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BIDIRECTIONAL NEURON-GLIOMA INTERACTIONS: EFFECTS OF GLIOMA CELLS ON SYNAPTIC ACTIVITY AND ITS IMPACT ON TUMOR GROWTH

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Gliomas grow in a neuronal environment, but little is known on the functional changes in peritumoral neurons during tumor development. Moreover, investigations on the role of neural activity in glioma progression have yielded contradictory results. Here, we monitored longitudinal changes in network activity by recording visual evoked potentials (VEP) and local field potentials (LFP) after transplant of GL261 glioma cells in mouse visual cortex. We detected a progressive deterioration of VEP amplitudes in glioma-bearing mice, accompanied by an increase in slow network oscillations. At the cellular level, the analysis of microdissected peritumoral neurons showed alterations in both pre- and post-synaptic markers. To investigate whether glioma-driven alterations in synaptic function may impact on tumor growth, we manipulated levels of afferent cortical activity in glioma-bearing mice. Specifically, we tested blockade of synaptic activity via botulinum neurotoxin A (BoNT/A), visual deprivation (dark rearing, DR) and visual stimulation (VS). Replicating cells were quantified via immunostaining for BrdU and Ki67. We found that manipulation of cortical activity bidirectionally regulated glioma proliferation. Silencing of cortical synapses with BoNT/A and DR increased tumor proliferation while daily VS had the opposite effect.

These findings demonstrate reduced responsiveness of peritumoral neurons which may in turn stimulate tumor cell proliferation, thus triggering a vicious loop that exacerbates glioma progression. Physiological stimulation of neural activity appears to restrain glioma proliferation so it could be implemented in the clinical setting as an adjuvant therapy.

MULTI-COLOUR LINEAGE TRACING TO ASSES INTRA-TUMOUR HETEROGENEITY IN GLIOBLASTOMA MULTIFORME

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BACKGROUND: Glioblastoma multiforme (GBM) represents nearly 50% of all malignant brain tumours. Molecular and genomic diagnostics are beginning to unravel the variation between individual tumours. However, there is growing evidence that cellular heterogeneity exists within a single malignancy. Singe cell analysis has demonstrated the presence of subpopulations corresponding to distinct expression profiles. OBJECTIVE AND EXPERIMENTAL APPROACH: characterisation of the intratumor heterogeneity is essential to understand biological behaviour and therapy response. Through combining a genetically labelled mouse model (rosa26-confetti lineage tracing locus) with genetically engineered GBM model we can label distinct cellular lineages. RESULTS: For three-dimensional imaging of these fluorescently labelled tumours we have optimised tissue clearing protocols. Fluorescence activated sorting of genetically labelled tumour cells identifies distinct populations within single tumours. With these techniques can now interrogate the spatial organisation of clones across large areas and we can compare distinct tumour lineages. OUTLOOK: Currently, we are engineering human glioblastoma cell lines with genetic fluorescent labels for lineage tracing. Several genetically characterised human cell lines are available for which novel therapeutic targets have been identified. We will apply our lineage tracing approach to investigate the clonal effects of these tailored therapeutics.

A MECHANICALLY-ENGINEERED SPRAY TO INCREASE BRAIN PENETRATION OF CHEMOTHERAPEUTIC NANOPARTICLES IN THE TREATMENT OF HIGH-GRADE GLIOMAS

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Design and implementation of innovative local drug delivery systems (DDS) may overcome current limitations in GBM treatment, such as the lack of therapeutic drug concentrations reaching residual GBM cells following surgery.

Here we describe a novel DDS which utilises a bespoke mechanically engineered spray device, designed for safe surgical use, to deliver a mucoadhesive hydrogel containing chemotherapeutic nanoparticles (NPs) into the tumour resection margins. The overall aim is to spray a NP and polymer solution onto the resection cavity and potentially increase penetration of anti-cancer drugs within the 2 cm reoccurrence zone beyond the infiltrative margin.

The mucoadhesive gel of choice, pectin, is currently used in other *in vivo* applications; however we have repurposed this for the brain. Pectin is biocompatible with GBM and human astrocyte cells *in vitro* and showed neither toxicity nor inflammation for up to 2 weeks upon orthotopic brain injection. Pectin is biodegradable in artificial CSF and is capable of being sprayed from the engineered device.

A panel of polymeric, oil-based and polymer-coated NPs have been developed and optimised to maximise drug encapsulation of etoposide and olaparib as proof-of-concept for combination drug delivery. Etoposide/olaparib was chosen due to cytotoxicity from 5 GBM cell lines, including primary lines isolated from the invasive tumour margin (Mean IC_{50} of 1.1 μM and 8.3 μM respectively). The optimal NP/drug formulation (based on drug encapsulation, spray capability and bio-adhesiveness) will ultimately be assessed for tolerability and efficacy using orthotopic allograft and xenograft high-grade glioma models.

THE METALLOPROTEINASE ADAMDEC1 MAINTAINS A NOVEL GROWTH FACTOR SIGNALLING LOOP IN GLIOBLASTOMA CANCER STEM CELLS

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Glioblastomas (GBM) are lethal brain tumours where poor outcome is attributed to cellular heterogeneity, therapeutic resistance, and a highly infiltrative nature. These characteristics are preferentially linked to GBM cancer stem cells (GSCs), but how GSCs maintain their stemness is incompletely understood and the subject of intense investigation. Here, we identify a novel growth factor signalling loop that induces and maintains GSCs. This loop consists of an atypical metalloproteinase, a disintegrin and metalloproteinase domain-like protein decysin 1 (ADAMDEC1), secreted by GSCs. ADAMDEC1 solubilizes fibroblast growth factor-2 (FGF2) in the tumour microenvironment. We find that GSCs exclusively express FGF receptor 1 (FGFR1), which upon binding of FGF2 induces upregulation of Zinc finger E-box-binding homeobox 1 (ZEB1). ZEB1 is a regulator of stemness and tumour initiation, and therefore ADAMDEC1-FGF2-FGFR1 signalling promotes malignancy in GBM. We further show that ZEB1 regulates ADAMDEC1 expression, creating a positive feedback loop. Genetic or pharmacological targeting of components of this axis attenuates self-renewal and tumour growth. These findings reveal a new signalling axis for GSC maintenance and highlight ADAMDEC1 and FGFR1 as potential therapeutic targets in GBM.

POLYCOMB-MEDIATED REPRESSION OF EPHRINA5 PROMOTES GROWTH AND INVASION OF GLIOBLASTOMA

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INTRODUCTION: The epigenetic regulator Bmi1 is essential for the self-renewal of neural stem cells (NSC), and highly expressed in glioblastoma (GBM) stem/initiating cells (GIC), where knockdown significantly reduces tumour growth in xenograft models. We have used a combined genome-wide and target gene-driven approach to identify EphrinA5 (EfnA5) as a