumor spread within the CSF pathways.

HGG-12. HUMAN IPSC-DERIVED H3.3K27M NEUROSPHERES: A NOVEL MODEL FOR INVESTIGATING DIPG PATHOGENESIS AND DRUG RESPONSE

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Diffuse intrinsic pontine glioma (DIPG) is a subset of high-grade glioma that occurs predominantly in children and has no cure. Up to 80% of DIPG harbor a heterozygous point mutation that results in a lysine 27 to methionine substitution in histone variant H3.3 (H3.3K27M). Existing DIPG models have provided insight into the role of H3.3K27M but have limitations: genetically engineered murine models often rely on overexpression of the mutant histone to form tumors; patient-derived xenografts (PDX) are more genetically faithful but preclude examination of the effect of individual mutations on pathogenesis. To address these shortcomings and better recapitulate the genetics of human tumors, we designed a novel DIPG model based on human induced pluripotent stem cells (iPSC) edited via CRISPR to express heterozygous H3.3K27M. Edited iPSC were chemically differentiated into neural progenitor cells, which upon implantation into the brainstems of immunodeficient mice formed diffusely invasive tumors that were histologically consistent with high-grade glioma. Further, neurospheres cultured from primary tumors formed secondary tumors upon reimplantation with more diffuse invasion, suggesting *in vivo* evolution. To validate this model's relevance to DIPG transcriptionally, we performed RNA-sequencing on a cohort of primary and secondary tumor neurospheres (termed primary and secondary iDIPG) and compared them to published RNA-seq data from pediatric PDX and patient tumor samples. Hierarchical clustering and principal component analysis on differentially expressed genes ($P<0.05$) showed that H3.3K27M iDIPG cluster with H3.3K27M PDX and patient tumors. Further, ssGSEA showed that H3.3K27M iDIPG are enriched for astrocytic and mesenchymal signature genes, a defining feature of H3.3K27M DIPG. Finally, we found that primary H3.3K27M iDIPG neurospheres are sensitive to panobinostat, an HDAC inhibitor shown to be effective against H3.3K27M DIPG cells *in vitro*. Overall, these data suggest that H3.3K27M iDIPG are a promising tool for investigating DIPG biology and new therapeutic strategies.