## EXCESSIVE NEW ORIGIN FIRING UNDERLIES SELECTIVE GLIOMA STEM CELL CYTOTOXICITY INDUCED BY REPLICATION STRESS RESPONSE INHIBITION

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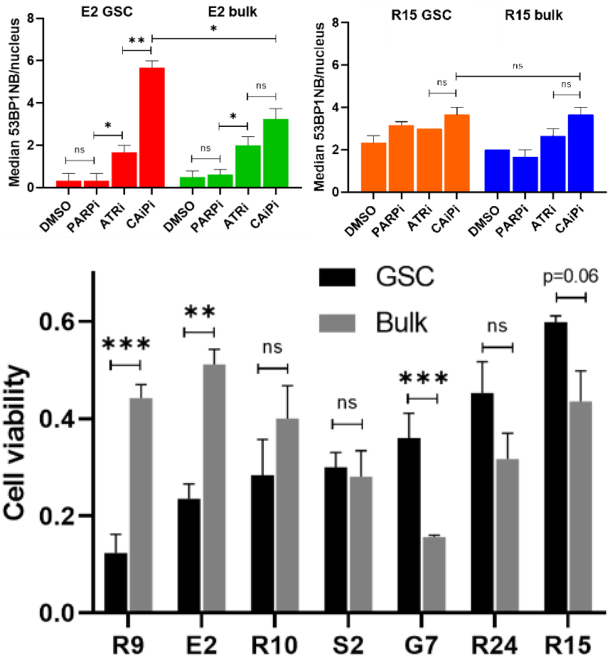
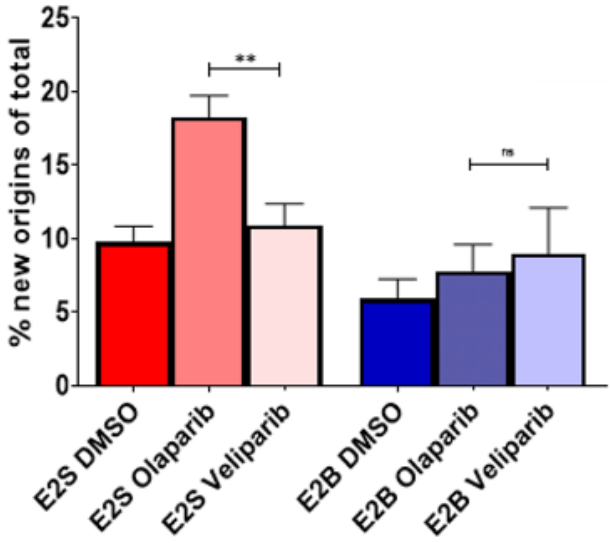
AIMS: Glioblastoma (GBM) is a treatment refractory cancer of extreme unmet need which exhibits treatment resistance due to a subpopulation of GBM cancer stem cells which have constitutive DNA damage response activation driven by elevated replication stress (RS). RS response inhibition is potently cytotoxic to GSC, however mechanistic understanding will be key to biomarker discovery and successful clinical translation. We investigated response to combined ATR and PARP inhibition (CAiPi) to gain mechanistic insight and inform biomarker development. METHOD: A panel of patient-derived GBM cell lines were cultured as stem enriched (GSCs) or stem depleted (bulk), to characterise response to combined ATR inhibition (VE821 5μM) and PARP inhibition (Olaparib 1μM), by CellTiter-Glo viability assay. Mechanistic investigations included immunofluorescence of 53BP1 nuclear bodies and DNA fibre analysis. Studies into the importance of PARP trapping included another PARPi Veliparib (1μM), and investigations into inhibition of origin firing used the CDK inhibitor Roscovitine. RESULTS: Responses to CAiPi in a panel of primary paired GBM GSCs vs differentiated progeny were heterogenous. CAiPi is selectively GSC cytotoxic in a subpopulation of tumours. DNA fibre analysis identified increased new origin firing with PARPi, which was correlated with increased PARP trapping. Inhibition of origin firing by exposure to roscovitine rescued the CAiPi cytotoxic phenotype, suggesting origin firing has an important role in selective GSC cytotoxicity. A population of treatment-sensitive GSCs with increased numbers of 53BP1 nuclear bodies in G1 phase with CAiPi were identified, indicative of under-replication of DNA in S phase. CONCLUSION: Selective GSC cytotoxicity is induced by CAiPi via dysregulation of replication, by both DNA under-replication resulting in DNA lesions, and the novel finding of increased new origin firing in GSC due to PARPi.




## PROTEOMIC ANALYSIS OF GENETICALLY STRATIFIED LOW-GRADE MENINGIOMA

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AIMS: Meningioma is the most common primary intracranial tumour. Although ~80% are benign WHO grade I and show high rates of recurrence. Surgery is the main therapeutic approach, yet location can hamper complete resection and chemotherapies are ineffective. Moreover, accurate biomarkers for clinical management are lacking. Approximately 60% sporadic meningiomas harbour mutations in the NF2gene, while mutations in genes including TRAF7, KLF4, AKT1, SMO and PIK3CAhave been identified majority in the NF2-positive low grade-tumours. Moreover, mutations in TRAF7 mostly co-occur with a KLF4K409Q or with AKT1E17K mutation. The mutations and their molecular manifestations consequently affect the signalling pathways at the protein level. The molecular mechanisms behind meningioma tumourigenesis are still obscure and the identification of specific biomarker is necessary to enable their implementation in routine diagnostics and therapeutics. Therefore, we aim to identify novel biomarkers and therapeutic targets of genetically stratified low-grade meningioma by characterising the proteomic landscape. METHOD: Frozen tumour samples have already been analysed for NF2-/- by next generation sequencing and genotyped for common mutational hotspots in non-NF2 meningioma such as TRAF7, KLF4 and AKT1 and grouped in to three different mutational groups: AKT1E17K/TRAF7, KLF4K409Q/TRAF7and NF2-/- and all these mutations will be compared to normal healthy meninges. For global proteomics, 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 (1.6.2.10) and Perseus software (1.6.1.3). 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) v6.8 against all human proteins. Potential candidates from expression data analysis will be validated via Western Blot and immunohistochemistry. RESULTS: 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. Comparative analysis showed 10 proteins were

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