Predictive Biomarker DPY19L1 Reduces Survival in Glioma Patients

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Abstract

Background: Glioblastomas have poor survival outcome. Identifying biomarker for survival differentiation is beneficial for glioma patients. This study aims to investigate the survival outcome and pathological grading of DPY19L1 in human gliomas.

Methods: We analyzed the DPY19L1 mRNA expression and survival of human gliomas from the Cancer Genome Atlas (TCGA) and Chinese Glioma Genome Atlas (CGGA) databases. We validated gene expression of DPY19L1 in glioma by quantitative RT-PCR and Western blot. Further validation via immunohistochemical staining (IHC) of human glioma samples was performed from the Human Protein Atlas. We also investigated in Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) to explore the protein-protein interaction of DPY19L1.

Results: Overexpression of DPY19L1 mRNA level was associated with higher glioma grade. Moreover, high expression of DPY19L1 reduced survival in patients with glioma. Human glioma samples further found that the labeling index of DPY19L1 was higher in high-grade than in low-grade gliomas.

Conclusion: Elevation in DPY19L1 expression correlates with higher WHO pathological grading and unfavorable prognosis. DPY19L1 is a novel predictive biomarker and potential therapeutic target in glioma treatment.

Introduction

Gliomas have the highest prevalence in primary malignant brain tumor and are categorized into 4 grades based on histologic appearance according to World Health Organization (WHO). Glioblastoma multiforme (GBM) is classified as WHO grade IV and accounts for 82% of gliomas [1]. Further classification includes various molecular features such as chromosome 1p/19q co-deletion, IDH mutation, EGFR amplification etc [2]. GBM manifests as highly invasive brain tumor with poor prognosis. Although the treatment of GBM have been advancing, the overall survival remains unsatisfying [3]. Therefore, it is crucial to identify genetic biomarkers and investigate novel therapy for gliomas.

DPY-19-like 1 (DPY19L1) was acknowledged as a homolog of C. elegans dpy-19 [4], and four dpy-19-like genes (Dpy19l1-4) have been recognized to this point [5]. Abundant expression of DPY19L1 presented in glutamatergic neurons of the cerebral cortex. The product of the DPY19L1 gene incorporates a multi-transmembrane protein of 746 amino acids which plays a role in the regulation of neuronal migration [6]. Previous studies of DPY19L1 have been published in lung cancer and colon cancer [7–9]. Nevertheless, no investigation of DPY19L1 in human glioma has been established.

We investigated PREdiction of Clinical Outcomes from Genomic Profiles (PRE-COG; http://precog.stanford.edu) and Gene Expression Omnibus (GEO) profile for genes associated with human diseases [10, 11]. Furthermore, we analyzed survival outcomes through The Cancer Genome Atlas (TCGA) and Chinese Glioma Genome Atlas (CGGA, http://www.cgga.org.cn). Finally, quantitative

validation was confirmed by RT-PCR and Western blotting. To conclude, our findings supported that DPY19L1 might present prognostic value in human gliomas.

Materials And Methods

Investigation of public genomic databases

The institutional review board of Tri-Service General Hospital, National Defense Medical Center, Taipei, Taiwan, ROC approved this study (TSGHIRB No: B-111-21). Several researchers have demonstrated the process for investigating public genomic databases [12-14]. For the purpose of exploring the impact of DPY19L1 expression on pathological grading and overall survival, we conducted analysis of The Cancer Genome Atlas (TCGA) and the Chinese Glioma Genome Atlas (CGGA) [15]. The data from TCGA database was extracted through UCSC Xena (https://xenabrowser.net/) and GlioVis (http://gliovis.bioinfo.cnio.es/) [16, 17], consisting of 226 sheets grade II gliomas, 244 sheets grade III gliomas and 150 sheets grade IV gliomas. We additionally acquired 321 samples from the CGGA database (http://www.cgga.org.cn/) including 103 grade II gliomas, 79 grade II gliomas and 139 grade IV gliomas.

We used Kaplan-Meier survival curve to present survival outcome compared to different expression level from cohorts of TCGA and CGGA. According to statistical analysis, the cut-off point of DPY19L1 expression was considered as median value. Plots were produced by GraphPad Prism 5 software, and $P < 0.05$ was defined as statistical significance.

RNA isolation and quantitative real-time PCR

We extracted total mRNA via TRIzol™ Reagent (Thermo Fisher Scientific, Wal-tham, WA, USA) according to the manufacturer's protocol. Oligo dT primer with MMLV Reverse Transcriptase (Epicentre Biotechnologies, Madison, WI, USA) was applied for cDNA synthesis. We purchased normal brain cDNA from Origene Technologies (Rockville, MD, USA). Amplification and quantification of DPY19L1 expression were achieved by a StepOne™ Real-Time PCR System (Thermo Fisher Scientific, USA). We used the $2-\Delta\Delta Ct$ method to compare relative quantitative gene expression with GAPDH as an internal control. The primer pairs included presented below: DPY19L1 forward 5'- ACACCACCTCTCCGTGAAAGCT-3' and reverse 5'- GCAGAGTGCAATCAA-GCTTCCTC-3'; GADPH forward 5'-CTTCATTGACCTCAACTAC-3' and reverse 5'-GCCATCCACAGTCTTCTG-3'.

Cell culture

LN229, U118MG and U87MG cell lines were commercially available from American Type Culture Collection (ATCC), and we also purchased GBM8401 glioma cell line from Bioresource Collection and Research Center (BCRC number 60163, Hsinchu, Taiwan). LN229 and GBM8401 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) which comprises 2% fetal bovine serum (FBS), penicillin,

and streptomycin, and U87MG, U118MG, and LNZ308 cells were cultured in DMEM consisting of 10% FBS, penicillin, and streptomycin. Cells above were incubated in 37 °C and 5% CO2 condition.

Cell Lysate Preparation and Western Blot

RIPA buffer (100 mM Tris-HCl, 150 mM NaCl, 0.1% SDS, and 1% Triton-X-100) was applied for cell lysis at 4 °C for 10 min and centrifuged at 15,000 rpm for 10 min. Separation of proteins was achieved by 10% Sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Then we transferred the proteins to polyvinyl difluoride membranes (Millipore, MA, USA) which was blocked with 5% skim milk in TBST for 1 hour. DPY19L1 antibody (Atlas Antibodies, Stockholm, Sweden) and GADPH (Santa Cruz Biotechnology, Inc.) were incubated. The result was observed through enhanced chemiluminescence and ChemiDocTM MP Imaging System (BIO-RAD, Hercules, CA, USA).

Immunohistochemical Staining of Glioma Tissue Microarray

We acquired samples of high- and low-grade gliomas from the Human Protein Atlas (https://www.proteinatlas.org/ENSG00000173852-DPY19L1/pathology/glioma). 11 cases with 19 cores of tissue microarray were stained using DPY19L1 antibody (HPA059139) and immunohistochemical staining protocol.

Protein–protein network and signaling pathways analysis

To analyze interaction between DPY19L1 and associated proteins, we investigated the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database version 11.5 (http://string-db.org) [18].

Statistical Analysis

We performed single tail t test to clarify the significance of DPY19L1 expression in different glioma grades according to the databases (GDS1962 / 1560916_a_at, TCGA and CGGA). The Bonferroni method was conducted to accurate the P value and eliminate the possibility of type I error by R 3.0.1 software package.

Results

DPY19L1 Expression Had Positive Correlation with WHO pathological grading of Gliomas

We observed that the expression level of DPY19L1 elevated considerably in higher glioma pathological grade. According to the analysis of 620 samples in TCGA dataset, the expression level of DPY19L1 enhanced with increasing WHO grade, and there was significant difference comparing GBM with grade III (P = 9.56 x 10-33) and grade II gliomas (P = 5.39 x 10-57, P adjusted by Bonferroni method) (**Figure 1a**).

To solidify our finding, we also evaluated 329 glioma samples in the CGGA dataset. DPY19L1 expression level corelated significantly with higher glioma grade, and the difference between each subgroup revealed

statistical significance (**Figure 1b**). The same results were concluded from these studies that overexpression of DPY19L1 mRNA level positively corelated with higher glioma grade.

High DPY19L1 Expression Associated with Shorten Survival in Glioma Patients

To investigate the prognostic value of DPY19L1 in gliomas, we conducted the Kaplan-Meier survival analysis from two iconic databases, TCGA and CGGA, representing western and eastern population. The result demonstrated poor overall survival in patients with high DPY19L1 expression compared to low expression group with statistical significance (TCGA, n = 394, P <0.0001 by log-rank test, 95% confidence interval: 3.939 to 6.508, hazard ratio 5.063; CGGA, n = 166, P <0.0001 by log-rank test, 95% confidence interval: 1.832 to 3.856, hazard ratio 2.658) (**Figure 1c, 1d**).

Protein Expression of DPY19L1 Enhanced in Glioma Cells

Quantitative real-time PCR and Western blot were performed to evaluate DPY19L1 protein expression between normal brain tissue and U87MG, LN229, GBM8401, U118MG, LNZ308 glioma cell lines. The result showed that DPY19L1 protein expression was significantly intensified in entire glioma cells in comparison to normal brain tissue (**Figure 2a**). Similar outcome was also obtained through western blot (**Figure 2b**).

High-grade Gliomas revealed overexpression of DPY19L1 in Tissue Microarray Samples

We investigated DPY19L1 protein expression in glioma tissues via immunohisto-chemical (IHC) staining of public tissue microarray. The staining locations were prominently observed in cytoplasm and membrane. Comparison of low- and high-grade glioma micrographs revealed overexpression of DPY19L1 in high-grade samples (Figure 3).

DPY19L1 represents as Hub Protein in the Protein–protein Interactions

We applied the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) to investigate the protein–protein interaction (PPI) network of DPY19L1. The network revealed interactions between DPY19L1 and WD repeat-containing protein 7 (WDR7), Spermatogenesis-associated protein 5 (SPATA5), Putative E3 ubiquitin-protein ligase SH3RF2, Netrin receptor UNC5A, Transmembrane and tetratricopeptide repeat containing 4 (TMTC4), Olfactory receptor 5I1 (OR5I1), Protein MANBAL and Receptor expression-enhancing protein 1 (REEP1) (Figure 4).

Discussion

There is no present study investigating the role of DPY19L1 in gliomas. In our study, we validate that DPY19L1 expression significantly rises in GBM than lower grade gliomas. Furthermore, high expression of DPY19L1 related to shorten survival outcome in glioma patients, indicating the characteristic of oncogene. We also applied RT-PCR and Western blotting to confirm our findings.

DPY19L1 acts as a regulator in neuronal migration, and high expression was found in glutamatergic neurons. Knockdown of DPY19L1 leads to improper migration in development of the neocortex [6]. The protein encoded in DPY19L1 is distributed in the endoplasmic reticulum and nuclear envelope which has impact on cell metabolisms. In mouse cortex, DPY19L1-knockdown neurons showed impairment of neurite extension [19]. Recently, Ebbing et al. demonstrated that the C. elegans Q neuroblasts polarize asymmetrically through the mechanisms of multiple transmembrane proteins by MIG-21 - DPY-19, CDH-3 - CDH-4/Fat and UNC-40/DCC pathways. DPY-19 function as a key regulator in directing the course of the growing protrusion autonomously [20]. Thrombospondin type 1 repeats can be C-mannosylated by DPY19L1 which is crucial for the transportation of the netrin receptor UNC5A in cell adhesion [21]. Asada et al. performed a multi-omics analysis and recognized the correlation between DPY19L1 and survival outcome of lung adenocarcinoma [9]. Moreover, DPY19L1 has been identified as proadhesive genes in hepatic metastasis of colon cancer due to its silence condition in nonadherent cancer cells [7]. In this study, we firstly reported DPY19L1 as a predictor of prognosis in human gliomas.

We investigated in the protein-protein interaction (PPI) network of DPY19L1 and found the connections to SH3RF2 and UNC5A [18]. SH3RF2 implying SH3 Domain Containing Ring Finger 2 comprises three SH3 domains and a RING domain. SH3RF2 acts as a key regulator in inhibition of PAK4 ubiquitination, therefore, promoting tumor cell survival. Overexpression of SH3RF2 was found in patients of colon cancer and as-sociated with worse prognostic outcome [22]. In contrast, UNC5A activated caspase-3 and resulted in p53-dependent apoptosis in cancer cells [23]. Multiple researches had identified UNC5A as tumor suppressor in colon cancer, bladder cancer, breast cancer, neuroblastoma and glioblastoma [24–27]. Additional studies may clarify the relationships among DPY19L1 and interactive proteins.

Several limitations remained in our current study. Samples of non-tumor controls and lower grade gliomas were hard to collect clinically. A number of datasets including GEO profiles, TCGA, CGGA and UCSC Xena were used to validate the prognostic value of DPY19L1 through pathological grading and survival analysis [17, 28–30]. Moreover, the thorough mechanism of DPY19L1 in affecting the prognosis of glioma patients should be studied in the future.

Conclusions

Our study demonstrated that higher expression of DPY19L1 associated with increased WHO pathological grades of gliomas. Elevated DPY19L1 levels in high-grade glioma patients induces poor survival outcome. Consequently, we consider DPY19L1 to be a novel prognostic biomarker and potential therapeutic target in glioma treatment.

Abbreviations

DPY19L1: DPY-19-like 1; TCGA: the Cancer Genome Atlas; CGGA: Chinese Glioma Genome Atlas; IHC: immunohistochemical staining; STRING: Search Tool for the Retrieval of Interacting Genes/Proteins;

WHO: World Health Organization; GBM: Glioblastoma multiforme; PRECOG: PREdiction of Clinical Outcomes from Genomic Profiles; GEO: Gene Expression Omnibus; PPI: protein–protein interaction

Declarations

Ethics approval

This study was approved by the ethics Committee of Tri-Service General Hospital, National Defense Medical Center, Taipei, Taiwan, ROC (TSGHIRB No: B-111-21). All methods were carried out in accordance with relevant guidelines and regulations.

Consent for publication

All of the authors signed the consent for publication of this study.

Availability of data and material

The dataset used and/or analyzed during the current study are available from the corresponding author on a reasonable request.

Conflicts of Interest

All of the authors participated in this study declare that they have no conflict of interest.

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Author's Contributions

Conceptualization, Kuang-Chen Hung, Dueng-Yuan Hueng; methodology, Ssu-Han Chen; val-idation, Po-Chun Lin, Li-Chun Huang; formal analysis, Po-Chun Lin; investigation, Ming-Hsuan Chung, Peng-Wei Wang; resources, Dueng-Yuan Hueng; data curation, Po-Chun Lin; writ-ing—original draft preparation, Po-Chun Lin; writing—review and editing, Dueng-Yuan Hueng; visualization, Po-Chun Lin; supervision, Dueng-Yuan Hueng; project administration, Po-Chun Lin; funding acquisition, Kuang-Chen Hung, Dueng-Yuan Hueng.

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Figures

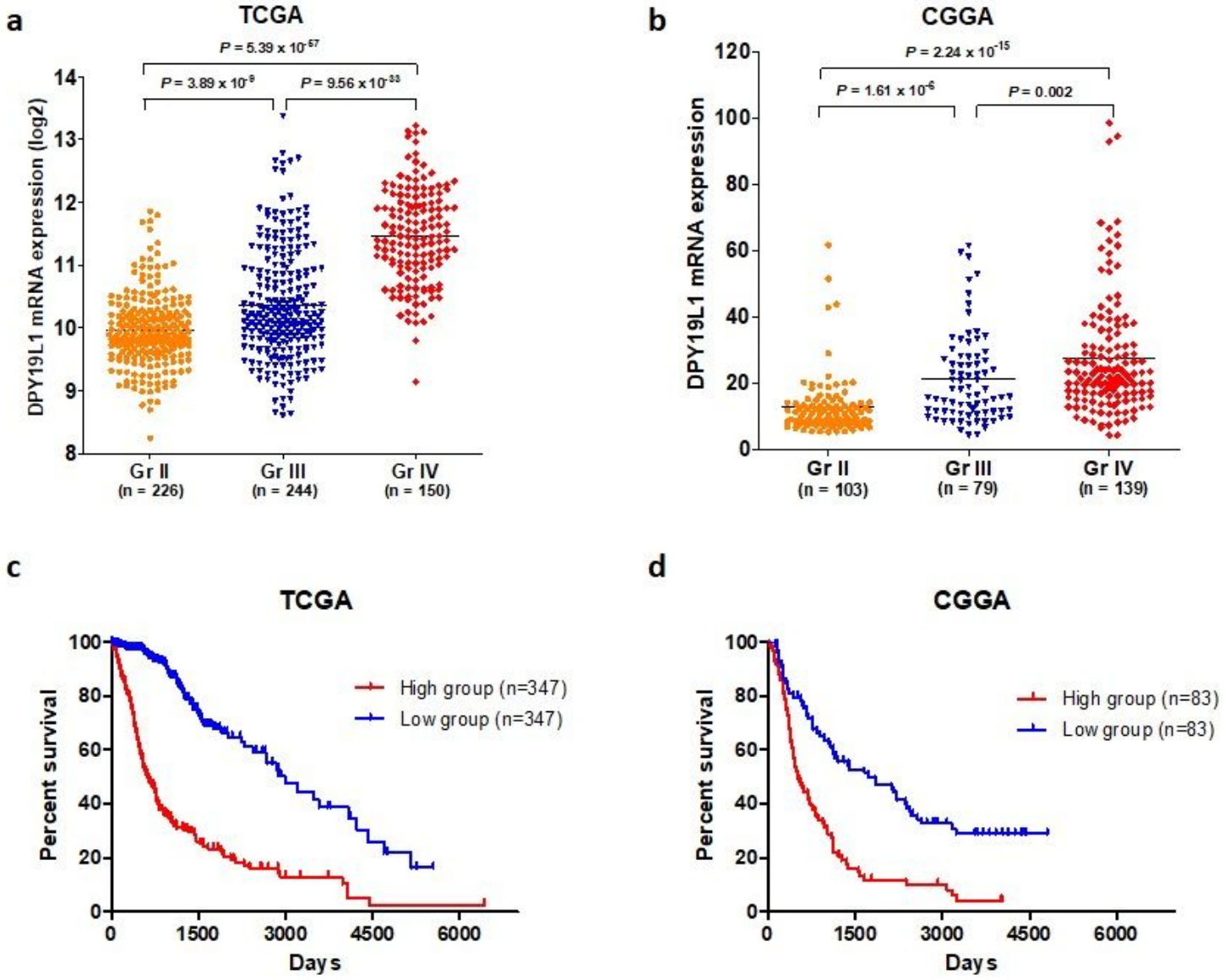

Figure 1

Increased DPY19L1 expression significantly correlated with higher glioma grade and unfavorable survival outcome. (a,b) The scattered plots demonstrated that DPY19L1 mRNA expression was associated with World Health Organization (WHO) pathological grading of gliomas in TCGA and CGGA datasets. (c,d) The Kaplan–Meier survival curves revealed worsen prognosis in high DPY19L1 expression group than those with low expression. (TCGA, n = 394, P <0.0001 by log-rank test, 95% confidence interval: 3.939 to 6.508, hazard ratio 5.063; CGGA, n = 166, P <0.0001 by log-rank test, 95% confidence interval: 1.832 to 3.856, hazard ratio 2.658).

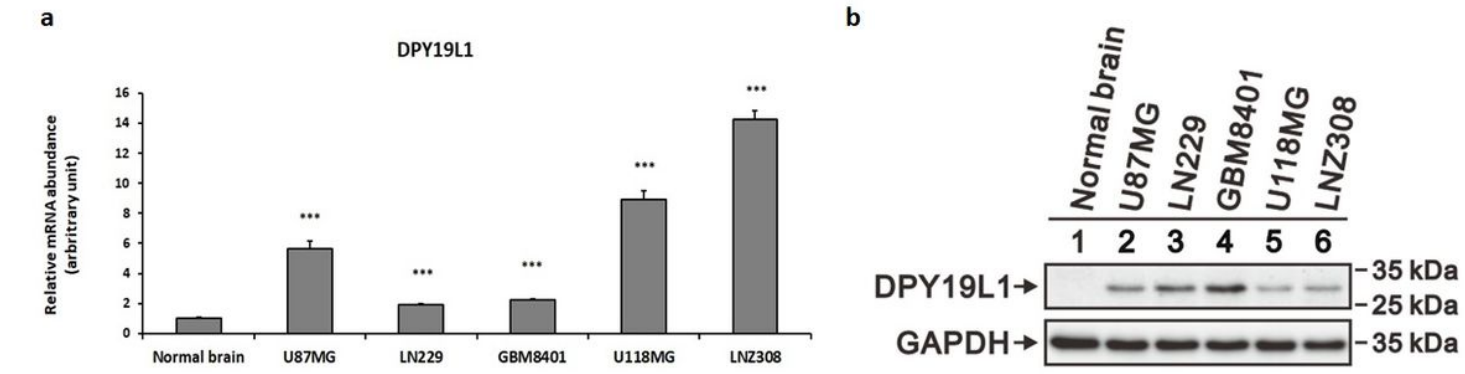

Figure 2

DPY19L1 mRNA and protein levels were validated by quantitative real-time PCR and western blot. (a) Normal brain tissue and glioma cell lines including U87MG, LN229, GBM8401, U118MG, and LNZ308 were analyzed by quantitative real-time PCR. *** $p < 0.001$. (b) DPY19L1 protein expression in normal brain and glioma cells was quantitated by western blot.

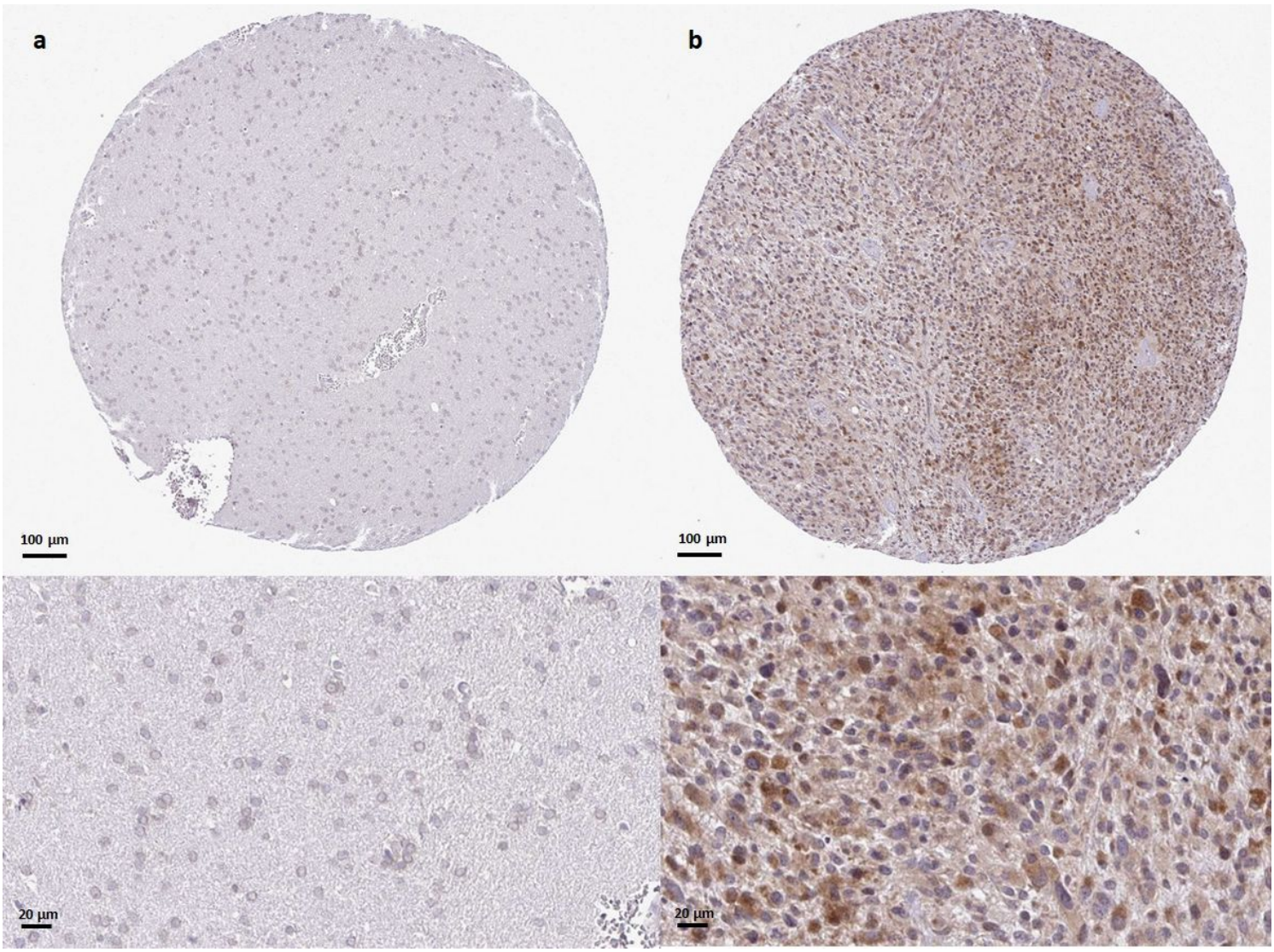

Figure 3

Enhancement of DPY19L1 expression in tissues of high-grade glioma. Immunohistochemical (IHC) staining of public tissue microarray from the Human Protein Atlas (https://www.proteinatlas.org/ENSG00000173852-DPY19L1/pathology/glioma) revealed moder-ate intensity in high-grade gliomas (a) and weak intensity in low-grade gliomas (b) respectively. DPY19L1 expression was mostly detected in cytoplasm and membrane through magnified mi-crographs below.

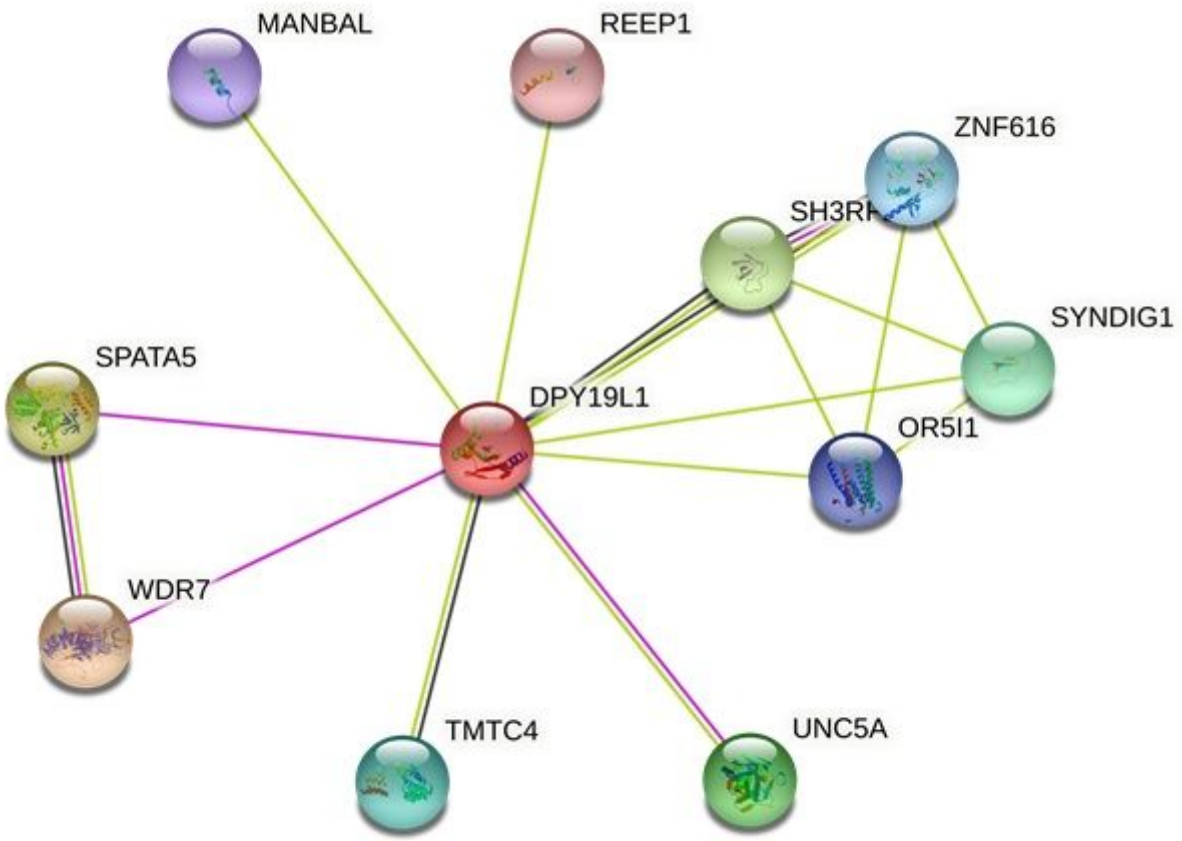

Figure 4

The DPY19L1 protein–protein interaction (PPI) network. The PPI network according to the STRING database showed that DPY19L1 manifests as a hub protein.