Caveolin-1, a driver of invasive phenotype in in-vitro 3D-spheroid assays comprised of high grade GBM cells association with an AKT-inhibited phenotype.

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INTRODUCTION

Glioblastoma multiforme (GBM) cells display a highly invasive phenotype, a hallmark which counters effective surgical and radiotherapy strategies. Caveolin-1 (Cav-1) is the main structural and functional component of caveolae. The impact of the expression of Cav-1 within a range of tumour and tumour-associated stromal cells is variable with both oncogenic and tumour suppressive roles reported which appear to be both disease-specific and context-dependent. Our hypothesis is that Cav-1 serves as promoter of invasion of GBM cells.

MATHERIALS AND METHODS

To investigate our hypothesis we used a lentiviral shRNA approach to silence Cav-1 in three GBM cell lines (U87, UP007, UP029) derived from adult brain tumours. We employed an in-vitro 3D cell-sprouting invasion assay with GBM cell spheres embedded in Matrigel. Quantification of invasion was undertaken using a novel image analysis tool or 3D systems, INSIDIA (ImageJ Macro for High-throughput Spheroid Invasion Analysis). Parallel migration and invasion studies were performed using a Boyden Chamber approach, as well as cell-cell adhesion assays. Activation of signalling pathways in 2D and 3D cultures were performed by proteomic array and Western Blot analysis.

RESULTS AND CONCLUSION

GBM cells expressing Cav-1 (Cav-1 +ve) displayed a higher invasive capacity compared cells where Cav-1 had been silenced Cav-1 –ve), the latter also showing increased cell-cell adhesion. A significant finding from the signalling analysis was an inverse association between Cav-1 silencing and activation of AKT evidenced by increased phosphorylation at both Ser473 and Thr308 sites. Ongoing studies are exploring this signalling axis and its relationship to the invasive phenotype.

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