ition to cycling populations and those associated with lineage-specificity, we identified 'aggressive' subpopulations defined by significant upregulation of immediate early response genes such as FOS/FOSL1/JUN, those associated with promotion of invasion-migration such as SERPINs and MMPs. These subpopulations could be mapped to isolated single-cell-derived subclones with highly proliferative or motile phenotypes, defined by comprehensive profiling of expressed and secreted proteins. Differential cis-regulation driving cell identity-tumorigenesis was found in one example to occur via a trans-histone mechanism mediated by an H4-lysine-methyltransferase, KMT5B. Application of functionally-defined interventional strategies aimed at disrupting the interactions between these subpopulations based upon evolutionary biology principles may offer a novel approach to treat these otherwise incurable tumours in children and young adults.

DIPG-42. DIFFUSE MIDLINE GLIOMAS, H3K27-ALTERED AS AN INTERDISCIPLINARY CHALLENGE

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INTRODUCTION: Diffuse midline gliomas represent a particular challenge in treatment. DIPG behave like highly malignant glioma with extremely poor prognosis due to location and inoperability. Molecular genetic studies, complementary to classical histopathology, have found an entity for midline gliomas, newly described ten years ago and entered the WHO classification in 2016. This group of childhood tumor with DMG's, H3K27-altered will be demonstrated in the following with 14 case reports from our clinic. METHODS: Clinical data of four patients with tectum/thalamic gliomas, six with diffuse intrinsic brainstem glioma, two with cerebellar, one with suprasellar, one with spinal glioma were retrospectively studied. MRI data, volume increase, contrast behavior was also analyzed. Tumor tissue was obtained by various surgical procedures and diagnostic workup included histopathology as well as genetics and epigenetics. RESULTS: 14 pediatric patients were treated from 2012 to 2021, median age 7,5 years. Leading symptoms were hydrocephalus, movement disorders, cranial nerve disorders. Four patients (29%) were partially resected, two (14%) received extended biopsy, seven (50%) were (stereo tactically) biopsied, one diagnosed by liquid biopsy (7%). Histological results revealed the presence of GBM in four cases (29 %). Subsequent methylome analyses confirmed that the tumors belonged to the group of diffuse midline gliomas, H3K27-altered. The other ten tumors (71%) were primarily assigned to this H3K27 group. CONCLUSION: The pediatric tumors of the brainstem, the further midline structures, including intraspinal manifestation show different MRI findings, histology, and clinical course. Complementary molecular genetic diagnosis is essential and a meaningful addition to the histological assignment. It is considered proven that the exclusivity of H3K27 – altered tumors of children and adolescents differs from that of IDH mutated gliomas and glioblastomas by their localization of hemispheric processes. Possible therapeutic approaches using targeted therapy require understanding of these oncological mechanisms.

DIPG-43. GLUCOSYLCERAMIDE SYNTHASE INHIBITORS INDUCE CERAMIDE ACCUMULATION AND SENSITISE H3K27 MUTANT DIFFUSE MIDLINE GLIOMA TO IRRADIATION

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BACKGROUND AND AIMS: Glycosphingolipids (GSL) are amphipathic lipids particularly abundant in the brain where their amount and expression patterns change drastically during the embryonic to postnatal stages and during tumorigenesis. The biosynthesis of GSL begins with the formation of glucosylceramide from ceramide, a step catalysed by the glucosylceramide synthase (UGCG). UGCG can be inhibited by eliglustat, which is used for treating children with Gaucher's disease. We have previously shown that the GSL composition is deregulated in H3K27M mutant diffuse midline glioma (H3K27M mut) and that eliglustat inhibits cell proliferation. Here we analysed the mechanism of action of eliglustat in H3K27M mut and its effect on irradiation. METHODS: The concentration of different components of the sphingolipid metabolism (ceramide, ceramide-1-Phosphate (CIP), sphingomyelin, Sphingosine and Sphingosine-1-Phosphate (S1P)) was assessed by mass spectrometry in the H3K27M mut cell line SF8628, before and after treatment with eliglustat. The combination of eliglustat with ionizing radiation was analysed by clonogenic assay. RESULTS: The treatment of H3K27M mut cells with eliglustat resulted in a significant increase in the concentration of ceramide, Sphingosine, C1P, but not S1P. The increase was concentration and time dependent and was not observed after longer incubation. Eliglustat treatment reduced the colony formation ability after irradiation. CONCLUSIONS: Ceramide is a known mediator of apoptosis involved in the molecular mechanisms underlying cellular response to irradiation. Increased endogenous ceramide levels, induced by blocking the synthesis of GSL, may sensitize H3K27M mut cells to irradiation. However, ceramide can be converted in C1P, a potent inhibitor of apoptosis and inducer of cell survival. Thus, the time and concentration dependent shift to ceramide and C1P requires further investigation in order to achieve an appropriate balance between the levels of these two metabolites and identify the optimal therapeutic window for combination with irradiation and potentially chemotherapy

DIPG-44. H3K27-ALTERED DIFFUSE MIDLINE GLIOMAS WITH SECONDARY DRIVER MOLECULAR ALTERATIONS

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INTRODUCTION: Large-scale sequencing led to the identification of driver molecular alterations such as FGFR1 and BRAF in occasional diffuse midline gliomas (DMGs) H3K27-altered, but their significance is not completely explored. We evaluated these associations in our institutional cohorts. MATERIALS AND METHODS: We searched our sequencing data base (2013-2020) for H3K27M-mutant gliomas and analyzed the co-occurring genetic alterations. The demographics, clinical information, and pathology were reviewed. Copy number profiles were evaluated using BioDiscovery's Nexus Copy Number software package. Oncoplots and Kaplan-Meier survival curves were generated with the maftools R package. RESULTS: We identified 77 patients (age range 2-68, median 26). The diagnosis was DMG (n=55), anaplastic astrocytoma/glioblastoma (n=19), low-grade glioma (n=1), low-grade glioneuronal tumor (n=1), and ganglioglioma (n=1). Recurrent alterations were seen in TP53 (n=42), ATRX (n=17), NF1 (n=15), PDGFRA (n = 4). Five cases had BRAF V600E (1 ganglioglioma; 4 DMG); twelve had FGFR1 mutations (9 DMG; 3 anaplastic astrocytoma/glioblastoma). The most common location in the BRAF group was the brainstem and in the FGFR1 was the thalamus. Survival ranged from 0 to 97 months, median 12.9 months (28.8 months for FGFR1 and 22.8 for the BRAF V600E). This was not significantly different from OS reported in the literature for DMG.The BRAF V600E ganglioglioma patient is alive 37 months after diagnosis. CONCLUSION: There was no significant difference in outcomes for patients with secondary molecular drivers when compared with conventional H3K27M DMG. The outcome of the BRAF V600E tumors seemed to correlate with the histology. These findings and the possibility of targeted therapy argue for comprehensive sequencing of H3K27-altered gliomas.

DIPG-45. RADIATION INDUCES A ROBUST INTERFERON RESPONSE IN DIFFUSE MIDLINE GLIOMA (DMG), IMPROVING THE POTENTIAL FOR COMBINATION IMMUNOTHERAPY

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Diffuse Midline Glioma (DMG), H3K27M altered, confers a dismal survival of 9-15 months and has a non-inflammatory tumor immune microenvironment (TIME). Radiation therapy (RT) is the mainstay treatment for DMG and has been shown in other cancers to recruit an immune component. However, the effect of RT on the DMG TIME has not been explored. In a syngeneic murine model of pontine DMG (PDGFB+, H3.3K27M, p53–/–), mice were treated with single fraction 15Gy RT or sham control, four mice per group. We performed single cell sequencing after CD45 isolation to evaluate the TIME 4 days post RT and compare to untreated tumor (sham control). Unsupervised clustering of 14,848 CD45+ cells revealed 16 immune cell subsets, most abundantly microglia at 75% of cells, with four subtypes representing a spectrum of homeostatic to activated. Microglia from RT are more concentrated in the activated subtypes with an upregulation of interferon response (i.e. Isg15, Ifit3) compared to untreated tumor with an increase in several interferon pathways using REACTOME. Consistent with