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Subtype-specific block of glioblastoma motility through dual inhibition of water and ion flux

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Comprising more than half of all brain tumours, glioblastoma multiforme (GBM) is a leading cause of brain cancer-related deaths worldwide (Engelhard et al., 2010, Holland, 2000). Identification of multiple diagnostic indicators has supported the classification of glioblastoma into proneural, neural, classical, and mesenchymal subtypes (Brennan et al., 2013, Verhaak et al., 2010). Classification of molecular markers associated with different glioblastoma subtypes has aided investigations into the genetic events underlying resistance to available clinical treatments but nonetheless, a major clinical challenge is presented by the capacity of glioma cells to rapidly infiltrate healthy brain parenchyma, allowing the cancer to escape control by localised surgical resections and radiotherapies, and promoting recurrence in other brain regions. Proposedly, therapies that target cellular motility pathways could be used to slow tumour dispersal, providing a longer time window for administration of frontline treatments needed to directly eradicate primary tumours.

Aquaporins (AQPs), synaptic receptors and ion channels are prime candidates as pharmacological targets to restrain cell migration in glioblastoma, given the diverse roles of these protein classes in cellular mechanisms associated with volume regulation, cell-cell and cell-matrix adhesions, cytoskeletal rearrangement and regulation of proteases and extracellular-matrix degrading molecules (Papadopoulos et al., 2008, Stroka et al., 2014, Cramer et al., 1997, Mattila and Lappalainen, 2008, Pollard and Borisy, 2003, Schwab et al., 2007, Weaver, 2006, Ridley et al., 2003, Vicente-Manzanares and Horwitz, 2011, Geiger et al., 2001, Martin et al., 2002, Ding et al., 2011, McCoy et al., 2010, McFerrin and Sontheimer, 2006).

According to the public GBM Bio Discovery Portal Database (Celiku et al., 2014), AQP1, and some classes of glutamate receptors, and potassium, sodium and calcium channels are enriched in GBM tumours. Increased levels of these membrane proteins may enhance invasion processes such as cell volume regulation and extracellular matrix degradation. The identification of optimal combinations of protein targets and highly specific inhibitory agents that allow effective intervention of invasion with minimal disruptions to the surrounding neuro-glial networks could overcome signalling pathway redundancy, a limitation inherent to current GBM treatment strategies that target individual cellular pathways.

Implementing Transwell invasion assays, glioblastoma cells exposed to control or drug treatments were applied atop a layer of extracellular matrix gel, providing an experimental setting that resembled components of a tumour microenvironment. Table 1 lists the pharmacological inhibitors of glutamate receptors and ion channels that were each tested individually and then in turn combined with novel AQP1 water channel inhibitor AqB013. The additive effects of these compounds on the ability of glioblastoma cells to migrate through the extracellular matrix barrier was evaluated.

Table 1: AQP1, glutamate receptor and ion channel blockers investigated as inhibitors of GBM invasion

Drug	Target(s) in cellular invasion
AqB013	Aquaporin-1 (AQP1)
Nifedipine	Voltage-gated Ca^{2+} channels
Amiloride	Acid-sensing ion channels
Apamin	Calcium-activated K^+ channels
4-aminopyridine (4-AP)	Voltage-gated K^+ channels
Cyanquixaline (CNQX)	AMPA/Kainate-type glutamate receptors

The additive effects of co-application of the pharmacological agents differed between U87-MG and U251-MG. In U87-MG, invasion was significantly blocked by each individual agent tested and furthermore, dual treatment with AqB013 and each drug yielded an additive block of invasion. In U251-MG, whilst invasion was impeded following monotreatment with each drug, additive block was only observed upon combination of apamin, 4-aminopyridine or nifedipine with AqB013. This idea prompted the prediction that the efficacies of pharmacological agents could correlate with the gene expression profiles and hence the GBM subtypes exhibited by the cell lines. Analysis of U87-MG and U251-MG using the open-access Cancer Cell Line Encyclopedia (Ghandi et al., 2019, Nusinow et al., 2020) revealed the presence of molecular markers for the proneural and classical subtypes in U87-MG and U251-MG respectively. A comparison of the transcript levels of the proteins of interest reported for each GBM subtype in the GBM Bio Discovery Portal and the observed drug sensitivities of U87-MG and U251-MG supported the classification of U87-MG as proneural and U251-MG as classical.

Tailoring clinical interventions to the genetic profiles of different glioblastoma subtypes through an optimised combination of additive or synergistic agents could improve methods for limiting glioblastoma motility with minimal cytotoxic side-effects. Enhanced understanding of the underlying molecular characteristics and proteomic landscape of glioblastoma is required to identify targeted therapies and combination regimens applicable to broader patient populations.