

# The impact of CSF and CHI3L1 expression on glioblastoma malignancy

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#### ABSTRACT

### INTRODUCTION

Glioblastoma (GBM) is the most common malignant primary brain tumor in adults [1]. Considering the invasive nature of the GBM cells complete surgical resection is nearly impossible to achieve [2,3]. The biological resemblance of Neural Stem Cells (NSCs) to Glioblastoma Stem Cells (GSC) indicates that they are potentially originating from the Subventricular Zone (SVZ) [6].

Tumor location has a major impact on the patients' prognosis. GBM tumors located proximally to the LV (Lateral Ventricle) wall results in lower OS (Overall Survival) and increased rate of distal recurrence comparing to the tumors located distally from the LV wall [6], due to the tumor being potentially exposed to cancer CSF [6]. Our laboratory has identified transcriptomic changes in GBM cells in response to CSF. Among the pathways that changed expression patterns upon CSF treatment, a common gene involved in them is CHI3L1. The aim of this project is to identify the role of

#### **METHODS**

Patient derived GBM cells were used to conduct all the experiments, particularly 965 WT, 120 WT, 1A WT cell lines. All three cell lines were treated for 72 hours with artificial (aCSF), noncancerous (ncCSF) and cancerous (cCSF) cerebrospinal fluid (CSF). RT-qPCR was performed to test the expression of genes CHI3L1, SERPINA3 and SPP1. Alamar Blue Assay was performed with different concentrations of CHI3L1 for 72 hours to evaluate its role on cell viability and proliferation in GBM cells. The same three cell lines were treated for 72 hours with +/-rhCHI3L1 (500 ng/mL). RT-gPCR was conducted to test gene expression of OPN/SPP1, Nestin, Vim, E-cad, CHI3L1, SERPINA3, CD44, MMP2, EGFR, FGF2, Sox2. Transwell Migration Assay was performed with the scope of evaluating the potential correlation between CHI3L1 and cell migration.

#### RESULTS

According to our RT-gPCR results, CHI3L1, SERPINA3, OPN/SPP1 have increased expression in the GBM cells treated with cCSF comparing to the control aCSF. There was an increase of OPN/SPP1, Nestin, Vim and E-cad regulation in GBM cells treated with CHI3L1. However, the expression of other genes such as CHI3L1, SERPINA3, CD44, MMP2, EGFR, FGF2, Sox2 did not increase comparing to the control. There were also significant results after performing the Alamar Blue Assay for 72 hours with different concentration of CHI3L1. We concluded that 500 ng/mL is the amount of protein that promotes cell viability the most. Finally, we did a Transwell Migration Assay for 24 hours to study the potential correlation between CHI3L1 and cell migration. We performed the assay twice. The results were significant, more cells that were treated with CHI3L1 for 24 hours migrated through the membrane, comparing to the nontreated group.



BACKGROUND





Figure 2. The correlation between the levels of CHI3L1 in GBM and probability of survival (data from TCGA)





#### **OBJECTIVES**

- Study the tangency between cCSF and CHI3L1 overexpression.
- Study the relationship between CHI3L1 and the overexpression of genes such as SPP1, SERPINA3 and CD44.
- Study the correlation between the gene CHI3L1 and GBM aggressiveness and invasion.







Figure 6. GBM cells treated with A, NC, C CSF qPCR. When GBM cells are in contact with cCSF, CHI3L1, SERPINA3 & SPP1 are overexpressed in the GBM cells

CSF and CHI3L1 gene expression on glioblastoma malignancy.

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# CONCLUSION

- · C CSF induces an increase in the expression of CHI3L1. SPP1 and SERPINA3
- CHI3L1 increases GBM cell viability
- CHI3L1 promotes GBM cell migration.
- Treatment with rhCHI3L1 alters gene expression of OPN/SPP1. Nestin. Vim and E-cad in GBM cells.

# **FUTURE OBJECTIVES**

- Measure expression of other genes such as VEGF.
- CD44 & CHI3L1 Gene silencing.
- Block CD44 receptor as a potential treatment.
- Study the Erk, Akt, and β-catenin signaling pathways activated by the CHI3L1 - CD44 complex.

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NT CHI3L1 CHI3L1 Figure 9. After the GBM cells were treated with CHI3L1 for 24 hours, the results demonstrate that CHI3L1 induces cell migration.

NT CHI3L1