present study we have knocked out HK2 using CRISPR in patient-derived cultures (n=3) and the established cell line U251MG, to determine changes in the rate of cell proliferation and drug sensitivity. Additionally downstream expression changes were investigated via Qiagen profiler arrays, across 84 key genes involved in the regulation and enzymatic pathways of glucose metabolism. A substantial growth rate reduction between 38 to 44% (p<0.007) was demonstrated in CRISPR-modified cultures after 7 days compared to non-CRISPR cultures. Sensitivity to metformin was also significantly (p<0.0001) increased in response to HK2 knockout, where average ID50 values were 60% lower in cultures. Additionally CRISPR modified cultures yielded greater synergistic (CI<1) and additive effects (CI=1), with metformin and temozolomide combination treatment. Array data revealed an extensive change in downstream gene expression in CRISPR-modified cultures, between 25 to 48 genes were downregulated compared to the corresponding non-CRISPR cultures. Furthermore CRISPR-modified cultures demonstrated a similar reduction in downstream expression when compared to GBM biopsy tissue, conversely a greater number of genes had unchanged expression levels compared to normal brain tissue. This study demonstrates the predominant role of HK2 within the glycolytic pathway, with overexpression potentially key in driving the genetic alterations downstream. HK2 knockout revealed considerable ubiquitous reductions in downstream gene expression compared to GBM biopsy tissue and non-CRISPR cultures. Additionally an increase in drug sensitivity was depicted with the loss of HK2 signifying the potential of HK2 inhibition as a novel therapy in a significant subset of GBM.

PATH-34. VENTRICULAR-SUBVENTRICULAR ZONE CONTACT BY GLIOBLASTOMA IS NOT ASSOCIATED WITH MOLECULAR SIGNATURES IN BULK TUMOR DATA

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Whether patients with glioblastoma that contacts the stem cell niche of the ventricular-subventricular zone (V-SVZ+GBM) have a distinct survival profile from V-SVZ-GBM patients independent of other known predictors or molecular profiles is unclear. Using multivariate Cox analysis to adjust survival for widely-accepted predictors, hazard ratios (HRs) for overall (OS) and progression free (PFS) survival between V-SVZ+GBM and V-SVZ-GBM patients were calculated in 170 single-institution patients and 254 patients included in both The Cancer Genome (TCGA) and Imaging (TCIA) atlases. A multivariable analysis adjusted for age, Karnofsky performance score, IDH1 mutation, MGMT promoter methylation status, chemotherapy, radiation therapy, and extent of surgical resection revealed that V-SVZ contact was independently associated with decreased survival in both datasets (institutional patients: OS HR 1.55 [95% CI 1.03–2.33], P=0.037; PFS HR 1.57 [1.08–2.28], P=0.018; TCIA/TCGA patients: OS HR 1.69 [1.28–2.24], P<0.001; PFS HR 1.24 [0.91–1.7], P=0.18). Thorough TCGA molecular data analyses were conducted using differential molecular feature extraction, gene expression network construction, clustering methods, and dimensionality reduction. All analyses revealed that V-SVZ contact by GBM was independent of mutational, DNA methylation, gene expression, and protein expression signatures in the bulk tumor. Therefore, while survival of GBM patients is independently stratified by V-SVZ contact, with V-SVZ+GBM patients displaying a poor prognosis, the V-SVZ+GBMs do not possess a distinct molecular signature at the bulk sample level. Focused examination of the interplay between V-SVZ cytoarchitectural features, microenvironmental factors, and cancer cells within glioblastomas using subpopulation- or single-cell-based approaches is warranted.

PATH-35. FREQUENCY AND CHARACTERISTICS OF H3K27M-MUTATION IN ADULTS WITH RADIOGRAPHICALLY-DETERMINED MIDLINE GLIOMAS

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BACKGROUND: H3F3A mutations define the entity of Diffuse Midline Glioma, which was added to the WHO 2016 classification. There have been several reports describing the clinical, prognostic, and histopathological implications of this mutation. It is unclear, however, what proportion of adults with gliomas occurring in the midline have an H3 K27M mutation. We set out to define this in a single-institution, retrospective cohort study. METHODS: From 850 consecutive gliomas in adults we identified 163 cases with radiographically-determined midline gliomas (brainstem, thalamus, basal ganglia, corpus callosum, spinal cord, or cerebellum). Clinical cases were reviewed in accordance with IRB guidelines. FFPE tissue was obtained from 120 cases and stained for H3 K27M. RESULTS: A H3 K27M mutation was identified in 18 of 120 cases (15%). As compared to non-H3 K27M mutated tumor, average age was 45.1 ± 12.8 versus 53.1 ± 16.7 years (p=0.2). 56% were female (p=0.3). 83% had contrast enhancement on MRI (p = 0.79). All H3 K27M mutant tumors were WHO grade III or IV on histology, while non-mutant tumors encompassed all four grades (p = 0.08). The most common locations to have H3 K27M-mutated tumors were midbrain (2/2; 100%), pons (4/10; 40%), cerebellum (6/22; 27.3%), spinal cord (2/13; 15.4%), and thalamus (3/30; 10%). Median survival was 16 ± 6.0 months as compared to 8.1 ± 3.6 months in non-mutated midline high grade gliomas (p = 0.15). CONCLUSIONS: H3K27M mutated tumors are common in gliomas located along the midline and this molecular subtype should be considered in adults of all ages and grades with midline tumors, regardless of tumor location or contrast enhancement. Survival was not significantly different from non-H3 K27M mutated tumors, though a larger dataset will be necessary for confirmation.

PATH-36. IDH AND TERT PROMOTER MUTATIONS IN NON-DIAGNOSTIC BIOPSIES FROM GLIOMA PATIENTS

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BACKGROUND: Non-diagnostic biopsies are a recurrent issue in patients with a suspected brain tumor. Herein, in order to explore the utility of molecular testing in this setting, we determined whether IDH and TERT promoter (pTERT) mutations can be detected in non-diagnostic biopsies from glioma patients. METHODS: Using Snapshot PCR, we retrospectively assessed IDH and pTERT mutation status in 28 adult glioma patients in whom a first non-diagnostic biopsy had led to perform a second biopsy. RESULTS: Median age at diagnosis was 65 years and median delay between the first and second biopsy 21 days. The first biopsy consisted of not characterizable infiltrated glial cells (n=19), hemorrhage (n=4), necrosis (n=2) or normal tissue (n=3). The second biopsy demonstrated an IDH-wildtype glioblastoma (n=22), an IDH-wildtype astrocytoma (n=4), an IDH-mutant oligodendroglioma (n=1) and an IDH-mutant astrocytoma (n=1). A pTERT mutation was present in 21 cases. Retrospectively, the same IDH and pTERT mutations were identified in the non-diagnostic biopsies of the 2 patients with an IDH-mutant glioma and of 12 out of 21 patients with a pTERT-mutant glioma (57%). Overall an IDH and/or a pTERT mutation were detected in the non-diagnostic biopsies of 13 out of the 22 IDH-mutant and/or pTERT-mutant gliomas (59%) and in 13 out of the 28 cases included in the present series (46%). CONCLUSION: IDH and pTERT mutations can be detected in a high percentage of non-diagnostic biopsies from glioma patients supporting a role for molecular testing in the interpretation of non-diagnostic biopsies from patients with a suspected brain tumor.

PATH-37. LIQUID BIOPSY FOR IDENTIFICATION OF NEWLY DIAGNOSED GLIOMA

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INTRODUCTION: In patients with newly diagnosed intracerebral lesions based on MRI, gliomas are often suspected, but MRI is rarely definitive thus necessitating biopsy. For non-enhancing lesions involving eloquent or deep-seated structures, diagnosis can be especially challenging as biopsy may be relatively risky or undesirable to the patient. In this study, analysis of plasma isolated cell-free DNA and exosome mRNA and miRNA from newly diagnosed glioma patients and from cancer-free volunteers was used to predict disease. METHODS: Blood was drawn from 40 patients with newly diagnosed gliomas (28 high grade glioma (HGG), 12 low grade (LGG)) and 10 healthy volunteers without documented history of cancer. High quality DNA and RNA was isolated and sequenced using Next Generation Sequencing and Digital Droplet PCR was used for detection and verification of trace molecular artefacts. Multianalyte processing yielded data that was harmonized and interpreted through an Artificial Intelligence

based algorithm to assess for possible glioma and to assign grade. EGFRviii and IDH1 mutations were also analyzed and compared to molecular testing from tumor specimens. RESULTS: 97.5% (39 of 40) of glioma patients were deemed to have gliomas by plasma testing. 96% of HGG patients and 67% of the LGG patients were correctly graded. Of the 10 healthy controls, 8 were concluded to be cancer-free. Two of the patients were suspicious for malignancy, of which one was possible glioma. IDH1 and EGFRviii mutation had concordance at 74 % (26/35) and 59% (12/16), respectively. CONCLUSIONS: Analysis of plasma cell free tumor derived DNA and RNA was highly sensitive for detecting glioma with high agreement in grading as well. In patients with newly diagnosed intracerebral lesions, this may be a useful screening test to determine the need for more invasive testing, i.e. biopsy/resection. Further testing in blinded samples from brain tumor patients and healthy subjects will follow.

PATH-38. CORRELATION OF ALTERATION OF HLA-F EXPRESSION AND CLINICAL CHARACTERIZATION IN 593 BRAIN GLIOMA SAMPLES

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BACKGROUND: Human gliomas are highly fatal tumor with a significant feature of immune suppression. The immune system in glioma is gradually revealed, and immunotherapy is expected to improve the survival of glioma patients. With a deep understanding of the immune microenvironment of glioma, immunotherapy of gliomas has been increased exponentially in recent years. Searching for key regulators of immune response in glioma will provide clinical targets for immunotherapy. In our research, we focus on human leukocyte antigen (HLA) system, responsible for regulating the immune system, and discovered the relationship between HLA-F expression and clinical prognosis in gliomas. METHODS: A total of 593 gliomas patients are concluded in our research, 325 patients from Chinese Glioma Genome Atlas (CGGA) and 268 patients from GSE 16011 set. Kaplan-Meier (KM) analysis is performed to explore the prognostic value of HLA-F. T test analysis is used to find the distribution difference in various groups. R language packages are used for other statistical computations and figures drawing. RESULTS: HLA-F was significantly negatively correlated with overall survival (OS) in all grade gliomas and glioblastoma (GBM). Moreover, HLA-F was enriched in GBM and IDH1 wild-type group, and HLA-F was a mesenchymal subtype marker. Pearson correlation test showed that HLA-F was correlated with other HLA-I molecules. CONCLUSION: HLA-F expression was positively with malignant phenotype and negatively with OS indicating that HLA-F could predict the immune state in glioma, and might be a clinical target of glioma immunotherapy. Key Words: HLA-F; glioma; immunotherapy; OS

PATH-39. ASTROCYTOMA OF THE SPINAL CORD: A GENETIC CHARACTERIZATION AFTER MICROSURGICAL RESECTION

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INTRODUCTION: The revised version of the WHO classification system (2016) introduced molecular markers being of prognostic importance in gliomas. Primary spinal cord astrocytomas are very rare. Aggressive surgical resection is believed to be critical for extending progression free and overall survival. However, the prognostic significance of molecular variables remains unclear for these tumors. Herein we investigate molecular chances of spinal gliomas, which may allow more accurate risk stratification. METHODS: We performed genome sequencing in 10 spinal astrocytomas undergoing surgical resection between 2000 and 2017. These spinal astrocytomas include glioblastomas (WHO grade IV), anaplastic astrocytomas (WHO grade III), diffuse astrocytomas (WHO grade II) and pilocytic astrocytoma (WHO grade I). RESULTS: We identified 5 spinal glioblastomas, 1 anaplastic astrocytoma, 2 diffuse astrocytomas and 2 pilocytic astrocytomas. Median overall survival (OS) was 6 months (range: 2–14 months) for grade IV tumors, 33 months (range: 30–136 months) for grade II and III tumors and 95 months (range: 49–141 months) for grade I tumors, respectively. One grade II and one grade I tumor were carrying the IDH1 and IDH2 mutation, all other tumors were IDH wild type (OS: 93 vs. 10 months). Gross total resection was not achieved in any patient. 9 patients received adjuvant radiotherapy. The most current findings in spinal GBM were H3F3A mutations (5/5) and ATRX mutation (3/5). H3F3A mutation was observed in 1 WHO grade II tumor with a OS of 33 months. Mutation in H3F3A and WHO grade was associated with shortened OS in univariate analysis. WHO grade II tumors were found to have mutations in CCND2, DDX3X, EGFR, HIST1H3B, KIT, MYC, PDGFRA, PTCH2, SMARCA4 and TSC2. CONCLUSION: Genomic analysis of spinal astrocytomas provides an opportunity to identify potential clinically relevant information. These data indicate an association between H3F3A mutation and a shortened overall survival in spinal astrocytomas.

PATH-40. TARGETED NEXT GENERATION SEQUENCING (NGS) OF YOUNG ADULTS WITH ISOCITRATE-DEHYDROGENASE WILD-TYPE GLIOBLASTOMA (IDH-WT GBM) REVEALS NEGATIVE PROGNOSTIC IMPACT OF EPIDERMAL GROWTH FACTOR RECEPTOR AMPLIFICATION (EGFRAMP)

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BACKGROUND: Young adults with IDH-WT GBM represent a rare, understudied population compared to pediatric, IDH-mutant, or typical (elderly) GBM. We aimed to explore clinically detected genomic alterations in this population and their prognostic impact. METHODS: We identified patients ages 21 45 with newly diagnosed, previously untreated IDH-WT GBM whose tumors underwent NGS at our institution. Patients with hereditary cancer syndromes were excluded. The NGS panel detects pathogenic variants by targeted exome sequencing of 47 (2014–2016) or 153 (2016-present) genes. Clinical characteristics and overall survival (OS) were collected. These data were also collected from a contemporaneous cohort of older (>=65) patients with newly diagnosed, IDH-WT GBM. RESULTS: 28 young and 30 older patients were included. In young patients, 12 (43%) had an EGFR alteration [2 (7%) EGFR mutation, 7 (25%) EGFR amplification (EGFRamp), and 3 (13%) both EGFRamp and EGFRvIII]. Other mutations detected in 2 young patients were TP53 in 7 (25%), BRAF (V600E) in 3 (11%), RB1 in 3 (11%), PTEN in 2 (7%), SETD2 in 2 (7%), and DNMT3A in 2 (7%). Differences detected in older vs. younger patients were more frequent PTEN mutations (27% vs. 7%, p=0.049) and MGMT methylation (50% vs. 25%, p =0.06). In young patients, median OS was 19.5 months (95% CI 15.9–24.4), and EGFRamp was associated with inferior median OS (16.3 vs. 23.5 months, p=0.047). There was no difference in OS by EGFRamp in older patients. CONCLUSIONS: EGFRamp was associated with inferior OS in this contemporary cohort of young adults with IDH-WT GBM, whereas no association was detected in older patients. This suggests a potential role for targeting EGFR specifically in this population. In addition, consistent with prior studies, we found that MGMT methylation is less common in young patients with IDH-WT GBM, highlighting the need for alternatives to temozolomide.

PATH-41. PLASMA CELL-FREE DNA (cfDNA) CONCENTRATION IS INDEPENDENTLY ASSOCIATED WITH RADIOGRAPHIC TUMOR BURDEN IN NEWLY DIAGNOSED GLIOBLASTOMA (GBM) PRIOR TO INITIAL SURGICAL RESECTION

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BACKGROUND: Distinguishing between radiographic pseudoprogression and true tumor progression is challenging in patients with GBM. Recent data suggests that plasma cfDNA concentration may serve as a viable surrogate for tumor burden in other malignancies. We performed a pilot study to determine the feasibility of detecting cfDNA in patients with newly diagnosed GBM and explored its correlation with radiographic tumor burden and other relevant clinical variables. METHODS: We collected blood in Streck cfDNA tubes from patients with radiographically suspected high-grade glioma prior to planned initial surgical resection. Plasma was isolated using a 3-step centrifugation protocol. cfDNA was extracted from using a QIAamp Circulating Nucleic Acid Kit. cfDNA concentration (ng DNA/mL) was determined by quantitative real-time PCR (qPCR) for the ALU repeat element. Tumor burden was defined as the sum of products of diameters (SPD) of target enhancing lesions plus the SPD of the T2 FLAIR signal abnormality on preoperative MRI. RESULTS: 22 preoperative patients were enrolled and diagnosed histopathologically with GBM. The median cfDNA concentration was 10.9 ng/mL (IQR 7.2–23.6, range 0.48–37.6). There was a significant correlation between radiographic tumor burden and cfDNA concentration (Spearmans rho = 0.46, p = 0.03). In a multiple linear regression model, cfDNA concentration remained significantly associated with radiographic tumor burden (beta coefficient 1.87, p=0.03) after adjusting for age, sex, tumor Ki-67 proliferation rate, weight, and glomerular filtration rate (GFR). GFR was also independently (negatively) associated with cfDNA concentration. CONCLUSIONS: In this small pilot study, we demonstrated that plasma cfDNA is easily detected and quantified in patients with newly diagnosed GBM prior to initial surgical re-