Identification of Genes Driving Glioblastoma Stem Cell Phenotype

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Abstract

Glioblastoma multiforme (GBM) is classified as a grade IV tumor by the World Health Organization (WHO). As the deadliest type of adult brain tumor, only 5% of the GBM patients survive 5 years or more after diagnosis. Its high motility rates are attributed to stem-like cancer cells within GBM, which result in tumor initiation, therapeutic resistance, and even tumor recurrence. By exploring the underlying mechanism of GBM stem cell induction and maintenance, the aim is to identify human genes that are induced in GBM cancer stem cells. The study will utilize real time-quantitative polymerase chain reaction (RT-qPCR) and protein quantification immunoblotting assays (Western blotting) employing RNA and protein samples extracted from human brain tumor cells for analysis. The results of this study through a quantitative approach would benefit people who want to focus on the aspects of brain tumorigenesis and the identification of genes induced in GSCs (Glioblastoma stem cells).

Introduction

Glioblastoma Multiforme (GBM) is a grade IV glioma and a highly malignant brain tumor. Due to its high malignancy and significant risk of recurrence, only a small percentage of patients achieve a five-year survival. Safari and Khoshnevisan (2015) suggests that GBM's resistance to therapy is primarily caused by a small population of cancer stem cells. These cells, con ontribute resistant to treatment and high malignancy rates. In studying the relationship between GBM and glioblastoma stem cells (GSCs), preliminary data from RNA sequencing analyses showed the induction of two genes, *SERPINF1* and *FOXS1* (Hawkins et al., 2024).

The primary goal of this study was to identify genes that are altered in GBM cancer stem cells (GSCs). A quantitative experimental research approach was employed, focusing on the analysis of two human genes, *SERPINF1* and *FOXS1.* The experiments were conducted in the Department of Biological and Health Sciences, Texas A&M University-Kingsville to collect gene expression data. Our study aims to investigate how *SERPINF1* and *FOXS1* are regulated in GSCs, which may serve as potential biomarkers for future therapeutic targets and stem cell maintenance.

Results

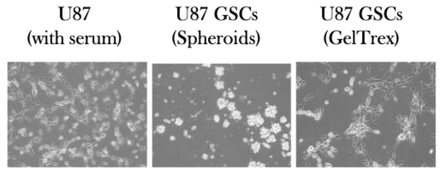

Figure 1. U87 and U87 glioblastoma stem cells. To enrich and establish GSC cell lines, U87 cells were grown in StemFlex (ThermoFisher) media without serum using either Ultra-Low Attachment plates (Corning) or GelTrex basement matrix (ThermoFisher). Cells were passaged more than 30 times. Representative images of U87 cells, U87 GSC spheroids, and U87 GSCs grown on GelTrex matrix.

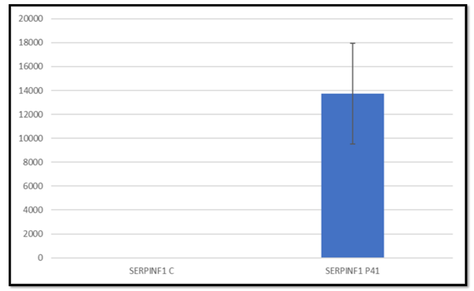

Figure 2. qRT-PCR Analysis. *SERPINF1* in the GSC P41 sample shows a 13,745-fold induction compared to the differentiated cell control.

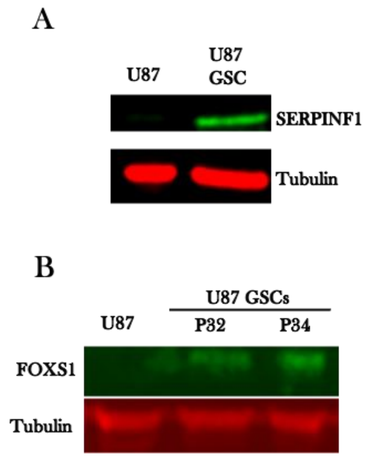

Figure 3. Immunoblotting analysis. U87 and U87 GSCs were subjected to Western blotting using anti-FOXS1, anti-SERPINF1, and anti–Tubulin antibodies. SERPINF1 (**A**) and FOXS1 (**B**) are induced in U87 GSCs.

Methods and Materials

***Establishment of U87 stem cells*:**
U87 monolayer glioblastoma cells were cultured in EMEM with 10% FBS. To enrich and establish GSC cell lines, U87 cells were grown in StemFlex (ThermoFisher) media without serum using either Ultra-Low Attachment plates (Corning) or GelTrex basement matrix (ThermoFisher).

***qRT-PCR Analyses*:**
qRT-PCR assays were conducted using RNA from control and GSC cell lines, the Luna One-step qRT PCR kit (New England Biolabs), and a Bio-Rad CFX Opus 96 real-time PCR cycler. Relative gene expression levels were determined using the delta-delta Ct method, with Aldolase serving as the normalization control.

***Immunoblotting Analyses*:**
U87 and U87 GSC cell lysate samples were prepared with RIPA lysis buffer, and they were subjected to electrophoresis. The separated proteins were transferred to a nitrocellulose membrane using the iBlot 2 transfer system (Invitrogen). The membrane was incubated with primary antibodies against FOXS1, SERPINF1, and Tubulin. After washing, the membrane was incubated with secondary antibodies. Finally, the membrane was scanned using a Li-Cor Odyssey Fc scanner.

Discussion

To identify the genes responsible for GSC induction and maintenance, we have performed RNA-seq, qRT-PCR and immunoblotting assays. Among the altered genes from initial RNA-seq analyses, we have determined that *SERPINF1* and *FOXS1* are induced in GSCs which may provide underline mechanisms of GSC induction and maintenance. While the Notch signaling pathway is suggested to control *SERPINF1* expression (Song et al., 2023), the direct regulators of *SERPINF1* involved in GSC stemness have not yet been identified.

Conclusions

Our study focused on identifying genes whose expression is altered in GSCs. *SERPINF1* and *FOXS1* were found to be significantly induced in GSCs, although their functional roles in the induction and maintenance of GSCs have not yet been determined. Ongoing knockdown and protein-interaction studies are being conducted to identify their roles and binding partners, which will help us better understand the mechanisms driving GSCs and may lead to the development of therapeutic markers for targeting GSCs.

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References

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