Orthogonal Arrays of Particles alter cytoskeleton and cell invasion dynamics in GBM and glioma cells

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Background: Fluid homeostasis plays a pivotal role in cytoskeletal rearrangements and changes in cell shape during adhesion, migration, invasion and tumour growth. A water channel protein, Aquaporin-4 (AQP4) is in charge of controlling this balance in brain glial cells playing a key role during migration. AQP4-M23 is the Orthogonal Arrays of Particle forming isoform (AQP4-OAPs) while AQP4-M1 (AQP4-tetramer) is not able to aggregate into OAPs. The role of AQP4 in malignant gliomas is still unclear. It has been shown that knocking down AQP4 expression could result in specific and massively impaired invasion and migration of glioblastoma cells.

Methods: To analyse the physiological role of AQP4-OAPs in glioma cytoskeletal dynamics, astrocyte primary cultures (prepared from AQP4-null mice), TNC (immortalized astrocytes), U87 (glioblastoma, GBM), GL95 (GBM primary cell line), C6 (glioma), B16F10 (melanoma) cell lines were transfected with AQP4-tetramer or AQP4-OAPs. Immunofluorescence and Western blot were used to analyse cell shape, actin cytoskeleton dynamics and apoptosis. Invasiveness was assessed by matrigel assay using 3D scaffold and Boyden chamber assays and metalloproteinase-9 (MMP) analysis. Mutagenesis was used to identify the aminoacids involved the alteration observed.

Results: Results have shown that AQP4-OAPs trigger cell shape changes only in U87 and C6. Changes were characterized by a stellate morphology and the appearance of cytoplasmic protrusions compared to cells transfected with AQP4-tetramers. These changes were associated with increased F-actin content and a major increase in AQP4-OAPs and F-Actin physical interaction. Moreover, AQP4-OAPs expression in U87 cells induced apoptosis, assessed by AnnexinV staining, and decreased invasion ability. Several AQP4-OAP mutants at C-terminus tail and extracellular loops were therefore generated to investigate the OAP sequence responsible for the observed changes. Results indicated two prolines (254 and 296) at C-terminus tail as the key for AQP4-OAP effects in GBM and glioma cells.

Conclusion: All together these results indicate a different effect of AQP4-OAPs and AQP4 tetramers in GBM and glioma cells. In particular, AQP4-OAPs induce morphological changes and actin cytoskeleton reorganization associated with apoptosis and decreased invasiveness. This study supports the idea that modulation of AQP4 isoform expression is a potential target for brain cancer therapy.