

Bioinformatics Analysis of the Mechanisms by Which NAGLU Regulates Glioblastoma Cell Development

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Abstract: To investigate the expression of N-acetyl- α -glucosaminidase (NAGLU) in glioblastoma (GBM) and to understand its relationship with disease progression and prognosis, we screened for differentially expressed genes in GBM progression using the QuickGO and GEPIA 2 online databases and identified the target gene *NAGLU*. Further analysis was conducted using bioinformatics tools, including the STRING, TIMER 2.0, and UALCAN databases, to analyze the differential expression of *NAGLU* across various cancer types and its correlation with GBM progression, as well as to predict its association with prognosis. We used the miRNet database to analyze its target miRNAs and lncRNAs and employed Cytoscape v3.10.0 to visualize the potential ceRNA regulatory network. The results showed that 144 genes related to cell developmental processes were retrieved from online databases. Additionally, 7,648 genes were differentially expressed in GBM. Sixty-two differentially expressed genes were involved in the biological processes of GBM cell senescence. Among them, *NAGLU* was significantly overexpressed in multiple cancers, including GBM, and high *NAGLU* expression was statistically associated with a significantly worse prognosis in GBM patients. The *NAGLU* gene encodes 10 interacting proteins, primarily involved in 3 signaling pathways (KEGG), 3 molecular functions (MF), 12 cellular components (CC), and 6 biological processes (BP). We predicted 1 miRNA and 44 lncRNAs targeting *NAGLU*. The study results indicate that *NAGLU* is highly expressed in various tumors, including GBM. Its expression also showed significant differences based on patient gender, age, ethnicity, and TP53 mutation status. In GBM progression, *NAGLU* may function via the lncRNA/miR-1-3p/*NAGLU* axis, serving as both a prognostic marker and a potential therapeutic target.

Keywords: N-acetyl- α -glucosaminidase; Cell Development; Glioblastoma; Bioinformatics.

1. Introduction

In China, the proportion of GBM among primary brain tumours ranges from about 12% to 16%, with an annual incidence rate of roughly 3 to 5 cases per 100,000 population, and GBM exhibits an extremely high degree of malignancy, with an average median survival of only 12 months and an extremely low 5-year survival rate of no more than 5%, following pancreatic and lung cancers in the ranking of systemic malignant tumours, with an extremely poor prognosis [1]. The challenge in the treatment of GBM mainly stems from the high proliferation rate and aggressive growth pattern of tumour cells, which making it difficult to achieve complete elimination of tumour cells by surgery alone, while postoperative adjuvant therapies are unable to ensure the eradication of residual tumour cells, which results in inevitable recurrence of the tumour [2,3]. In view of this, it is particularly important to explore new therapeutic means or therapeutic targets for the biological characteristics of GBM, aiming to inhibit tumour growth and delay the recurrence process more effectively. Tumorigenesis is closely related to the cell development process, with many characteristics of tumor cells (e.g., dedifferentiation, migration, invasion, stem cell properties, etc.) representing abnormal activation or dysregulation of the cell development program. The cell differentiation process of GBM, the most important factor determining malignancy degree and promoting invasion/metastasis, shows an inverse correlation with

aggressiveness; the higher the differentiation degree of GBM cells, the lower their malignancy and metastatic potential [4]; conversely, the lower the differentiation degree, the higher the malignancy, invasion/metastasis likelihood, and poorer patient prognosis [5], though its specific mode of action remains insufficiently clear.

N-acetyl- α -aminoglucosidase (NAGLU) is a lysosomal hydrolase essential for degrading glycosaminoglycan (GAG) heparan sulfate (HS) [6,7]. Defects in the *NAGLU* gene cause mucopolysaccharidosis type IIIB (MPS-IIIB, Sanfilippo syndrome B), a disorder characterized by lysosomal accumulation and urinary excretion of heparan sulfate [8]. Lysosomes, located at the endpoints of endocytosis, phagocytosis, and autophagy pathways, mediate cellular transport and play a key role in maintaining cholesterol homeostasis. Due to the blood-brain barrier, GBM cells depend on cholesterol supplied by the tumor microenvironment (TME), making their high lysosomal demand a potential Achilles heel of GBM [9,10]. Given its essential role in lysosomal hydrolysis, we hypothesized *NAGLU*'s involvement in GBM development. Here, we analyzed *NAGLU*-targeting miRNAs and lncRNAs via bioinformatics to explore its expression and regulatory mechanisms in GBM.

2. Materials and Methods

2.1. Acquisition of Cell Development-related Genes and GBM Differentially Expressed Genes

In the QuickGO database (<https://www.ebi.ac.uk/QuickGO/>), we searched for genes by entering "glial cell development" in the search box and selecting the "taxon" filter in the results page. We then entered "glial cell development" in the search box, selected "Homo sapiens" in the "taxon" tab of the search results, and clicked "Apply" to retrieve and download genes associated with glial cell development processes. Next, we accessed the GEPIA2 database (<http://gepia2.cancer-pku.cn/#index>) and selected "Differential genes" from the dropdown menu. For the dataset option, we chose "GBM" with "Chromosomal Distribution" set to "Both," then downloaded the differentially expressed genes for GBM. Finally, the resulting gene sets were analyzed using a Venn diagram tool (<https://bioinformatics.psb.ugent.be/webtools/Venn/>) to identify co-expressed genes.

2.2. Unraveling Prognostic Signatures and Protein Interaction Networks

The co-expressed genes were imported into the STRING online database (<https://cn.string-db.org/>) to construct protein-protein interaction networks, and the interacting proteins of the co-expressed genes were identified. TIMER2.0 (<http://timer.cistrome.org/>) and UALCAN database (<https://ualcan.path.uab.edu/index.html>) were employed to analyze the differential expression of co-expressed genes in GBM and predict their prognostic value and survival correlation in GBM.

2.3. Mapping Protein Interaction Networks and Functional Enrichment Profiles

Co-expressed genes were analyzed using STRING, a database for protein-protein interaction analysis, to identify their interacting proteins. The co-expressed genes and their interacting partners were then submitted to the DAVID database (<https://david.ncifcrf.gov/>) for functional annotation.

2.4. Analysis of ceRNA Regulatory Network of Co-expressed Genes

The co-expressed genes were input into the "Genes" module of the miRNet database (<https://www.mirnet.ca/mirnet/home.xhtml>), with the species specified as "Homo sapiens (human)", the ID type as "official gene symbol", and the target type as "miRNA"; the gene list was then submitted. The target miRNAs were subsequently used to identify their interacting lncRNAs in the database. Finally, the co-expressed genes along with the identified miRNAs and lncRNAs were imported into Cytoscape v3.10.0 to construct a ceRNA network.

3. Results

3.1. 62 Differentially Expressed Genes Involved in the Biological Process of GBM Cell Development

A total of 144 genes related to biological processes

associated with cell development were retrieved from the QuickGO database. Additionally, 7,648 GBM-related differentially expressed genes were obtained from the GEPIA2 online database (Supplementary Table 1). Co-expression analysis of these two gene sets identified 62 differentially expressed genes (including *NAGLU*) that were significantly associated with GBM cell senescence (Fig. 1), suggesting their potential roles in GBM tumorigenesis and progression.

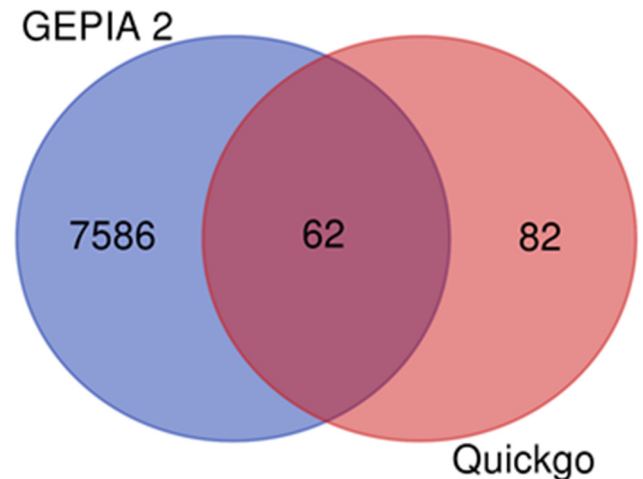


Figure 1. Venn diagram of cell senescence gene in Quick Go base and DEGs in GEPIA2 of GBM

3.2. The Expression of *NAGLU* in Pan-cancer

Analysis using the TIMER2.0 database revealed that *NAGLU* showed significantly higher expression in multiple cancer types. By integrating TCGA clinical data with UALCAN analysis, we found that *NAGLU* was highly expressed in GBM and other tumors, with significantly elevated mRNA levels in GBM specimens compared to normal brain tissues ($p < 0.01$) (Figure 2-3), suggesting *NAGLU*'s involvement in tumorigenesis and its potential as a therapeutic target. We summarized the abbreviations of various tumors in Table 1.

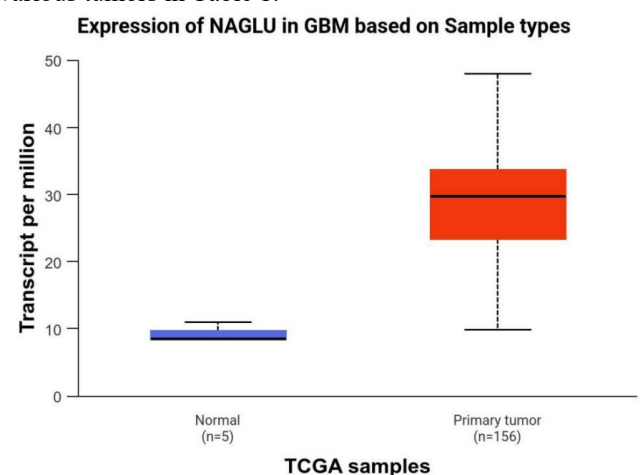


Figure 2. The expression of *NAGLU* in GBM in UALCAN database

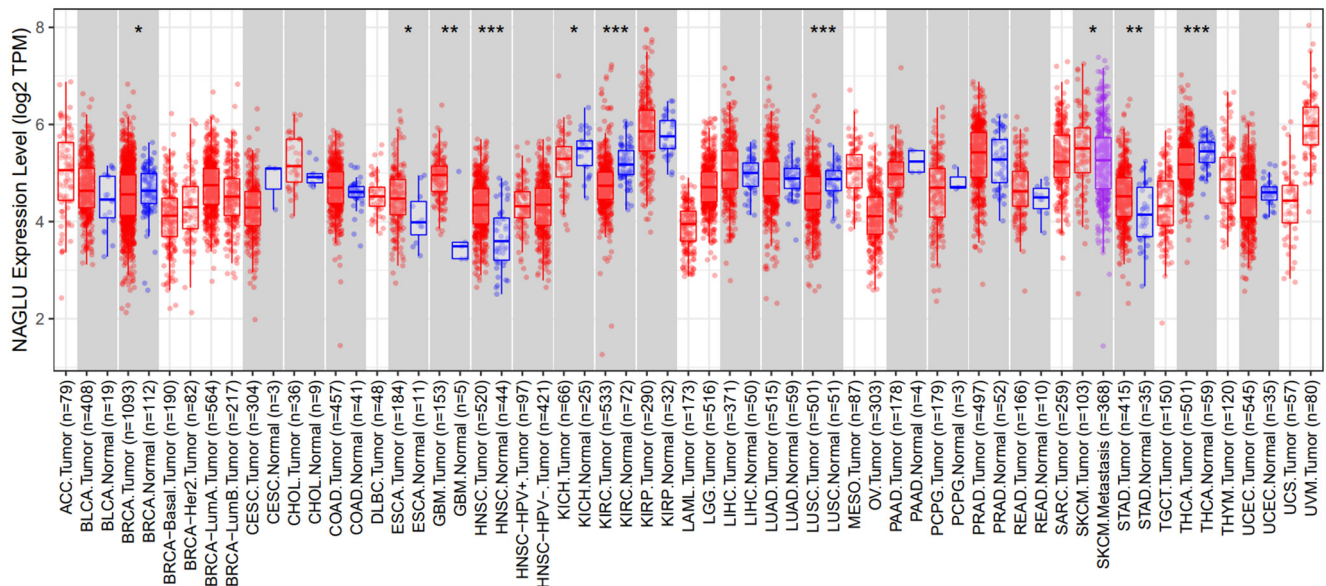


Figure 3. Expression of *NAGLU* in a variety of tumors in TCGA database

Table 1. Abbreviations and full name of TCGA cancer

abbreviation	full name	abbreviation	full name	abbreviation	full name
ACC	Adrenocortical carcinoma	KIRC	Kidney renal clear cell carcinoma	PRAD	Prostate adenocarcinoma
BLCA	Bladder Urothelial Carcinoma	KIRP	Kidney renal papillary cell carcinoma	READ	Rectum adenocarcinoma
BRCA	Breast invasive carcinoma	LAML	Acute Myeloid Leukemia	SARC	Sarcoma
CECS	Cervical squamous cell carcinoma and endocervical adeno carcinoma	LGG	Lower Grade Glioma	SKCM	Skin Cutaneous Melanoma
CHOL	Cholangio carcinoma	LIHC	Liver hepatocellular carcinoma	STAD	Stomach adenocarcinoma
COAD	Colon adenocarcinoma	LUAD	Lung adenocarcinoma	TGCT	Testicular Germ Cell Tumors
DLBC	Diffuse Large B-cell Lymphoma	LUSC	Lung squamous cell carcinoma	THCA	Thyroid carcinoma
ESCA	Esophageal carcinoma	MESO	Mesothelioma	THYM	Thymoma
GBM	Glioblastoma multiforme	OV	Ovarian serous cystadenocarcinoma	UCEC	Uterine Corpus Endometrial Carcinoma
HNSC	Head and Neck squamous cell carcinoma	PAAD	Pancreatic adenocarcinoma	UCS	Uterine Carcinosarcoma
KICH	Kidney Chromophobe	PCPG	Pheochromocytoma and Paraganglioma	UVM	Uveal Melanoma

3.3. Interacting Proteins and Functional Analysis of *NAGLU* Gene

The *NAGLU* gene encodes 10 interacting proteins, including *GNS*, *TMED9*, *GALNS*, *TMED4*, *TMED10*, *HSD17B1*, *LAMP2*, *HGSNAT*, *SGSH*, and *IDUA* (Figure 4). Functional enrichment analysis using the DAVID database revealed these genes were primarily associated with: three KEGG pathways (glycosaminoglycan degradation, lysosome, and metabolic pathways); three molecular functions (sulfuric ester hydrolase activity, hydrolase activity, and syntaxin

binding); 12 cellular components including lysosomal lumen, COPII-coated ER-to-Golgi transport vesicle, lysosome, and endoplasmic reticulum-Golgi intermediate compartment; and six biological processes including heparan sulfate proteoglycan catabolic process, glycosaminoglycan catabolic process, and endoplasmic reticulum-to-Golgi vesicle-mediated transport (Figure 5).

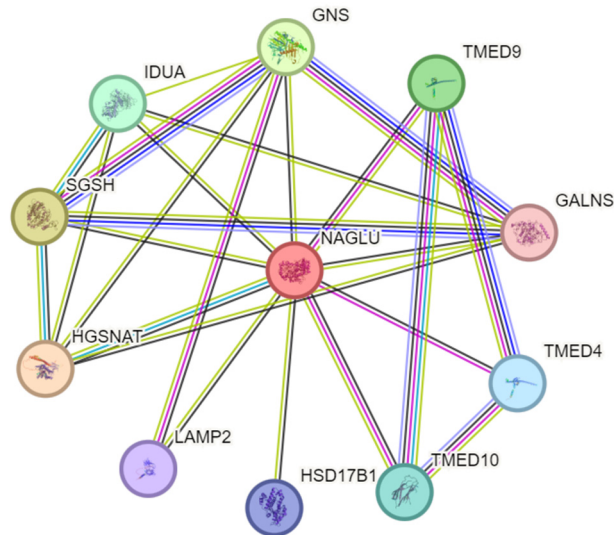


Figure 4. The PPI of *NAGLU*

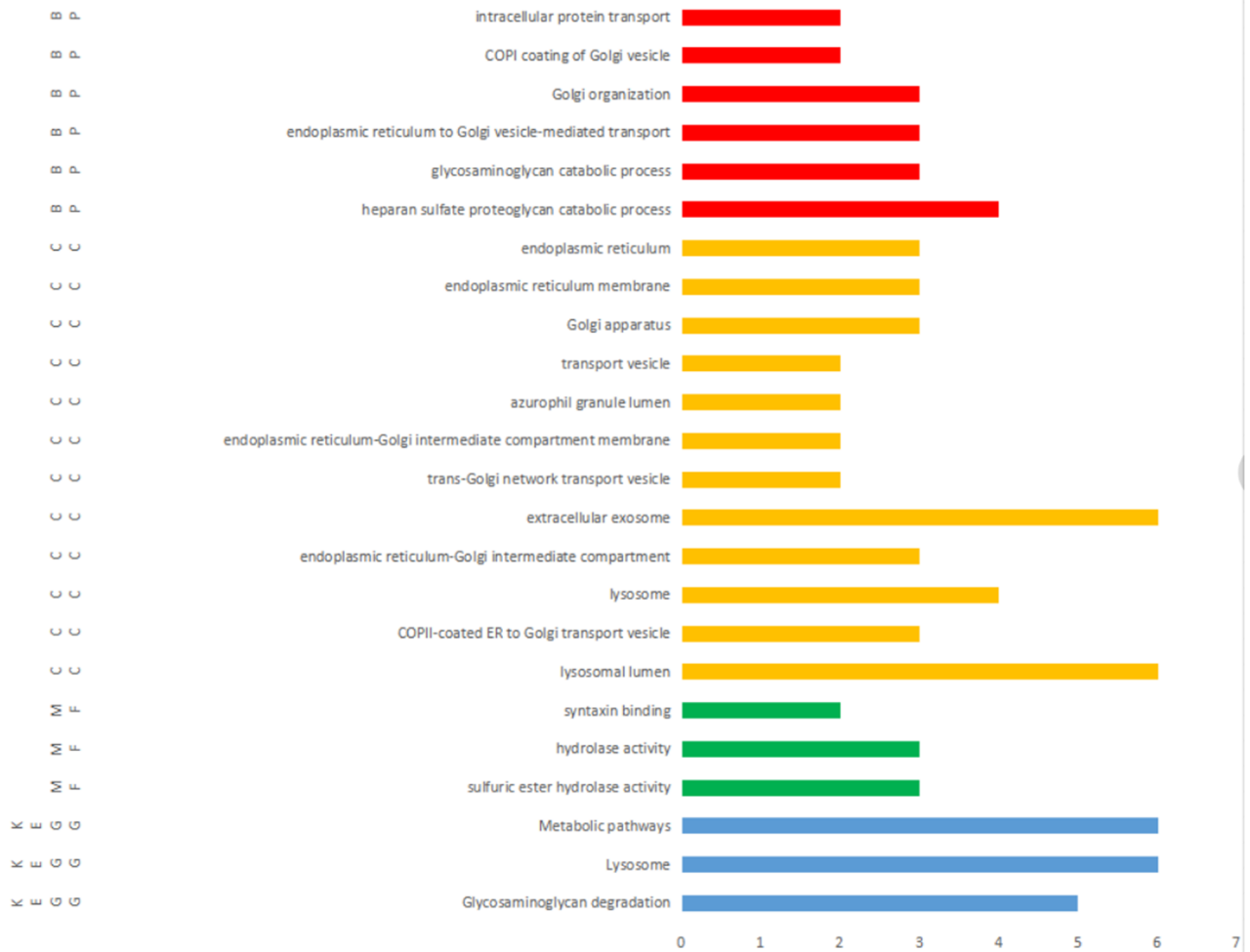


Figure 5. Functional enrichment analysis of *NAGLU* gene and its related genes

3.4. Value of *NAGLU* and GBM Disease Treatment and Prognosis

Analysis using the UALCAN database revealed significant differential expression of *NAGLU* in GBM. *NAGLU* expression levels significantly correlated with patient sex (Normal vs Male, $p < 0.001$; Normal vs Female, $p < 0.05$), age (Normal vs 21-40 years, $p < 0.05$; Normal vs 41-60 years, $p < 0.01$; Normal vs 61-80 years, $p < 0.001$; Normal vs 81-100 years, $p < 0.05$), TP53 mutation status (Normal vs TP53-

mutant, $p < 0.001$; Normal vs TP53-nonmutant, $p < 0.001$), and ethnicity (Normal vs Caucasian, $p < 0.01$; Normal vs African American, $p < 0.05$; Normal vs Asian, $p < 0.05$) (Fig. 6). The GEPIA2 database analyses yielded consistent results. As shown in Figure 7, prognostic survival curve analysis demonstrated that patients stratified into low- and high-expression groups based on *NAGLU* expression levels showed significantly different survival outcomes, with the low-expression group exhibiting longer survival time ($p = 0.012$). These findings suggest *NAGLU* may serve as a

potential biomarker for GBM treatment and prognosis

assessment.

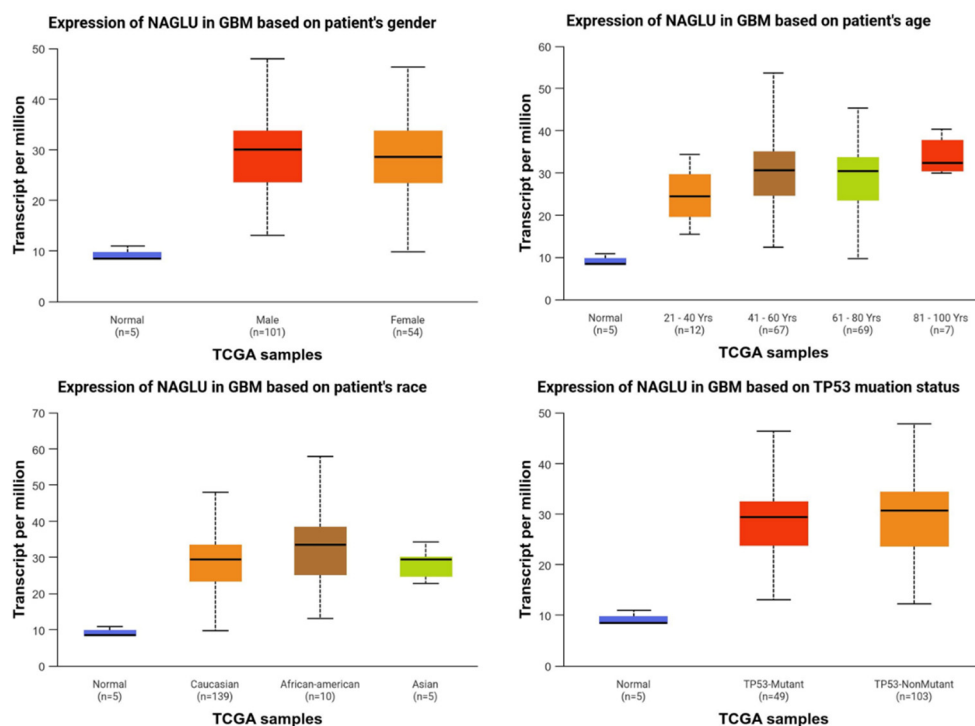


Figure 6. Comparison of *NAGLU* in different states of GBM

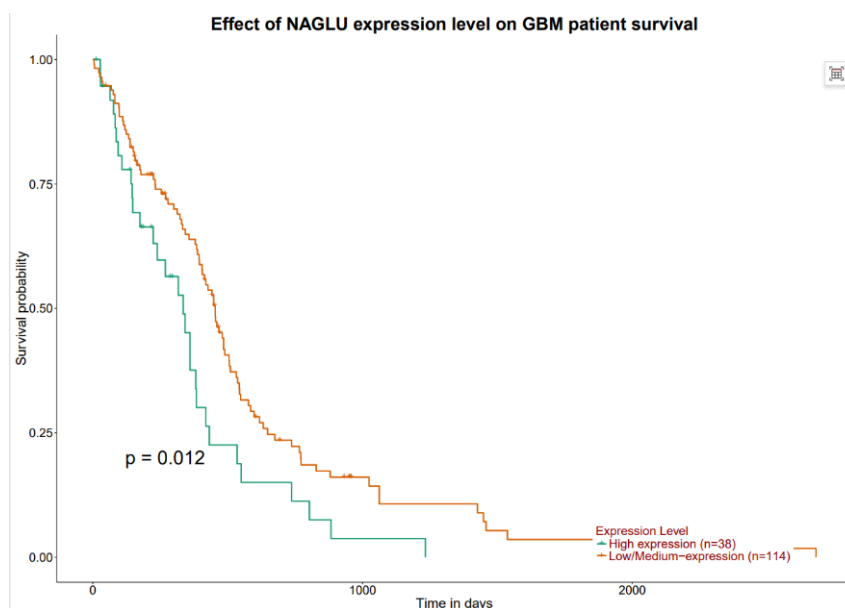


Figure 7. Prognostic survival analysis of *NAGLU* in GBM

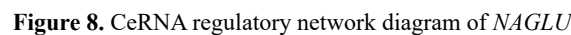
3.5. *NAGLU* gene ceRNA Regulatory Network Map

NAGLU was analyzed using the miRNet database for target RNA prediction, which identified one targeting miRNA (hsa-miR-1-3p) that was predicted to regulate 44 lncRNAs. These interaction networks were imported into Cytoscape software to construct the *NAGLU* regulatory network (Figure 8). These findings suggest *NAGLU* may be regulated via the lncRNAs/miR-1-3p/*NAGLU* axis in GBM.

4. Discussion

GBM is the most common primary malignant brain tumor characterized by aggressive behavior and poor patient survival [11]. Owing to its marked tumor heterogeneity, GBM

treatment remains clinically challenging [12,13]. Standard treatments including surgical resection, radiotherapy, and chemotherapy fail to prevent GBM recurrence [14,15]. While immunotherapy has shown limited success in improving GBM patient outcomes [16], significant interpatient variability limits its universal efficacy [17-19]. GBM typically presents with insidious onset, demonstrates rapid progression, and frequently develops treatment resistance, culminating in dismal prognosis. Cellular developmental processes are fundamentally linked to tumorigenesis, wherein tumors essentially represent developmental aberrations. Specifically, dysregulation of normal developmental control mechanisms results in uncontrolled proliferation, differentiation defects, and functional impairment. Therefore, targeting developmental pathways may offer novel therapeutic opportunities for tumor intervention.



In summary, *NAGLU*, a lysosomal hydrolase essential for heparan sulfate glycosaminoglycan degradation, was significantly upregulated in GBM compared to normal tissues and significantly correlated with gender, age, TP53 mutation status, and ethnicity in GBM patients. Database analysis demonstrated that elevated *NAGLU* expression correlated with poorer prognosis in GBM patients. Functional enrichment analysis indicated *NAGLU*'s protein-protein interactions and its involvement in multiple cellular components (CC), biological processes (BP), and molecular functions (MF). *NAGLU* may regulate lysosomal activity and cell development in GBM through the lncRNA-miR-1-3p-*NAGLU* axis. Collectively, these findings position *NAGLU* as a potential contributor to GBM pathogenesis and a promising therapeutic target and prognostic biomarker.

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