AJAP-1 Reduces Migratory Capacity and Neurite Outgrowth in U87 Glioblastoma Cells

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INTRODUCTION

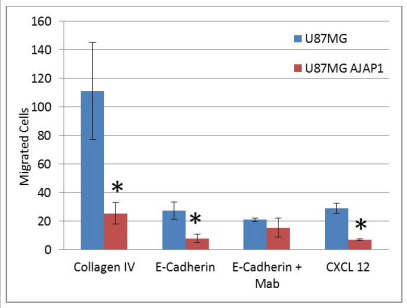
Glioblastoma Multiforme (GBM) is the most prevalent and invasively aggressive primary brain tumor. Adherens Junctional Associated Protein 1(AJAP1) is a transmembrane protein implicated in adherens junction formation in epithelial cells through interaction with the E-Cadherin-catenin complex. We recently found AJAP-1 is under-expressed in GBM cells and primary tissue, and hypothesized it to be migration related. This study aimed to understand the effect of AJAP1 on in-vitro migration of GBM cells in relation to chemoattractants that could modulate AJAP1's migratory effect.

METHODS

Migratory capacity was assayed using U87MG GBM cells and U87MG cells transfected with AJAP1. In a Transwell migration assay, cells were loaded into wells with 8 µm pores. The pores were coated with the chemoattractants collagen IV, E-Cadherin, E-Cadherin coupled with a specific antibody, or CXCL-12. Additionally, for an adhesion assay, cells were loaded into wells spotted with the attractants and a BSA control.

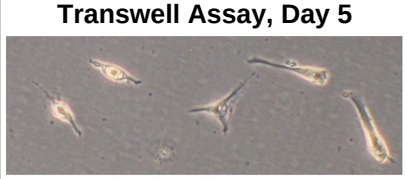
RESULTS

AJAP1 decreases glioma migration in the presence of chemoattractants.



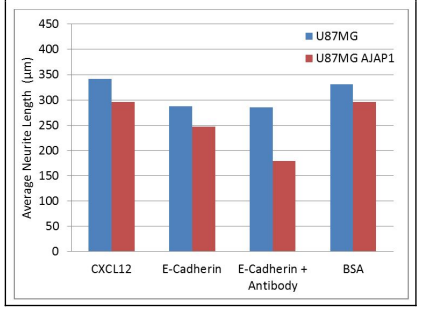
The Transwell assay found significantly decreased migration of U87MG AJAP1 cells through the chemoattractant-coated membrane compared to U87MG tumor cells without AJAP1 (*p<0.01).This effect in the presence of E-Cadherin was reversed with the addition of an antibody to E-Cadherin, with migratory capacities approximately equal for both U87MG and U87MG-AJAP1 cells.

Transwell Assay, Day 5



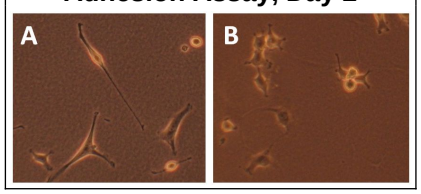
Representative picture of U87MG cells that have actively migrated through the Transwell pores.

AJAP1 impedes astrocytic extensions in U87MG glioma cells.



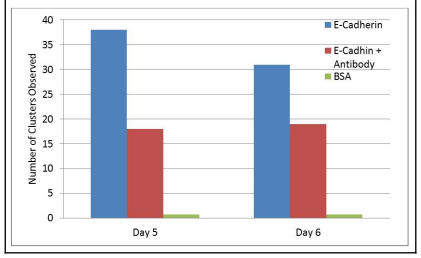
The adhesion assay found that U87MG AJAP1 cells showed significantly decreased astrocytic outgrowth in comparison to U87MG cells (p=0.02). Astrocytic extension length was approximately constant for the U87MG cells across the chemoattractants and control (p=0.12); however, for the U87MG-AJAP1 cells, addition of the antibody significantly decreased astrocytic extension length in comparison the other chemoattractants and BSA control (p=0.04).

Adhesion Assay, Day 2



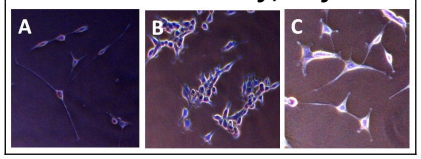
Representative image of astrocytic extensions in the presence of E-Cadherin in A) U87MG cells, and B) U87MG AJAP1 cells.

Adherent AJAP-1 cells demonstrate increased clustering in response to E-Cadherin.



On Day 5 and Day 6, the adhesion assay found no significant clustering in U87MG cells, while U87MG AJAP1 cells were found to grow in clusters upon interaction with E-Cadherin. This interaction was somewhat reversed with the addition of an antibody to the E-Cadherin, and was completely unobservable for the cells plated on the control BSA (p<0.01).

Adhesion Assay, Day 3



Representative pictures of A) U87MG cells plated with E-Cadherin show no clustering B) U87MG AJAP1 cells plated with E-Cadherin show increased clustering, and C) U87MG AJAP1 cells plated with E-Cadherin coupled with antibody show decreased clustering.

CONCLUSIONS

The results of this study thus implicate AJAP1 as a migratory inhibitor; loss of AJAP1 as in GBM cells is implicated in their increased migratory capacity. Both blockage of E-Cadherin with an antibody and under-expression of AJAP1 as in U87 tumor cells increased migratory capacity. Thus interaction of AJAP1 with E-Cadherin appears to be involved in maintaining a decreased migratory potential through an unknown mechanism.

REFERENCES

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