FINAL CATEGORY: OMICS IN MOLECULAR PATHOLOGY AND TUMOR CLASSIFICATION

OPTC-1. PROTEOMIC ANALYSIS OF GENETICALLY STRATIFIED LOW-GRADE MENINGIOMA

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INTRODUCTION: Meningioma are the most common primary intracranial tumour. According to WHO, ~80% tumours are benign grade I. Although, some grade I tumour clinically show aggressive behaviour. Radio-surgery are the main therapeutic approaches, chemotherapies are ineffective. Accurate biomarkers for clinical management are lacking. The mutational profile of low-grade meningioma is well-defined, with non-*NF2* mutated tumours harbouring recurrent mutations in genes including *TRAF7*, *KLF4*, *AKT1* and *SMO*. Here, we aim to identify novel biomarkers and therapeutic targets of genetically stratified low-grade meningioma by characterising the proteomic landscape. MATERIALS AND METHODS: Meningioma specimens were stratified according to mutational background: *AKT1E17K/TRAF7*, *KLF4K409Q/TRAF7* and *NF2-/-*. Proteins were separated by SDS-PAGE followed by in-gel tryptic digestion and sample preparation for LC-MS/MS analysis. Raw mass spectrometry data files were processed by MaxQuant and Perseus software. Quantitative phospho-proteomics was performed using TMT-10plex labelling approach followed by motif analysis using motif-X algorithm. GO enrichment analyses were performed using DAVID against all human proteins. RESULTS AND CONCLUSIONS: We have quantified 4162 proteins across all mutational meningioma subgroups and normal meninges (n=31). Hierarchical clustering analysis showed distinct proteomic profiles of mutational subgroups revealing clusters of differentially expressed proteins (DEPs). Comparative analysis showed 10 proteins were commonly significantly upregulated (log2 fold-change≥1; p<0.05) among all mutational subtypes *vs*. normal meninges, indicating proteomic landscapes of mutational subtypes to be highly variable. In contrast, 257 proteins were commonly significantly downregulated (log2 fold-change≤-1; p<0.05) and enriched with molecular functions including aldehyde dehydrogenase and oxidoreductase. Mutational subtype-specific analysis identified 162 proteins significantly upregulated in *AKT1E17K/TRAF7 vs*. remaining sample groups to be enriched in the oxidative phosphorylation pathway. Lastly, analyses of 6600 phospho-sites (n=8) predicted regulatory kinases including EGFR and PKCα. Several of these up-regulated proteins and kinases already verified via WB. Further validation and functional verification will allow us to identify potential drug targets/biomarkers for meningioma.

OPTC-2. IDENTIFICATION OF ENRICHED GENES AND PATHWAYS ASSOCIATED TO HYPOXIA INDUCED MIGRATION IN PATIENT-DERIVED GLIOMA STEM CELL LINES BY RNASEQ

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Glioblastomas (GBM), the most prevalent and lethal primary brain tumors, are characterized by high intertumoral heterogeneity, diffuse infiltration, and resistance to conventional therapies. Notably, the ability of tumor cells to invade surrounding tissues is one of their most damaging characteristics, it not only causes resistance to therapies such as surgery and radiotherapy but is ultimately the primary cause of death. Therapies that cause hypoxia (e.g. anti-angiogenic therapies) have been shown to increase invasiveness, leading to resistance to the therapy itself and further complications for the patients. Using patient-derived glioma stem cell lines (GSCL) we have discovered cell lines that display heterogeneous migratory behavior in response to hypoxia. As expected we observed that four GSCLs studied had increased migration in hypoxia. Strikingly, two other cell lines studied showed decreased migration in hypoxia. This unforeseen result reflects the heterogeneous nature of GBM and the difference between these GSCLs could be key to understanding this variable. To delve into the molecular context that could explain these differences we performed an exploratory RNAseq analysis on four of the GSCLs, two that showed hypoxia-induced migration and two with decreased migration in hypoxia, and evaluated genes differentially expressed in hypoxia versus normoxia. We also carried out gene ontology and pathway enrichment analysis to discover molecular and pathway patterns consistent with the migratory behaviors observed in each group of GSCLs. The results show how that a similar migratory response to hypoxia coincides with particular sets of enriched genes and pathways. Specifically, we found NOTCH and WNT signaling pathways upregulated in GSCLs which showed increased migration in hypoxia while the IFN-gamma pathway upregulated in GSCLs with decreased migration in hypoxia. Knowing the individual molecular mechanisms responsible for the migratory behavior could allow for tailor-made therapies that reduce the dissemination of these tumors.

OPTC-3. DIFFERENTIAL SYNAPSE-RELATED GENE EXPRESSION IDENTIFIES GLIOMA SUBTYPES AND PREDICTS PROGNOSIS

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Synaptic connectivity between gliomas and adjacent brain was recently implicated in tumorigenesis. However, the interaction of synapse-related genes (SRGs) with patient survival and with established clinical and molecular glioma subtypes merit further study in a large patient cohort. We characterized differential expression of SRGs in gliomas and investigated SRG expression as putative clinical biomarkers in a large glioma cohort. Expression of 1189 SRGs was interrogated via RNA sequencing analysis in 603 gliomas (LGG n=451, GBM n=152) of The Cancer Genome Atlas. SRG expression patterns partitioned gliomas into two clusters that were more distinctive than priorly identified glioma subtypes. The two glioma clusters showed significantly low (14.9 months) or high median survival (105.1 months; Hazard Ratio = 7.1, 95% CI: [5.32, 9.78], p = 1.29e-46 using a log-rank test). The high survival cluster showed overrepresentation of known pro-neural and neural subtypes and IDH-mutated gliomas (p<0.00001). The mesenchymal and classic GBMs and IDH-wild type gliomas were overrepresented in the low survival cluster (p < 0.00001). In addition, an Elastic Net Cox Regression model identified 34 SRGs whose expression significantly predicted differential survival and a prognostic Synapse Gene Score (SGS) was created. Using an accelerated failure time model, SGS predicted survival in the overall gliomas after adjusting for IDH-1 mutation (HR = 2.51, 95% CI: [2.27, 2.7496], p<0.00001), and in the IDH-1 mutant cohort after adjusting for 1p/19q co-deletion (HR = 2.05, 95% CI: [1.73, 2.37], p<0.00001). Our analysis shows that gliomas can be distinctively clustered by differential SRGs expression, suggesting that synapse-related proteins may contribute to tumorigenesis via multiple mechanisms. Furthermore, the potential utility of SRGs as clinical glioma biomarkers is supported by our creation of a prognostic SGS. Future studies elucidating interactions between tumorigenesis and synaptic mechanisms may reveal additional insights for glioma biology and therapeutic targeting.

OPTC-4. BIOINFORMATIC ANALYSIS OF COLXIA1 GENE EXPRESSION AND ITS ALTERNATIVE SPLICING REGULATION IN PAEDIATRIC DIFFUSE INTRINSIC PONTINE GLIOMAS (DIPGS).

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Paediatric Diffuse Intrinsic Pontine Glioma (DIPGs), is a rare aggressive childhood malignancy that arise in a region and age specific manner, with no cure and children seldom survive 2 years after diagnosis. There has been significant advances in genomic and molecular identification of driver genes as well as signature mutations that help with diagnosis. A multi-methodological approach to begin to profile the DIPG/host landscape in the context of developing brainstem was conducted. Bioinformatic analyses of a DIPG dataset and normal developing brain dataset to help separate expression associated with development from tumour was conducted. In parallel; fixed-formalin paraffin embedded DIPG tissue was obtained from post-mortem brain for focused RNA arrays and RNAseq. Two of several overlapping genes that were overexpressed in DIPG was tenascin C and the collagen XI (α1) gene (COL11A1). A common feature of these genes is that they are alternatively spliced. Therefore, the aim of this study was to focus on COL11A1, further explore its expression in diffuse midline gliomas (DMGs), to determine association with H3K27M alterations and to explore the use of bioinformatic programmes that could potentially predict the specific Col11α1 isoform expression in DIPG. The following programs were used to determine if a bioinformation approach could aid in our understanding of alternative splicing in development and DIPG: R2*: Genomics Analysis and Visualization Platform, SnapGene program, Basic Local Alignment Search Tools (BLAST), RNA Interactome Database (RNAInter). In silico analysis highlited* an RNA-binding protein *eIF4A3 as a candidate of alternative splicing regulator of* COL11A1 gene. EIF4A3 gene was shown to be upreguated in DIPG using publicly available datasets. Results shows that isoform A is the most likely isoform present in DIPG. The data obtained will help inform future *in vitro* and *in vivo* investigations into the potential role of COL11A1 and its isoforms in DIPG.