Clinical and Experimental Health Sciences

Unveiling the Expression of UNC13C in Healthy Brain and Glioblastoma Cells

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Received: 25.10.2024 **Accepted:** 25.11.2024

ABSTRACT

Objective: Glioblastoma (GBM) is the most aggressive type of brain tumor, accounting approximately half of malignant central nervous system tumors. Median overall survival remains below 15 months post-diagnosis. Current treatments include surgical resection, radiotherapy, and chemotherapy, primarily with temozolomide, yet the median overall survival remains below 15 months post-diagnosis. Understanding the molecular mechanisms of GBM is essential for developing novel therapeutic approaches. Among the implicated genes, the UNC13 protein family, particularly UNC13C, is of interest. While UNC13A and UNC13B have been linked to various neurological disorders, UNC13C has been less studied despite its involvement in neurotransmitter release and potential tumor-suppressive effects in other cancers. Our previous work indicated low expression levels of UNC13C in glioblastoma cell lines compared to healthy brain tissue, suggesting a role in GBM pathogenesis. In this study, we aimed to comprehensively evaluate UNC13C expression using web based bioinformatics tools and experimental approaches.

Methods: We analyzed UNC13C expression across various tissues via Correlation Analyzer, confirming in glioblastoma tissues compared to healthy brain samples using the GEPIA and UALCAN databases. Additionally, we assessed UNC13C levels in glioblastoma cell lines (LN-18, A-172, U-87), human microglia (HMC3), and healthy astrocytes through quantitative real-time polymerase chain reaction (qRT-PCR).

Results: Our findings reveal that UNC13C expression is notably reduced in glioblastoma cells, with the highest expression observed in healthy astrocytes, albeit at low levels. These results underscore the importance of UNC13C in GBM and highlight the need for further investigation into its role in tumor development and progression.

Conclusion: This study provides the first report of UNC13C expression detailed in human cell lines both normal and glioblastoma, emphasizing its significance from a developmental perspective.

Keywords: UNC13C, gene expression, glioblastoma, brain cells

1. INTRODUCTION

Glioblastoma (GBM) is the most aggressive type of brain tumor, accounting for 14.5% of all and 48.6% of malignant central nervous system tumors (1). The incidence of GBM ranges from 3.19 to 4.17 (cases per 100,000 people per year, and it is 1.58 times more common in men than in women (2). The current standard treatment for glioblastoma includes surgical resection of the tumor, followed by radiotherapy and chemotherapy (3). Since its FDA approval in 2005, temozolomide, an alkylating agent, has been the primary chemotherapeutic used in GBM treatment (4) Despite these multimodal treatment strategies, the median overall survival remains less than 15 months post-diagnosis. Therefore, identifying the pathophysiology of GBM is crucial for developing novel therapeutic approaches.

Understanding the molecular mechanisms underlying the central nervous system is crucial for elucidating the pathogenesis of neurological diseases. In the quest to identify the pathogenesis of glioblastoma (GBM), numerous studies are ongoing, leading to the discovery of various implicated genes. Among these, the UNC13 (uncoordinated-13) protein family, comprising the evolutionarily conserved members UNC13A, UNC13B, and UNC13C, UNC13D plays a pivotal role. These proteins are essential regulators of synaptic vesicle priming and are also involved in modulating immune responses (5,6).

UNC13A plays a crucial role in neurotransmitter release at nerve terminals and has been implicated in neurodegenerative diseases (7,8). UNC13B is involved in priming and fusing synaptic vesicles, with polymorphisms in this gene being

https://doi.org/10.33808/clinexphealthsci.1573250

linked to diabetic kidney disease, ALS, and epilepsy. UNC13D regulates immune cell function, and mutations in this gene are associated with familial hemophagocytic lymphohistiocytosis (FHL) (9,10). In contrast, UNC13C is less studied than its counterparts, though it is known to be involved in neurotransmitter release. The tumor-suppressive effect of UNC13C has been demonstrated in oral squamous cell carcinoma and hepatocellular carcinoma (11–13). Notably, UNC13C was previously reported by our group to have low expression levels in glioblastoma (GBM) compared to healthy brain tissue samples. This differential expression indicates a possible role for UNC13C in GBM pathogenesis, highlighting the need for further research into its involvement in tumor development and progression (14).

In this study, we will comprehensively evaluate the expression profile of the UNC13C gene using web-based bioinformatics tools. Additionally, we will put forward the expression levels of UNC13C in glioblastoma cancer cell lines, neural stem cells and healthy brain cell lines.

2. METHODS

2.1. Expression Profiling via web based tools

We examined UNC13C expression across multiple tissue types, including both cancerous and healthy tissues, using the Correlation AnalyzeR web tool (https://gccri.bishop-lab.uthscsa.edu/shiny/correlation-analyzer/) (15). To validate its differential expression in glioblastoma tumors and healthy tissues, we utilized the GEPIA database (http://gepia.cancer-pku.cn/), which is commonly used for analyzing data from The Cancer Genome Atlas (TCGA). Additionally, we employed the UALCAN database (http://ualcan.path.uab.edu/index.html) to confirm UNC13C expression levels in relation to different clinical characteristics.

2.2. Cell Culture

To assess the expression of the UNC13C gene, in addition to LN-18, A-172, and U-87 glioblastoma cell lines, healthy immortalized astrocytes, human microglia cells (HMC3), and neural stem cells were utilized. All cell lines were cultured in complete DMEM medium (DMEM, Gibco, New York, USA) supplemented with 10% fetal bovine serum (Gibco, New York, USA) and 1% Penicillin-Streptomycin (Gibco, New York, USA). Cultures were maintained at 37°C in a humidified atmosphere containing 5% CO2. Regular checks for mycoplasma contamination were performed. Two different passages of each cell line were used as biological replicates. Cells were trypsinized upon reaching 80% confluence, and the pellets were stored at – 80°C for further analysis.

2.3. RNA Isolation and quantitative real-time polymerase chain reaction (qRT-PCR)

RNA was isolated from the cell pellets using TRIzol Reagent (Invitrogen, Carlsbad, CA, USA) following the manufacturer's instructions. The isolated RNA was then reverse-transcribed into complementary DNA (cDNA) using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's protocol. The quantitative PCR reactions were performed using Universal Master Mix, TaqMan probe specific for UNC13C and the synthesized cDNA. The reactions were run on the StepOnePlus Real-Time PCR System (Applied Biosystems, Carlsbad, CA, USA) to accurately quantify the gene expression levels. GAPDH was used as the reference gene as a control of cDNA. The experiment was performed in triplicate, and the mean CT values with error bars were presented.

2.4. Statistical Analysis

The box plot illustrates the differential expression of UNC13C between cancerous and normal tissue samples across various tissue types. Statistical significance was assessed using the Wilcoxon rank sum test (15), The significance of differences performed by UALCAN in expression levels between normal tissues and primary tumors was evaluated using Welch's t-test (16). GEPIA used Differential gene expression analysis by conducting one-way ANOVA, as the independent variable to assess variations in expression levels (17). (significance levels in figures denoted as follows: *p < .05, **p < .01, ***p < .001, ****p < .0001).

3. RESULTS

UNC13C expression was analyzed across multiple tissue types, including cancerous tissues by Correlation Analyzer. Elevated levels of UNC13C mRNA were identified in the pancreas, intestine, immune cells, respiratory tissues, muscles, mammary glands, kidney, stomach, bone, skin, adipose tissue, and brain compared to cancerous samples (Figure 1).

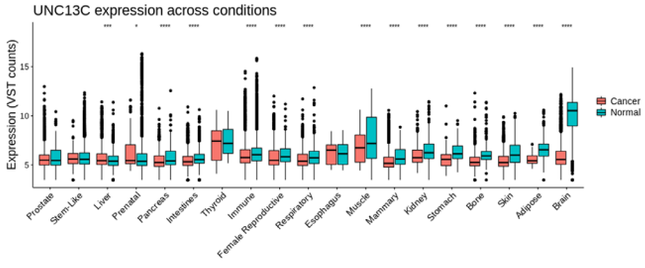

Figure 1. *UNC13C expression across multiple tissue types *: p < .05, **: p < .01, ***: p < .001, ****: p < .0001*

Since our focus was on UNC13C expression in glioblastoma and brain tissue, we specifically investigated its expression in glioblastoma tissues and healthy brain samples using GEPIA analysis, based on data from the TCGA database.A significant decrease in UNC13C expression was observed in glioblastoma tissues, as demonstrated by the boxplot analysis (Figure 2a). This finding was further validated through UALCAN analysis,

which confirmed the reduction of UNC13C expression at both the gene and protein levels (Figure 2b, c).

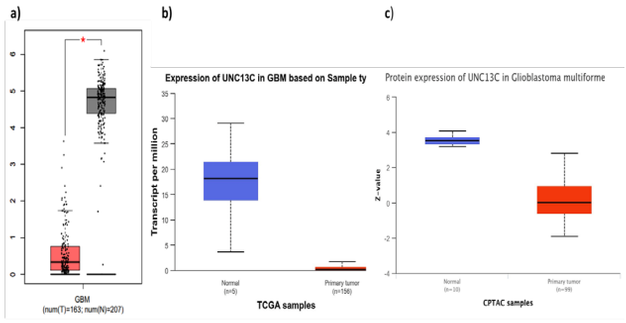

Figure 2. *UNC13C Expression in Glioblastoma Tissues. a) Boxplot from GEPIA analysis showing decreased UNC13C mRNA levels in glioblastoma tissues compared to healthy brain tissues ($p < .05$). b) Boxplot from UALCAN analysis confirming reduced UNC13C mRNA levels in glioblastoma tissues. c) Protein expression analysis from UALCAN further demonstrates reduced UNC13C protein levels in glioblastoma tissues compared to healthy brain tissue.*

We further assessed the expression of the UNC13C mRNA in glioblastoma cell lines U87, A172, and LN18, alongside healthy brain cells such as immortalized astrocytes and Human Microglia Cells (HMC3). Notably, late Ct values were observed in U87 and LN18 cells, and no expression was detected in the A172 cell line. Interestingly, the Ct values for HMC3 cells were very close to those of U87, indicating reduced UNC13C gene expression in both glioblastoma cells and microglia. In healthy astrocytes, UNC13C mRNA was detectable, although the Ct values were over 30, suggesting only basal expression levels. GAPDH was used as a control gene to validate the quality of cDNA from the cell lines (Figure 3b).

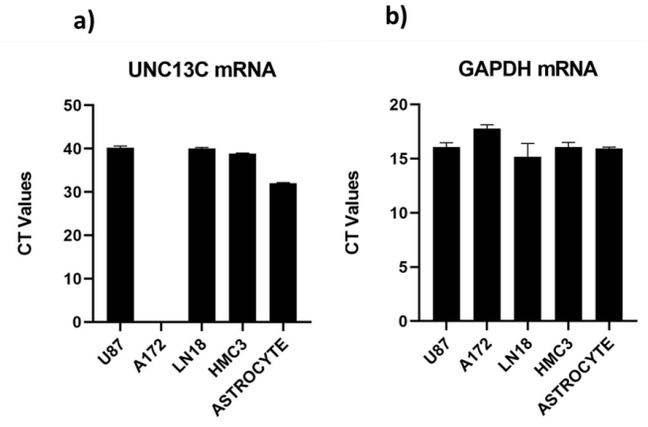

Figure 3. *UNC13C gene expression in cell lines a) The mean CT values of UNC13C in cell lines b) The mean CT values of control GAPDH gene in cell lines*

4. DISCUSSION

UNC13C, the mammalian homolog of Munc13 in mice, is evolutionarily conserved and orthologous to the human UNC13A variant. Mice deficient in UNC13C exhibit complete paralysis and die before birth, highlighting the gene's critical role in early development. Munc13-3 deletion mutants specifically demonstrate increased paired-pulse facilitation at parallel fiber–Purkinje cell synapses, which impacts synaptic plasticity. While these mutants display normal spontaneous motor activity, they show significant impairment in learning complex motor tasks, underscoring the importance of UNC13C in motor coordination and learning (18). Meunier et al. demonstrated that UNC13C plays a critical role in positional or molecular superpriming, which enhances the activation of calcium (Ca^{2+}) release (19) . This process is essential for efficient synaptic transmission, as the elevated Ca^{2+} levels facilitate the release of neurotransmitters, thereby contributing to proper synaptic function and signaling. Recently, a transcriptomic study on mouse astrocytes revealed that UNC13C exhibits high expression levels, highlighting its potential significance in astrocyte function (20).

In our previous study, we demonstrated a decreased level of UNC13C gene expression in the A172 and U-87 human glioblastoma cell lines (14). To gain a more comprehensive understanding, we expanded our analysis to evaluate the gene's expression across various tissue types using transcriptomic data. The highest expression of UNC13C was observed in healthy brain samples compared to tumor tissues. This finding was further validated by UALCAN and GEPIA analyses, both of which showed a significant decrease in UNC13C expression in glioblastoma samples at both the mRNA and protein levels.

To assess the mRNA levels of UNC13C in glioblastoma, we conducted RT-PCR, analyzing its expression in A172, U87, and LN18 glioblastoma cell lines, as well as HMC3 microglia cells and immortalized astrocytes. Consistent with the transcriptomic data, UNC13C expression was undetectable in A172 cells, while very late CT values indicated low expression in U-87 and LN18 cell lines. Wang et al. previously demonstrated that synaptic plasticity in the hippocampus is compromised in C6 glioma-bearing rats, providing support for our findings (21).

Among all the cell lines tested, human astrocytes showed the highest expression, although the CT values did not suggest early expression. While this aligns with transcriptomic data, we had anticipated higher levels of expression. The discrepancy may be due to the differences between cell lines and tissue samples. It could also be influenced by several other factors. These may include variations in the microenvironment, cellular differentiation states, or the influence of external factors such as culture conditions and passage number. Interestingly, basal levels of UNC13C expression were detected in HMC3 cells, a cell line established from 8-10 week-old embryos (22). We hypothesize that the basal UNC13C expression observed in HMC3 cells may reflect a developmental stage, with expression potentially increasing as astrocytes mature in adulthood. While

our findings are significant, the use of a healthy cell line rather than actual brain tissue presents a limitation. Future research that incorporates a broader range of cell lines and samples would yield more comprehensive insights into the role of UNC13C.

5. CONCLUSION

In conclusion, this study provides the first report of UNC13C expression in LN18, HMC3, and healthy astrocytes. Our findings underscore the importance of UNC13C from a developmental perspective.

Acknowledgements: *We would like to thank Marmara University for providing access to academic databases.*
Funding: *The author(s) received no financial support for the research.*
Conflicts of interest: *The authors declare that they have no conflict of interest.*
Peer-review: *Externally peer-reviewed.*
Author Contributions:
Research idea: DS
Design of the study: DS
Acquisition of data for the study:
Analysis of data for the study: DS
Interpretation of data for the study: DS
Drafting the manuscript: DS, ABD
Revising it critically for important intellectual content: DS, ABD, ÖFB
Final approval of the version to be published: DS, ABD, ÖFB,

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How to cite this article: Seven D, Dalan AB, Bayrak ÖF. Unveiling the Expression of UNC13C in Healthy Brain and Glioblastoma Cells. Clin Exp Health Sci 2025; 15: 198-202. DOI: 10.33808/clinexphealthsci.1573250