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Replication repair deficiency (RRD) is an important driving mechanism of pediatric high grade glioma (pHGG) occurring predominantly in the context of germline mutations in RRD-associated genes. Although pHGG present specific patterns of DNA methylation corresponding to driving oncogenic processes, methylation patterns have not been well studied in RRD tumors. We analyzed 52 RRD pHGG using either 450k or 850k methylation arrays. These arrays were compared with 234 PHGG driven by other genetic or epigenetic mechanisms and 10 additional pHGG samples known to be hypermutant. RRD pHGG displayed a methylation pattern corresponding to specific secondary mutations such as IDH1 and H3K27M. Strikingly, RRD pHGG lacking these known secondary mutations largely clustered together with a poorly described group previously labelled Wild type-C. Most of the hypermutant tumors clustered in a similar location suggesting undiagnosed RRD may be a driving force for tumors clustering in this location. Analysis of methylation patterns revealed that RRD pHGG displayed a unique CpG Island Demethylator Phenotype in contrast to the Methylator Phenotype described in other cancers. This effect was most concentrated at gene promotors. Prominent demethylation was observed in genes and pathways critical to cellular survival including cell cycle, gene expression, cellular metabolism and cellular organization. These data suggest that methylation profiles may provide diagnostic information for the detection of RRD pHGG. Furthermore, our findings highlight the unique natural selection pressures in these highly dysregulated, hypermutant cancers and provide novel impact of hypermutation and RRD on the cancer epigenome.

HGG-21. GERMLINE MUTATIONS IN *MSH2* GENE IN PEDIATRIC PATIENTS WITH CONGENITAL AND SPORADIC GLIOBLASTOMA

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INTRODUCTION: Glioblastoma (GBM) remains one of the biggest therapeutic challenges in neuro-oncology. In spite of multimodal treatment approaches the prognosis of GBM is extremely poor, median survival is estimated about 12–16 months. Although GBM is one of the most common and malignant primary brain tumors, pediatric glioblastoma, including congenital is a very rare tumor, with an incidence of about 1.1–3.4 per million live births. Moreover, the mode of presentation, behavior, response to therapy and molecular background of pediatric glioblastomas differs from adult type of GBM. Until now, about ten patients with congenital glioblastoma have been described and in none of them germline markers were examined. Here we report two patients with GBM, one with congenital tumor with germline mutations in *MSH2* gene. METHODS: Targeted Next-Generation Sequencing (NGS) of the probands DNA extracted from leucocytes was performed using the TruSight One sequencing panel on an Illumina HiSeq 1500. Applied gene panel investigated the coding sequence and splice sites of 4813 genes associated with known disease phenotypes. The NGS data were analyzed using an in-house procedure. Identified variants were validated by Sanger sequencing. RESULTS: NGS analysis of patients constitutional DNA revealed know, pathogenic variants c.940C>T and c.942 + 3A>T in *MSH2* gene (NM_000251.3) associated with MMR-dependent hereditary cancer syndromes. CONCLUSION: Molecular analysis are heavily needed for better understanding of pediatric GBM etiology and new treatment modality implementation. Identification of this oncogenic driver may provide insight into the pathogenesis of GBM, including congenital cases. Funded by National Science Centre, Poland (2016/23/B/NZ2/03064 and 2016/21/B/NZ2/01785).

HGG-24. HIGH-GRADE GLIOMA WITH A NOVEL FUSION GENE OF VCL-ALK

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A previously healthy 2-year-old boy presented with status epilepticus following intermittent vomiting. Computed tomography scan showed a 7 cm mass on the left occipital lobe with midline shift, inferior cerebellar herniation, and diffuse cerebral edema. The extensive dissemination to bilateral cerebral hemispheres, brain stem, and optic nerve was also observed. He underwent brain biopsy from the lesion on his left occipital lobe. The histopathological diagnosis determined the diffuse or epithelial proliferation of astrocytic tumor cells with high mitotic rate, positive for p53 and glial fibrillary acidic protein positive staining consistent with high-grade glioma. The progressive tumor led to communicating hydrocephalus, that was favorably controlled by cerebrospinal fluid shunting. The data from the FoundationOne CDx cancer genome profile disclosed a novel VCL-anaplastic lymphoma kinase (ALK) fusion in the tumor cells of the patient. ALK rearrangement was determined to be positive for the tumor cells assessed by fluorescence *in situ* hybridization. Only 4 pediatric cases of glioma with ALK-rearrangement have ever been reported. All of them received subtotal or gross total resections and then survived with or without chemotherapy. This is the first case of glioma harboring *VCL* as a novel partner of ALK fusion gene. After the favorable response to the first-line chemotherapy, subsequent irradiation therapy has now been scheduled. The molecular classification of high-grade glioma may help to expand the targeted therapy for unresectable advanced brain tumor.

HGG-26. H3G34V MUTATION AFFECTS GENOMIC H3K36 METHYLATION IN PEDIATRIC GLIOMA

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BACKGROUND: Histone H3.3 mutation (*H3F3A*) occurs in 50% of cortical pediatric high-grade gliomas. This mutation replaces glycine 34 with arginine or valine (G34R/V), impairing SETD2 activity (H3K36-specific trimethyltransferase), resulting in reduced H3K36me on H3G34V nucleosomes relative to wild-type. This contributes to genomic instability and drives distinct gene expressions associated with tumorigenesis. However, it is not known if this differential H3K36me3 enrichment is due to H3G34V mutant protein alone. Therefore, we set to elucidate the effect of H3G34V on genomic H3K36me3 enrichment *in vitro*. METHODS: Doxycycline-inducible short hairpin RNA (shRNA) against *H3F3A* was delivered via lentivirus to established H3G34V mutant pediatric glioma cell line KNS42, and H3G34V introduced into H3.3 wild type normal human astrocytes (NHA). Transfections were confirmed by western blot, fluorescent imaging, and flow cytometry, with resulting H3.3WT and H3K36me3 expression determined by western blot. H3.3WT, H3K36me3, and H3G34V ChIP-Seq was performed to evaluate genomic enrichment. RESULTS: Complete knockdown of H3G34V was achieved with DOX-induced shRNA, with no change in total H3.3, suggesting disproportionate allelic frequency of genes encoding H3.3 (*H3F3A* and *H3F3B)*. Modest increase in H3K36me3 occurred after *H3F3A*-knockdown from KNS42, suggesting H3G34V alone impacts observed H3K36me3 levels. Distinct H3K36me3 genomic enrichment was observed with H3G34V knock-in. CONCLUSIONS: We demonstrate that DOX-inducible knockdown of *H3F3A* in an H3G34V mutant pediatric glioma cells and H3G34V mutation transduction in wild-type astrocytes affects H3K36me3 expression. Further evaluation by ChIP-Seq analysis for restoration of wild-type genomic H3K36me3 enrichment patterns with H3G34V knockdown, and mutant H3K36me3 patterns with H3G34V transduction, is currently underway.

HGG-27. ANTI-CANCER POTENTIAL OF ARGINASE FOR HIGH-GRADE GLIOMA *IN VITRO* & *IN-VIVO*

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BACKGROUND: High-grade glioma is currently incurable. It was reported that glioma may be auxotrophic to arginine due to the lack of urea cycle genes expressions, suggesting arginase may be a potential agent for high grade glioma. AIM: We investigated the efficacy of pegylated arginase I (pegArg-I) or in combination with other anti-cancer drugs for high-grade glioma *in vitro* and *in vivo*. METHODS: 4 high-grade glioma cell lines (U87, U373, U138, D54) were treated with pegArg-I *in vitro*. The molecular mechanism of pegArg-I-induced cytotoxicity was tested in U87. The ultramorphological changes of pegArg-I-treated U87 was investigated by both scanning and transmission electron microscopy. Orthotopic glioma xenograft model with luciferase-transfected U87 cell line was tested for anti-cancer efficacy of peg-Arg I *in vivo*. RESULTS: We showed that pegArg-I

induced significant cell death in all 4 cell lines *in vitro*. Temozolomide, difluoromethyornithine and chloroquine (CQ) were then tested together with pegArg-I in U87 *in vitro*. We found that only CQ showed additive effect with pegArg-I against glioma *in vitro*. Such additive cytotoxic effect may be associated with enhanced autophagy and necrosis as shown in transmission electron microscopy and autophagy markers' expression by Western blotting. PegArg-I prolonged the survival of glioma mice, suggesting its possible anti-glioma efficacy. However, CQ+pegArg-I didn't show further significant anti-cancer efficacy *in vivo*. CONCLUSION: PegArg-I may be useful in slowing the progression of glioma, but additional drug candidate which works synergistically with pegArg-I remains to be explored.

HGG-29. A CASE OF CIRCUMSCRIBED HIGH-GRADE ASTROCYTOMA WITH ATRX AND CDKN2A/B ALTERNATIONS WHO WAS INITIALLY DIAGNOSED AS GLIOBLASTOMA AND HAS 20 YEARS SURVIVAL

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Pediatric high-grade gliomas are rare and often hard to classify, which grow locally and show longer survival than diffuse high-grade gliomas in adults. We report a case of circumscribed high-grade astrocytoma who was initially diagnosed as glioblastoma and has 20 years survival. A 7-year-old girl suffered from epileptic seizure due to a left occipital lobe tumor. The tumor was resected in another hospital and diagnosed as glioblastoma. The tumor disappeared after extended local irradiation and chemotherapy using nimustine hydrochloride (ACNU) and cisplatin (CDDP). Eighteen years after initial onset, first recurrence was confirmed as the intra-tumoral hemorrhage. The tumor was resected and diagnosed as anaplastic oligoastrocytoma. After 6 courses of temozolomide (TMZ), the tumor disappeared. Twenty years after initial onset, the second local recurrence was confirmed. Although gamma knife and TMZ was performed, the tumor did not disappear. The tumor was surgically resected. Histopathology showed localized growth with some infiltration and mitosis but lacked pseudopallisading and microvascular proliferation. The tumor was diagnosed as circumscribed high-grade astrocytoma. Immunostaining revealed ATRX nuclear loss and CDKN2A / B homozygous deletion. After 10 courses of TMZ, the third local recurrence was confirmed. The tumor was completely removed and has not occurred recurrence more than 3 months after the last operation. Circumscribed high-grade glioma is expected to survive longer than invasive glioma. Pediatric gliomas should differ from adult gliomas in the genes of tumorigenesis. Care should be taken for its diagnosis and treatments. We also need a new classification based on histology and gene profile. HGG-30, ANALYSIS OF PEDIATRIC GLIOMAS IN OUR INSTITUTE *Kaoru Tamura*, Mai Fujioka, Masae Kuroha, Motoki Inaji, Yoji Tanaka, Tadashi Nariai, and Taketoshi Maehara; Tokyo Medical and Dental University, Tokyo, Japan. PURPOSE: Recent advances in genetic interrogation of pediatric glioma increase the importance of molecular diagnosis using surgical specimen. However, surgical resection may be avoided to preserve quality of life, especially in brain stem glioma cases. We retrospectively examined diagnosis and treatment of pediatric gliomas in our hospital. METHODS: This study includes 14 consecutive glioma patients under the age of 18 who underwent initial treatment at our hospital from 2000 to 2019. Histopathological diagnosis, clinical course and molecular status such as IDH, H3F3A and BRAF were analyzed. RESULTS: 5 patients (1 pilocytic astrocytoma (PA), 3 diffuse astrocytomas, 1 oligodendroglioma were treated only by surgical resection (group A). 7 patients (1 PA, 1 anaplastic oligodendroglioma, 2 diffuse midline gliomas and 3 glioblastomas (GBM)) received radiation and/or chemotherapy after surgical resection (group B). 2 diffuse intrinsic pontine gliomas (DIPG) received radiation and chemotherapy without surgical resection (Group C). No IDH mutation was observed in all pathological specimen obtained cases. BRAF alteration was observed in all PA cases. 1 case of GBM had *BRAF V600E*mutation and the other had H3K27M mutation. During a median of 7.7 years of follow-up, group A patients have no recurrence. Group B includes various diagnosis and prognosis. 2 group C patients diagnosed DIPG by MRI showed different clinical courses. CONCLUSION: Pediatric gliomas include diverse biological subgroups and show broad range of clinical behavior. Since pediatric glioma has a low incidence and a wide variety of genetic mutations, multicenter study is important to improve the treatment of pediatric glioma.

HGG-31. UNIQUE BIOLOGICAL CHARACTERISTICS OF RADIATION-INDUCED GLIOMAS

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Radiation-induced gliomas (RIGs) are the most common secondary solid tumours with very unfavourable prognosis. We aimed to describe different clinical and molecular biological characteristic of RIGs from primary gliomas. We reviewed clinical data of ten patients with RIGs. In patients with available samples, we used the whole genome methylation array and performed targeted sequencing for specific mutations. Between 2000–2018, we diagnosed RIG in 10 patients (M/F 2/8) aged 5–12 years at primary diagnosis of different solid tumours and acute leukaemia. These patients developed RIG with a median 9.5 years (ranging 3–31) after primary diagnosis. Eight patients died within 1 year after diagnosis of RIG and 2 patients are still alive more than 4 years from this diagnosis. According to Heidelberg DNA methylation-based classification, most RIGs belong to the IDH-wild type glioblastoma subclass midline which biologically corresponds to diffuse midline glioma (DMG). However, compared to primary DMGs they do not carry the characteristic H3K27M mutation. One patient developed anaplastic ganglioglioma with BRAF-V600E mutation and methylation profile identical to pleomorphic xanthoastrocytoma (alive for 4 years after diagnosis of RIG). In half of the patients from the group DMGs IDH wild type, examined by methylation array, PDGFRA amplification was found. Our data shows that most RIGs are midline IDH-wild type glioblastomas with poor prognosis that are biologically different from primary DMGs. PDGFRA amplifications are potentially targetable by kinase inhibitors in order to order to prognosis of these patients.

HGG-32. UNCOVERING THERAPEUTIC VULNERABILITIES IN MISMATCH REPAIR-DEFICIENT GLIOMAS

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INTRODUCTION: We have observed that approximately 26% of recurrent gliomas acquire hypermutation following treatment with temozolomide (TMZ). Intriguingly, 91% of these tumors harbor mutations in mismatch repair (MMR) genes. Strategies to target MMR-deficient gliomas thus stand to impact a large number of patients. METHODS: We ablated the MMR genes *MSH2, MSH6, MLH1, and PMS2* using an all-in-one sgRNA-CRISPR/Cas9 expression vector to generate panels of isogenic MMR knockouts in patient-derived glioma cell lines. We have characterized the phenotype of these MMR-deficient glioma cells, and leveraged high-throughput drug screens to identify therapeutic vulnerabilities induced by loss of MMR. RESULTS: We demonstrate that sgRNA-CRISPR/Cas9 targeting of either *MSH2* or *MLH1* – the two obligatory components of the MutSα and MutLα complexes, respectively – also results in loss of protein expression of their respective binding partner MSH6 or PMS2. Moreover, we show that loss of each MMR component confers resistance to TMZ while maintaining sensitivity to the alkylating nitrosourea CCNU. Furthermore, we show that long-term TMZ treatment of *MSH2* and *MSH6* knockouts in an *MGMT*-methylated line induces hypermutation with enrichment of C > T mutations but not in MMR wild-type controls. Lastly, loss of MSH2 or MLH1 confers differential dependencies to small molecule inhibitors. CONCLUSIONS: CRISPR/Cas9 knockout of individual MMR pathway members allows us to systematically study the response of MMR-deficient cells to alkylating agents in an isogenic context. MMR deficiencies in glioma confer dependencies to small molecule treatment, which may inform future therapies for MMR-deficient tumors.

HGG-34. DETECTION OF ONCOGENIC FUSION EVENTS IN SUPRATENTORIAL GLIOBLASTOMAS OF YOUNG CHILDREN

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INTRODUCTION: Glioblastoma in infancy and early childhood is characterized by a more favorable outcome compared to older children, a stable genome, and the occurrence of tyrosine kinase gene fusions that may represent therapeutic targets. METHODS: 50 glioblastomas (GBM) with supratentorial location occurring in children younger than four years were