Modulation in the expression levels and protein localization of cell volume and actin cytoskeleton associated proteins by the Slit/ Robo signaling pathway in Glioblastoma multiforme.

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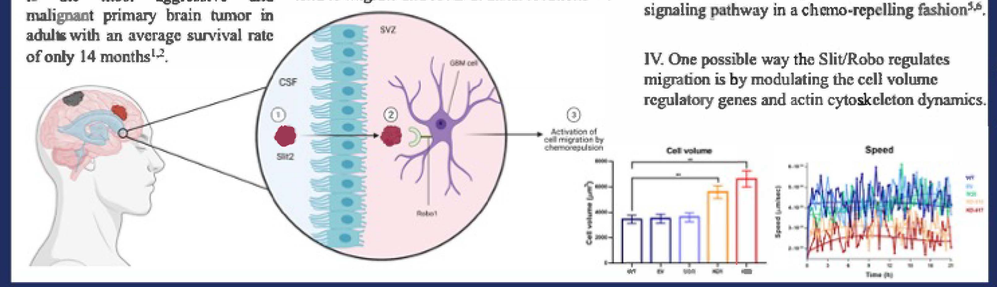
BACKGROUND

I. Glioblastoma multiforme (GBM) is the most aggressive and malignant primary brain tumor in adults with an average survival rate of only 14 months[1,2].

II. GBM tumors that contact the lateral ventricles tend to migrate and recur at distal locations [3,4].

III. Neural Progenitor Cell (NPC) migrate from the SVZ to the olfactory bulbs via the Slit/Robo signaling pathway in a chemo-repelling fashion[5,6].

IV. One possible way the Slit/Robo regulates migration is by modulating the cell volume regulatory genes and actin cytoskeleton dynamics.



HYPOTHESIS

The Slit/Robo signaling pathway modulates the expression levels and protein localization of cell volume and actin cytoskeleton associated proteins.

OBJECTIVES

- To determine the role of the Slit/Robo signaling pathway in modulating cell volume regulatory genes
- To determine the role of the Slit/Robo signaling pathway in actin cytoskeleton regulatory proteins, and the modulating the localization of cytoskeleton associated proteins.

METHODS

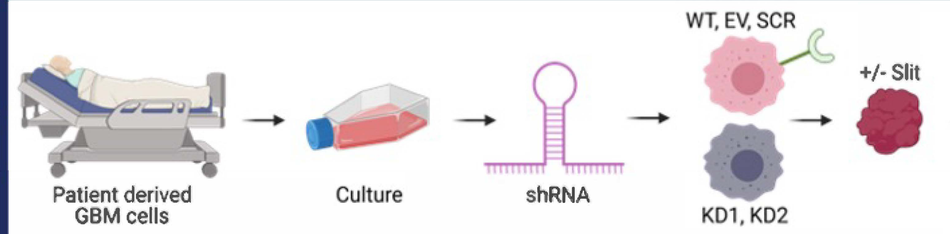


Figure 1. Graphical layout of the experimental design used to assess the role of the Slit/Robo signaling pathway in GBM cells. Patient derived GBM cells were cultured and treated with shRNA to decrease the expression of the Robo1 receptor prior to treatment with Slit2 protein. An empty vector (EV) and a scrambled (SCR) shRNA sequence were used to create two negative control cell variants. Analysis was conducted by RT-qPCR, Western Blot, and Immunocytochemistry.

R E S U L T S

1. Robo1 modulates cell volume regulatory genes

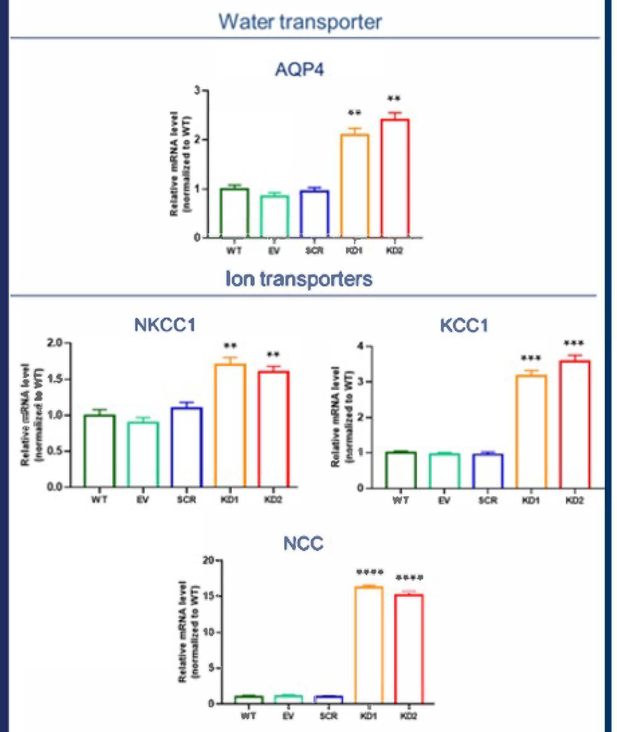


Figure 2. Changes in cell volume regulatory gene expression. Aquaporin-4 (AQP4) is a water channel transporter which showcased a significant increase in expression in the Robo1-KD cells. The expression of ion transporters NKCC1 (sodium potassium chloride transporter), KCC1 (potassium chloride cotransporter), and NCC (sodium chloride symporter) significantly increased in the Robo1-KD cells. These cell volume regulatory genes are directly affected by the Robo1 receptor. Where: * $p \leq 0.01$, ** $p \leq 0.001$, *** $p \leq 0.0001$, and **** $p \leq 0.00001$.

2. The Slit/Robo signaling pathway modulates actin cytoskeleton regulatory protein

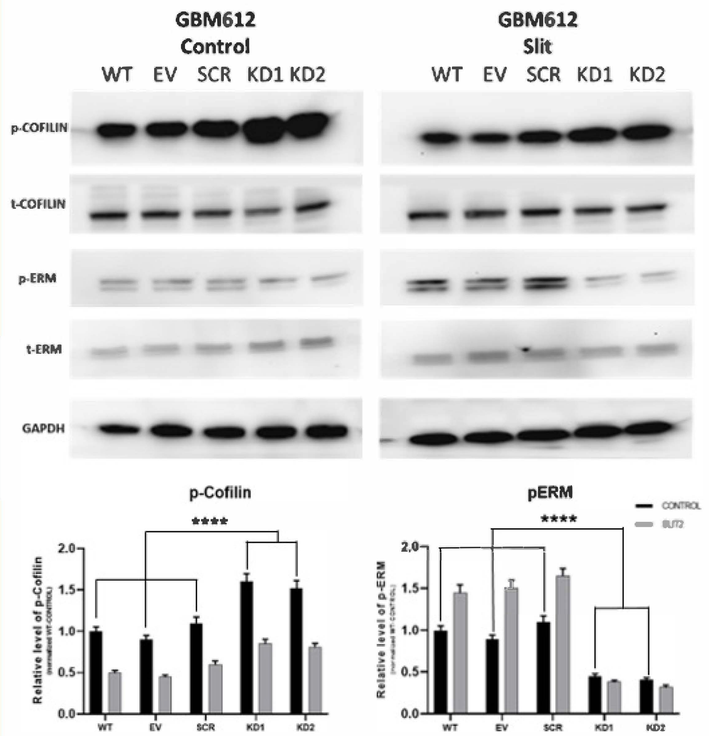


Figure 3. Western blot & western blot quantification of actin cytoskeleton regulatory proteins in GBM with and without Robo1 and Slit2. Cofilin promotes actin regeneration and is inactivated when phosphorylated. When all cells are exposed to Slit2, p-Cofilin expression decreases. However, an increase in p-Cofilin expression is seen in Robo1-KD cells. ERM cross-links actin to the plasma membrane and is active when phosphorylated. In WT, EV, and SCR cells, p-ERM expression increases in the presence of Slit2 protein. A decrease in expression of p-ERM is seen in the KD cells without Slit2 protein. Additionally, in the presence of Slit2, the Robo1-KD cells do not respond to the stimulus and p-ERM expression does not recuperate to basal conditions. **** $p \leq 0.00001$.

3. Focal adhesion complexes: Robo1 modulates actin cytoskeleton protein localization

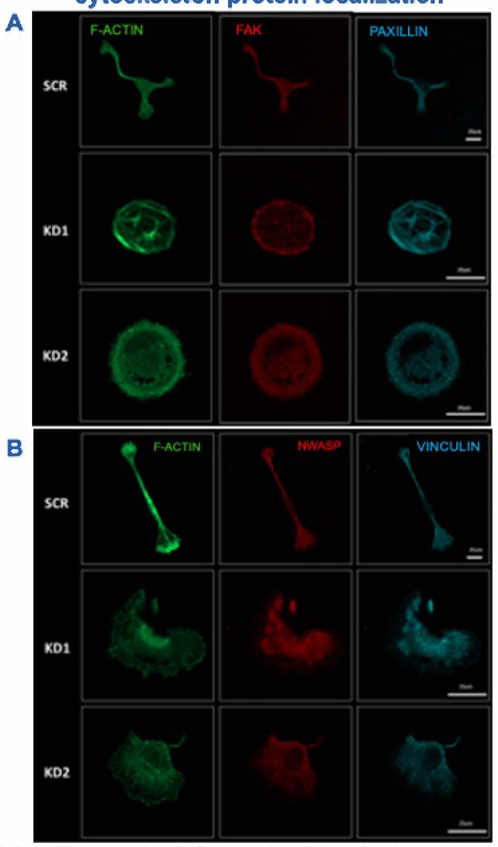


Figure 4A. ICC of actin cytoskeleton associated proteins. Focal adhesion kinase (FAK) aids in binding integrins to actin and is distrusted in a diffuse layout in the SCR cells. In the Robo1-KD cells, there is a different localization of this protein, where it is concentrated in a ring along the outer portion of the cell. This same pattern is seen in Paxillin which links cell surface receptors to actin.

Figure 4B. ICC of actin cytoskeleton associated proteins. N-WASP, which stimulates actin polymerization, and Vinculin, which links integrins to actin, primarily reside around the nucleus in Robo1-KD cells. This is unlike the equal distribution seen in the SCR cells.

CONCLUSIONS & FUTURE DIRECTIONS

- **The Slit/Robo signaling pathway increases the expression levels of genes that contribute to cell volume regulation in GBM cells.**
- **The mechanism by which the Slit/Robo signaling pathway causes the pro-migratory phenotype is by the activation of Cofilin and ERM.**
- **Future directions: Investigation of potential therapeutic drugs that could inhibit this pathway, thus reducing GBM migration and recurrence, increasing overall**

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