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THE POTENTIAL OF CRL4DCAF1 AND KSR1 AS THERAPEUTIC TARGETS IN MERLIN-DEFICIENT MENINGIOMA

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BACKGROUND: Merlin-deficient meningiomas are caused by mutations in the Neurofibromin 2 gene and occur in approximately 60% of sporadic meningiomas. Merlin loss is commonly associated with the genetic condition Neurofibromatosis type 2, leading to the development of multiple low grade tumours including schwannoma and meningioma. Currently, the only treatment for low grade meningioma is (radio)surgery therefore identification of novel drug targets is vital. Previous studies have shown that Kinase suppressor of Ras 1 (KSR1) is a potential therapeutic target in schwannoma and that the E3 ubiquitin ligase, CRL4DCAF1, binds to KSR1. The aim of this project is to investigate the interaction between CRL4DCAF1 and KSR1 to determine if targeting this protein complex in meningioma holds therapeutic value. METHODS: HEK293T cells were transfected with KSR1 constructs and interactions with CRL4DCAF1 were investigated by immunoprecipitation. Immunohistochemistry, cell fractionation and Western blot were used to analyse DCAF1 and KSR1 expression and localization in primary human meningioma. A shRNA construct was used to knock down DCAF1 and APS_2_79 was used to inhibit KSR1. RESULTS: KSR1 interacts via the N-term with DCAF1 and is ubiquitinated at the C-term in the NF+/+ model which may be DCAF1 dependent. Immunohistochemistry showed increased DCAF1 and KSR1 expression in Merlin-deficient meningiomas compared with normal meninges, whereas Western blot analysis showed variable protein expression. DCAF1 knockdown led to a reduction of nuclear pERK1/2 and a significant decrease in proliferation of meningioma cells but pERK1/2 and Cyclin D1 levels were unchanged. Combination of shDCAF1 and APS_2_79, a specific KSR1 inhibitor, reduced pERK and proliferation in both BenMen-1, a benign meningioma cell line and primary meningioma. Therefore, targeting both DCAF1 and KSR1 represents an attractive novel therapeutic strategy in meningioma.

5-ALA FLUORESCENCE BASED ISOLATION OF MINORITY POPULATION GBM CELLS IDENTIFIES PUTATIVE INVASION ASSOCIATED MOLECULAR CHANGES

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INTRODUCTION: Glioblastoma (GBM) is a highly pleomorphic polyclonal tumour with molecular abnormalities varying temporo-spatially (intra-tumour heterogeneity), one mechanism of therapy resistance. Fluorescence guided resection (FGR) is performed with prior administration of 5-aminolevulinic acid (5-ALA) leading to individually fluorescent tumour cells mixed within a background population of non-neoplastic neural cells in the invasive region beyond the "pure" tumour. We have isolated this invasive tumour population by fluorescence activated cell sorting (FACS) to allow the study of invasive tumour cells without an overwhelming background "normal" signal. We conducted genome wide gene expression of phenotypically distinct areas of the tumour and from fluorescent/non-fluorescent cancer/non-cancer cells purified from the invasive zone respectively.METHODS: We performed genome-wide gene expression analysis on 14 glioma samples from three different GBM patients including samples from tumour core, rim, invasive margin and GBM cells from the invasive margin that were isolated by 5-ALA assisted FACS. RESULTS: Statistical analysis by linear models for microarray data identified 325 differentially expressed genes between FACS positive cells and other tumour regions (adjusted P-value < 0.05), of these, 50 genes were upregulated in all comparisons. These transcriptomic changes orchestrate MAPK (DUSP1, DUSP2, DUSP10 and FOSB), chemokine signalling pathways (CXCL2, CXCL3, CCL20 and NFKB1) and negative regulation of cell proliferation (EREG and KLF6). In contrast, 29 downregulated genes in FACS positive cells were enriched with signal transduction (DDR2 and MTSS1L) and ECM-receptor interaction (COL4A1, COL4A2, and HSPG2). DISCUSSION: Residual cells responsible for GBM recurrence in the invasive zone are shown to be not only phenotypically different but also exhibiting activation of distinct molecular pathways and biological processes. These unique molecular features offer hope for developing more efficacious targeted therapies focusing on this population rather than the bulk tumour that has been the subject of most historical analyses.

AN EPIGENETICALLY CONTROLLED PML/SLIT AXIS AT THE ROOT OF CELL MIGRATION IN BOTH NORMAL AND NEOPLASTIC CELLS IN THE CNS

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In the central nervous system (CNS), regulation of nuclear function has been implicated in the control of cell cycle and migratory processes during neurogenesis. Alterations of these processes can lead to neoplastic transformation of neural stem cells (NSCs) and glioblastoma multiforme (GBM). The ability of GBM cells to migrate through the brain parenchyma represents a key factor underlying GBM aggressiveness and resistance to treatment. Notably, brain cancer cells use the same routes utilized by neuroblasts/immature neurons and NSCs, suggesting a neurobiological root of brain cancer migration. However, our understanding of potentially common mechanisms regulating cell migration/invasion during neurogenesis and brain tumourigenesis remains limited. Our previous work has implicated the Promyelocytic Leukaemia protein (PML), the essential component of the PML nuclear body (PML-NB), in regulation of embryonic neurogenesis via its ability to control proliferation in NSCs. We set out to investigate the role of PML in adult neurogenesis and GBM. Loss of PML leads to impaired NSC and neuroblast migration and to a smaller olfactory bulb in the adult mouse brain. A similar migration defect is observed in primary GBM cells where PML expression has been knocked down. Mechanistically, PML controls cell migration in both mouse NSCs and primary GBM cells via down-regulation of Slit genes, which are key regulators of axon guidance during development. Changes in Slits transcription upon PML kd are caused by global reduction of the repressive H3K27me3 epigenetic mark. This is associated with its redistribution to nuclear lamina-associated domains (LADs). Finally, PML controls tumor invasion and survival in an orthotopic animal model and inversely correlates with patient prognosis in GBM. Taken together, these findings support a model whereby PML-mediated modifications of chromatin structure and function regulate cell migration during normal neurogenesis and brain tumorigenesis, suggesting a neurobiological root of brain cancer invasion.

EPIGENETIC INACTIVATION OF ARGININE BIOSYNTHESIS GENES IN PAEDIATRIC INTRACRANIAL EPENDYMOMA

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Aberrant cellular metabolism is recognized as a major event in the growth and development of many cancers and the targetting of metabolic defects in tumour cells represents a new therapeutic opportunity. For example, cells that do not express sufficient levels of argininosuccinate synthetase-1 (ASS1) or argininosuccinate lyase (ASL) become auxotrophic for arginine and require exogenous supply. Arginine deprivation using arginine deiminase (ADI-PEG20) is currently under evaluation in clinical trials for adult GBM. In this study, we investigated the arginine biosynthesis pathway in paediatric intracranial ependymoma, comprising 24 fresh frozen biopsies and 17 short-term cell cultures of low passage (<10). The methylation status of ASS1 and ASL was assessed by methylation-specific PCR and gene expression levels were measured using real-time Q-PCR analysis. The response of ependymoma cell cultures in vitro to ADI-PEG20 was determined at various time points using the sulphorodamine B (SRB) assay. Promoter hypermethylation of ASS1 was present in 41.5% ependymoma (17/41 samples) and methylation correlated with down-regulation of ASS1 expression ($p<0.0001$, Fisher's exact test). Importantly, methylation and expression status was maintained in 6 patient-derived cell cultures for which paired biopsies were available. Conversely, methylation of ASL was not detected in any samples. Treatment with ADI-PEG20 inhibited proliferation of ependymoma cells only in those cultures with methylation-dependent silencing of ASS1. Our findings suggest that argininine depletion therapy may benefit a significant proportion of paediatric patients with intracranial ependymoma.