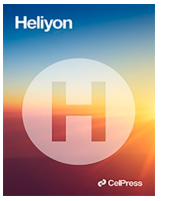
Heliyon 10 (2024) e27004

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

Potential role of POFUT1 as a prognostic predictor in low-grade gliomas: Immune microenvironment insights from a pan-cancer analysis

Fan Yu, Shuang Lou, Haihong He, Yiwen Zhou *

Centre of Clinical Laboratory Medicine, Shenzhen Hospital, Southern Medical University, China

ARTICLE INFO

Keywords:
POFUT1
Low-grade gliomas
Tumor immune microenvironment
Pan-cancer
Tumor-associated macrophages

ABSTRACT

The POFUT1 gene, known to be up-regulated in various tumor tissues and associated with tumor biology, has yet to be explored for its potential role in immune response regulation and tumor immune microenvironment. The normalized pan-cancer dataset (TCGA Pan-Cancer) was downloaded from the UCSC database, followed by analysis of POFUT1 expression in various tumors and functional enrichment analysis. The correlation between POFUT1 expression levels and patient prognosis was assessed. GSEA of POFUT1 based on low-grade glioma (LGG) samples and immune infiltration analyses of LGG and glioblastoma (GBM) were conducted. The correlation between POFUT1 expression levels and infiltration levels of 22 immune cells in LGG and GBM was examined, as well as the correlation between immune cell infiltration levels and LGG patient prognosis. Additionally, the relationship between POFUT1 expression levels and characteristic gene expression of identified immune cells was evaluated. Lastly, external dataset validation was performed using the integrated CGGA dataset. Significant differences were observed in POFUT1 expression levels across 20 tumor types. High POFUT1 expression correlated with poor prognosis in GBMLGG, and LGG patients. Enrichment analysis and GSEA of POFUT1 in LGG demonstrated involvement in tumor-related and immune-related pathways. A positive correlation was identified between POFUT1 expression levels and infiltration levels of resting memory $CD4^+$ T cells, as well as M2 macrophages or M2-like TAMs in the LGG immune microenvironment, potentially contributing to poor prognosis. External dataset validation revealed a positive correlation between M2 macrophages or M2-like TAMs and POFUT1 expression levels in LGG, and a negative correlation with LGG patient prognosis. POFUT1's negative impact on LGG prognosis may result from its influence on M2 macrophage and M2-like TAM infiltration levels within the immune microenvironment. This suggests its potential as a prognostic predictor and therapeutic target for LGG.

1. Introduction

The POFUT1 gene, located on chromosome 20, encodes protein O-fucosyltransferase 1, a 393-amino-acid protein that functions as a fucosyltransferase modifying the fucosylation of epidermal growth factor-like domains [1]. Dysregulation of POFUT1 has been implicated in the development of various pathological conditions, including malignant transformation [2]. Wan's study on breast

* Corresponding author: Yiwen Zhou. No.1333 Xinhu Road, Shenzhen, Guangdong, 518000, China.
E-mail address: yiwenzhou21@aliyun.com (Y. Zhou).

https://doi.org/10.1016/j.heliyon.2024.e27004
Received 4 August 2023; Received in revised form 31 January 2024; Accepted 22 February 2024
Available online 27 February 2024

cancer has demonstrated a correlation between high POFUT1 expression, lymph node metastasis, and advanced stage [3]. In the context of colorectal cancer, Du's study revealed that the suppression of POFUT1 leads to enhanced tumor cell apoptosis and suppressed cell proliferation [4]. Due to its distinct expression patterns across different cancer types, Multiple studies have proposed POFUT1 as a potential biomarker or anti-cancer target in various types of tumors, such as colorectal cancer and bladder cancer [5,6]. Currently, POFUT1's role in cells and tumors has been widely studied, with mechanisms promoting cell proliferation, migration, and invasion involving the activation and participation of NOTCH, MAPK, and PI3K/Akt signaling pathways [7]. Furthermore, findings from Cui's research have demonstrated that POFUT1 plays a significant role in promoting epithelial-mesenchymal transition (EMT), a critical process involved in cancer metastasis [8]. It is worth noting that recent studies have suggested that POFUT1 impacts immune cell development through the NOTCH signaling pathway, and its deletion may block the development of specific lymphocytes, including T cells, thereby inhibiting immune system response [9]. However, the effect of POFUT1 on the immune environment and immune response during tumor development remains unexplored. In this study, we first conducted a pan-cancer analysis of POFUT1 and provided a preliminary overview of its expression level changes across various tumor types and their correlation with patient prognosis. The association between POFUT1 and glioma patient prognosis prompted further investigation into POFUT1's role in glioma, particularly its potential impact on the immune microenvironment.

Glioma, one of the most prevalent malignant tumors of the central nervous system, accounts for approximately 30% of brain malignancies [10]. The World Health Organization classifies grades I and II gliomas as low-grade gliomas (LGG), primarily consisting of astrocytic tumors and oligodendrogliomas [11]. Despite advances in LGG diagnosis and treatment, surgical and radiation outcomes remain suboptimal due to the tumor's invasive growth characteristics and anatomical location. Additionally, LGG heterogeneity contributes to significant variations in patient prognosis. Notably, LGG patients are at risk of progressing to higher-grade glioma, with around 20% of glioblastomas (GBMs) emerging from preexisting LGGs [12]. Given these factors, identifying molecular markers as potential therapeutic targets or prognostic indicators may offer new opportunities for LGG diagnosis and treatment. Recent studies have observed increased POFUT1 expression in glioblastoma tissue, correlating with various tumor biological behaviors [13]. However, the specific role and mechanism of POFUT1 in LGG development, particularly its regulatory effects on the immune microenvironment, remain unexplored.

In this study, following our preliminary pan-cancer analysis of POFUT1 that indicated an association with glioma (including GBMLGG and LGG datasets), we further examined the correlation between POFUT1 and the glioma immune microenvironment to investigate potential mechanisms influencing prognosis. External dataset validation revealed a correlation between POFUT1 and the infiltration levels of M2 macrophages and M2-like tumor-associated macrophages (TAMs) in the LGG immune microenvironment. This correlation with M2 macrophage and M2-like TAM infiltration in the LGG immune microenvironment might represent one mechanism through which POFUT1 affects LGG patient prognosis. These findings may serve as a foundation for exploring the regulatory mechanisms of the LGG immune microenvironment and potential molecular targets for immunotherapy.

2. Method and material

2.1. Downloading and preprocessing of POFUT1 expression data

The normalized pan-cancer dataset (TCGA Pan-Cancer) was retrieved from the UCSC database (https://xenabrowser.net/). Tumor types with fewer than three samples were excluded, resulting in POFUT1 expression data for 26 tumor types. A log2(x+0.001) transformation was then applied to the sample expression values. For validation, the mRNAseq_693 dataset, containing 693 glioma samples with clinical data, and a non-glioma sample dataset were obtained from the CGGA database (http://www.cgga.org.cn/). The preprocessing and transformation methods applied to both datasets were consistent.

2.2. Analysis of POFUT1 expression in multiple tumors and enrichment analysis

R software (version 3.6.4) was employed to compute differences in POFUT1 expression levels across various tumor tissues, using the unpaired Wilcoxon rank-sum test for significance analysis to identify tumor types with significant disparities in POFUT1 expression. Subsequently, a protein-protein interaction network (PPI network) centered on POFUT1 was constructed using Cytoscape, based on the STRING database, and a gene set comprising selected POFUT1-related genes was established. Functional enrichment analysis of this gene set was then conducted using the clusterProfiler package, based on GO annotations from the org.Hs.eg.db package and the latest KEGG Pathway gene annotations obtained via the KEGG REST API. Results with $P < 0.05$ and FDR<0.1 were considered statistically significant.

2.3. Correlation analysis between POFUT1 expression level and prognosis of tumors

For the selected tumors, corresponding clinical data from TCGA were collected, and the coxph function in the survival package was utilized to analyze the correlation between gene expression and Hazard Ratio for various tumor patients. The log-rank test was subsequently conducted to identify tumor types in which POFUT1 expression levels were significantly correlated with patient prognosis (overall survival rate).

2.4. GSEA of POFUT1 based on LGG samples

To further explore the potential functional role of POFUT1 in LGG, we extracted LGG samples from the obtained pan-cancer dataset and divided them into high expression (≥50%) and low expression (<50%) groups based on POFUT1 expression levels, with the median value as the threshold. GSEA of POFUT1 in LGG samples was then performed using GSEA software (version 3.0), based on Hallmark gene sets, KEGG gene sets, Reactome gene sets, and Wikipathways gene sets from Human Collection 2 in the Molecular Signatures Database (MSigDB, http://www.gsea-msigdb.org/gsea/). Pathways with FDR<0.05 and |NES|>1 were considered statistically significant.

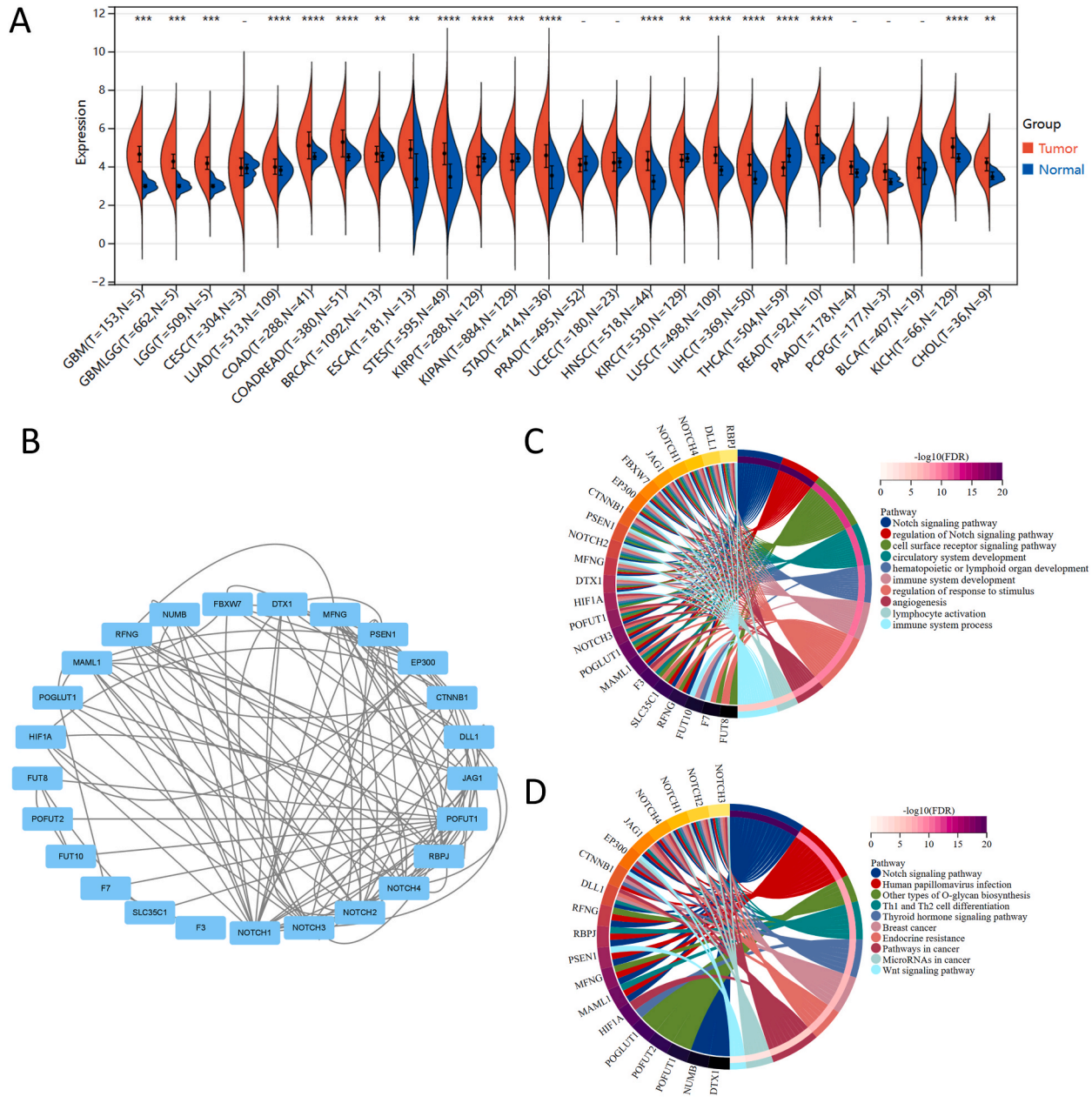


Fig. 1. **A.** Comparison of expression levels of POFUT1 in 26 types of tumor tissues and corresponding normal tissues(-: $p > 0.05$, *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$, ****: $p < 0.0001$). **B.** The 25-node protein-protein interaction network with POFUT1 as the core. **C.** Highly enriched GO terms of POFUT1 and its related genes. **D.** Highly enriched KEGG pathways of POFUT1 and its related genes.

2.5. Immune infiltration analyses of LGG and GBM

LGG and GBM samples were extracted from the obtained pan-cancer dataset, and the ESTIMATE package was employed to analyze stromal scores, immune scores, and ESTIMATE scores for both LGG and GBM. Subsequently, the corr.test function in the psych package was used to calculate the correlation between POFUT1 expression levels and immune infiltration scores in LGG and GBM.

2.6. Correlation analysis of POFUT1 expression level and infiltration level of immune cells in LGG and GBM

Using the extracted expression data of LGG and GBM samples from the pan-cancer dataset, infiltration levels of 22 immune cell types in both tumor types were calculated employing the CIBERSORT algorithm within the IOBR package. The correlation between infiltration levels of various cell types and POFUT1 expression levels was further analyzed using the corr.test function of the psych

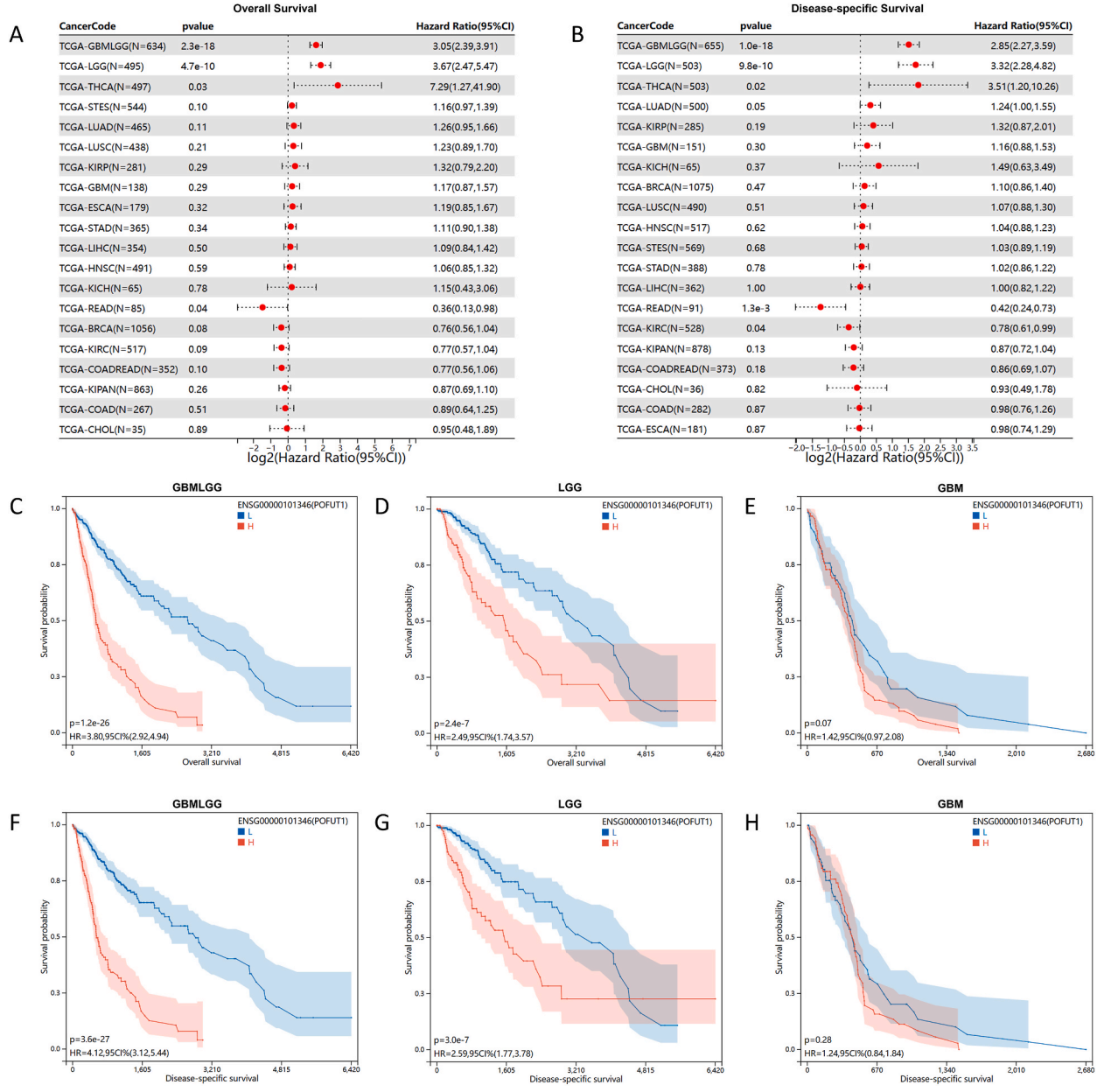


Fig. 2. A, B. Results of correlation analysis of POFUT1 expression level and overall survival and disease-specific survival of tumors. **C-E.** Kaplan-Meier curves of POFUT1 expression level and overall survival of GBMLGG, LGG and GBM. **F–H.** Kaplan-Meier curves of POFUT1 expression level and disease-specific survival of GBMLGG, LGG and GBM.

package. Cell types correlated with POFUT1 expression levels were subsequently identified.

2.7. Correlation analysis of infiltration level of immune cells and prognosis of LGG patients

Utilizing the Survival module of TIMER2.0 (http://timer.cistrome.org/), the correlation between infiltration levels of the identified immune cell types and the prognosis of LGG patients was analyzed in CIBERSORT-ABS mode. Cell types correlated with both POFUT1 expression levels and LGG patient prognosis were subsequently determined.

2.8. Correlation analysis of POFUT1 expression level and expression level of characteristic genes of identified immune cells

Characteristic genes of the identified immune cells were located by consulting relevant literature, and a correlation analysis between POFUT1 expression levels and various characteristic genes was conducted using the TIMER database (https://cistrome.shinyapps.io/timer/). This analysis aimed to further investigate the relationship between POFUT1 and the infiltration levels of the identified immune cells, as well as the potential mechanisms regulating the tumor immune microenvironment.

2.9. External dataset validation

The CGGA mRNAseq_693 dataset, containing 693 glioma samples, and the dataset of 20 non-glioma samples, were employed for validation. First, CIBERSORTx was used to analyze immune cell infiltration levels in all samples and further investigate infiltration levels of previously identified immune cell types in each sample group. After extracting POFUT1 expression data for each sample group, a correlation analysis was conducted on the infiltration levels of each cell type and POFUT1 expression levels. Lastly, correlations between overall survival rate, POFUT1 expression level, and immune cell infiltration levels were analyzed, focusing on LGG samples with complete clinical and survival data.

3. Result

3.1. POFUT1 expression level in various tumor and functional enrichment analysis

Among the 26 tumor types ultimately included in the analysis, POFUT1 expression levels in 20 tumor tissues exhibited significant differences ($p < 0.01$). Expression levels were significantly increased in 16 tumor tissues, while they were significantly reduced in 4 tumor tissues (Fig. 1A). Based on the PPI network centered on POFUT1, constructed using the STRING database (Fig. 1B), a POFUT1-related gene set comprising 25 genes was established. Functional enrichment analysis, based on GO annotations and KEGG Pathway annotations of the gene set, was performed separately, and pathways with extremely high enrichment (FDR<0.01) were selected for

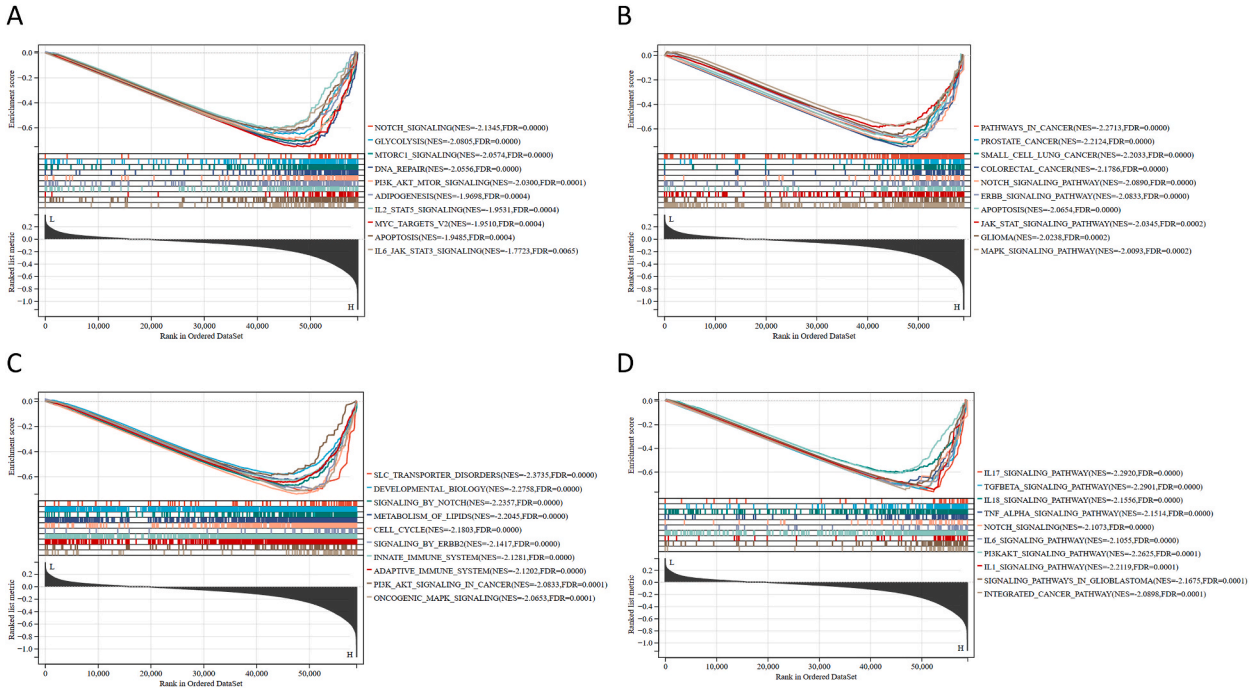


Fig. 3. Results of GSEA of POFUT1 in LGG. **A.** Hallmark gene sets. **B.** KEGG gene sets. **C.** Reactome gene sets. **D.** Wikipathways gene sets.

visualization (Fig. 1C and D).

The results of the functional enrichment analysis based on GO annotations revealed that the gene set was involved in various functional pathways closely associated with tumor development, tumor immune infiltration levels, and regulation of the body's immune response. These pathways included cell surface receptor transduction, response to stimulation regulation, NOTCH signaling, immune system development, immune process regulation, lymphocyte activation, angiogenesis, and hematopoietic or lymphoid organ and circulatory system development. KEGG Pathway functional enrichment analysis also demonstrated that POFUT1 and its associated genes were extensively involved in numerous cancer-related processes, including the NOTCH signaling pathway and the Wnt signaling pathway.

3.2. Correlation of POFUT1 expression level and prognosis of tumors

The correlation analysis of POFUT1 expression levels and prognosis for the 20 screened tumor types (Fig. 2A and B) revealed that high POFUT1 expression levels were significantly associated with poor prognosis in GBMLGG (comprising glioblastoma and low-grade glioma samples) and LGG (low-grade glioma samples) (Hazard Ratio >1, $p < 0.01$). However, no significant correlation was found between POFUT1 expression levels and prognosis in GBM (glioblastoma samples). Kaplan-Meier curves were employed to more intuitively display the correlation between POFUT1 expression levels and overall survival as well as disease-specific survival in GBMLGG Fig2C, F / Fig2D, G / Fig2E, H, respectively. The database website states that the GBMLGG dataset is a combination of LGG and GBM datasets. Therefore, in conjunction with the above results, POFUT1 is more likely to be related to the prognosis of LGG than GBM.

3.3. GSEA of POFUT1 based on LGG samples

LGG samples were divided into high (≥50%) and low (<50%) expression groups based on POFUT1 expression levels. The results of GSEA for POFUT1, based on Hallmark gene sets (Fig. 3A), KEGG gene sets (Fig. 3B), Reactome gene sets (Fig. 3C), and Wikipathways gene sets (Fig. 3D) from Human Collection 2 in MSigDB, indicated that in LGG, high expression of POFUT1 might be involved in cancer-promoting and immune-related pathways. These pathways include the NOTCH signaling pathway, PI3K-AKT-mTOR pathway, IL2-STAT5 signaling pathway, IL6-JAK-STAT3 signaling pathway, ERBB signaling pathway, and MAPK signaling pathway. Furthermore, it has been demonstrated that POFUT1 expression in LGG is related to cytokines such as IL1, IL6, IL17, IL-18, and TGF-β, as well as biological functions including DNA repair, apoptosis, lipid metabolism, and innate immune system processes.

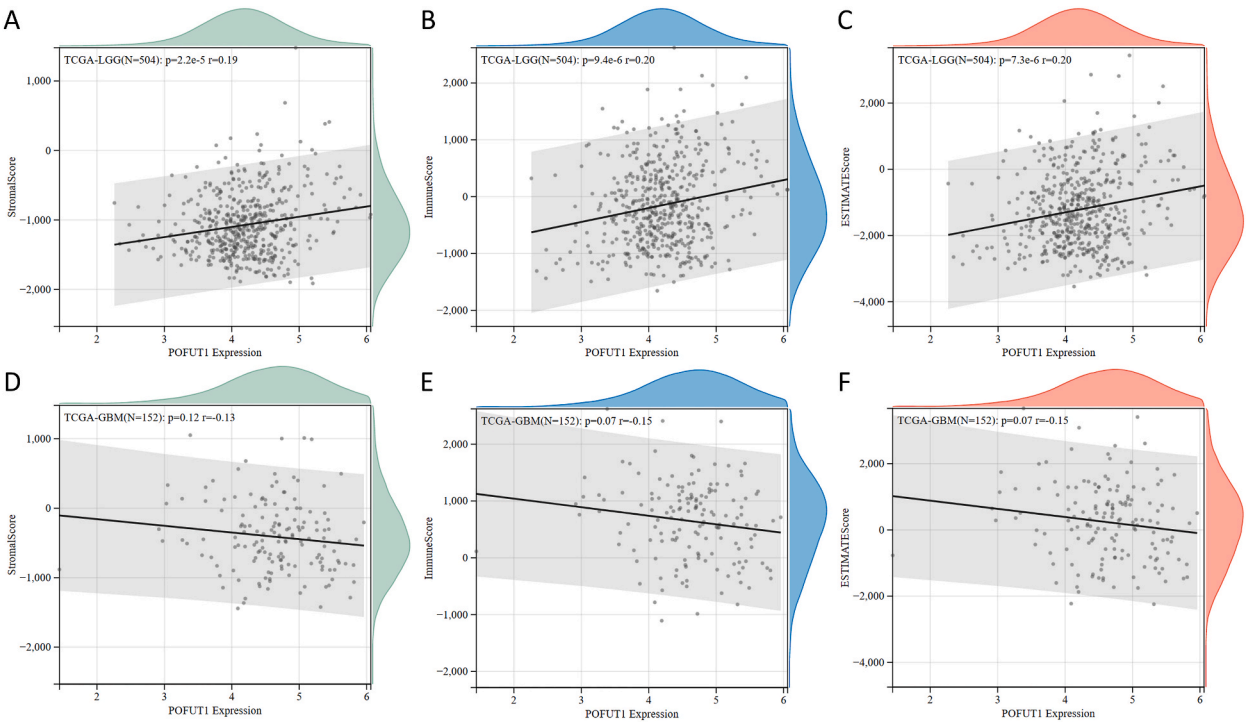


Fig. 4. Results of correlation analysis of POFUT1 expression level and stromal score, immune score and ESTIMATE score. **A-C.** LGG. **D-F.** GBM.

3.4. Correlation of immune infiltration and POFUT1 in LGG and GBM

Immune infiltration analyses of LGG were conducted using the ESTIMATE package, and the correlation between POFUT1 expression levels and immune infiltration scores, including stromal score, immune score, and ESTIMATE score, was calculated and visualized (Fig. 4A, B, C). For comparison, the same process was also performed in GBM samples (Fig. 4D, E, F). The results showed that in LGG, there was a weak positive correlation between POFUT1 expression levels and immune scores and ESTIMATE scores ($p < 0.01$ but $r < 0.30$), while no correlation was found between POFUT1 expression levels and each score in GBM.

3.5. Correlation analysis of POFUT1 expression level and infiltration level of immune cells in LGG and GBM

Infiltration levels of 22 types of immune cells in LGG and GBM, for comparison, were calculated. The correlation between infiltration levels of cells and POFUT1 expression levels was then analyzed and demonstrated (Fig. 5A). The results of this step showed that POFUT1 expression levels in LGG were positively correlated with resting memory $CD4^+$ T cells, regulatory T cells (Tregs), γδ T cells, resting mast cells, and macrophages (M0, M1, M2) ($p < 0.05$). However, γδ T cells were not included in further analysis because their proportion was 0 in 501 out of 504 LGG samples. The correlation between POFUT1 expression levels and the other six types of immune cells was further illustrated by scatter plots (Fig. 5B–G). It was found that, compared to other cell types, resting memory $CD4^+$ T cells

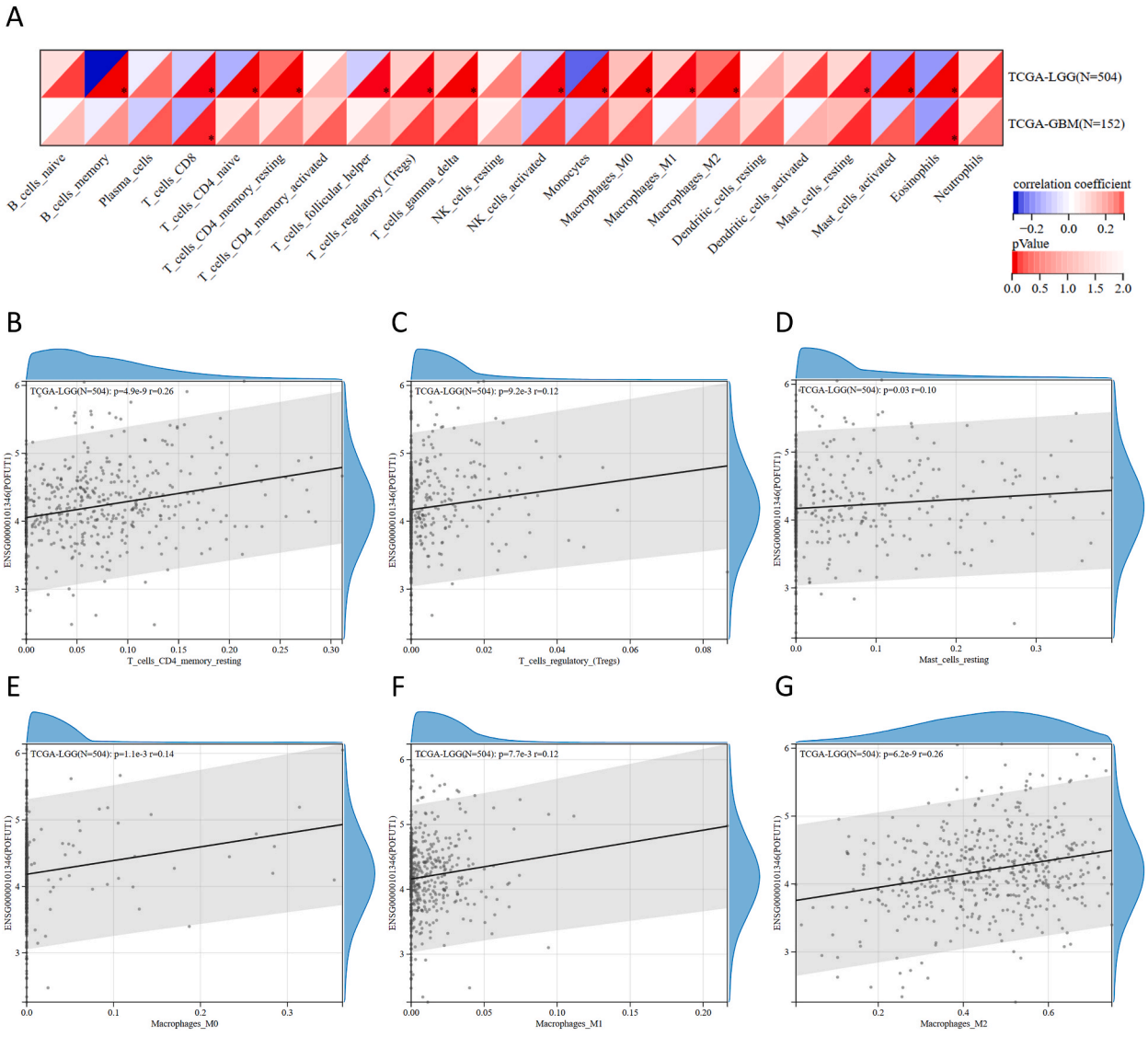


Fig. 5. A. Result of correlation analysis of POFUT1 expression level and infiltration level of 22 immune cells in LGG and GBM with CIBERSORT algorithm (*: $p < 0.01$). **B-G.** The scatter plots showing correlation of POFUT1 expression level and infiltration level of resting memory $CD4^+$ T cells, Tregs, resting mast cells, M0 macrophages, M1 macrophages and M2 macrophages.

and M2 macrophages had higher r values with POFUT1 expression levels, exhibiting a relatively significant correlation.

3.6. Correlation analysis of infiltration level of immune cells and prognosis of LGG patients

The correlation between the infiltration levels of the above six cell types (resting memory $CD4^+$ T cells, Tregs, resting mast cells, and macrophages (M0, M1, M2)) and the prognosis of LGG patients was analyzed (Fig. 6A–F). In combination with the results from the previous step, it was found that resting memory $CD4^+$ T cells and M2 macrophages were correlated with both POFUT1 expression levels in LGG and the prognosis of LGG patients.

3.7. Correlation analysis of POFUT1 expression level and expression level of characteristic genes of identified cell types

According to related literature, characteristic genes of M2 macrophages include CD163, MS4A4A, IL10, MMP2, MMP9, and VSIG4, while characteristic genes of resting memory $CD4^+$ T cells include PTPRC (CD45) and BTG1. The correlations between POFUT1 expression levels and each characteristic gene of M2 macrophages (Fig. 7A) and resting memory $CD4^+$ T cells (Fig. 7C) in LGG tissues were obtained from the TIMER database. It should be noted that M2-like TAMs present in the tumor immune microenvironment share some characteristic genes with M2 macrophages and usually cannot be distinguished by CIBERSORT. Therefore, we additionally analyzed the characteristic genes relatively unique to M2-like TAMs, including CD24, CD80, CD68, and CCL2 (Fig. 7B). As shown in the figures, POFUT1 expression levels have a significant positive correlation with multiple characteristic genes of M2 macrophages, M2-like TAMs, and resting memory $CD4^+$ T cells. This is consistent with the correlation analysis results of POFUT1 expression levels and cell infiltration levels.

3.8. External dataset validation

After reviewing the data, 159 LGG (WHO II) samples, 249 GBM samples in the CGGA mRNAseq_693 dataset, and 20 normal samples were selected for validation. The infiltration levels of immune cells in all samples were illustrated in a histogram (Fig. 8A). The infiltration levels of the six previously screened immune cell types in each group of samples were demonstrated with boxplots (Fig. 8B). POFUT1 expression levels in each group were also shown in a boxplot (Fig. 8C). The correlation between the infiltration level of each cell type and POFUT1 expression level was then analyzed (Fig. 8D). The results indicated that in the external validation dataset, POFUT1 expression levels exhibited a gradient increase in normal samples, LGG samples, and GBM samples. A significant positive correlation was found between POFUT1 expression levels and M2 macrophage infiltration levels, consistent with previous analysis results. However, an inconsistency was observed in the external validation dataset: POFUT1 expression levels were not correlated with

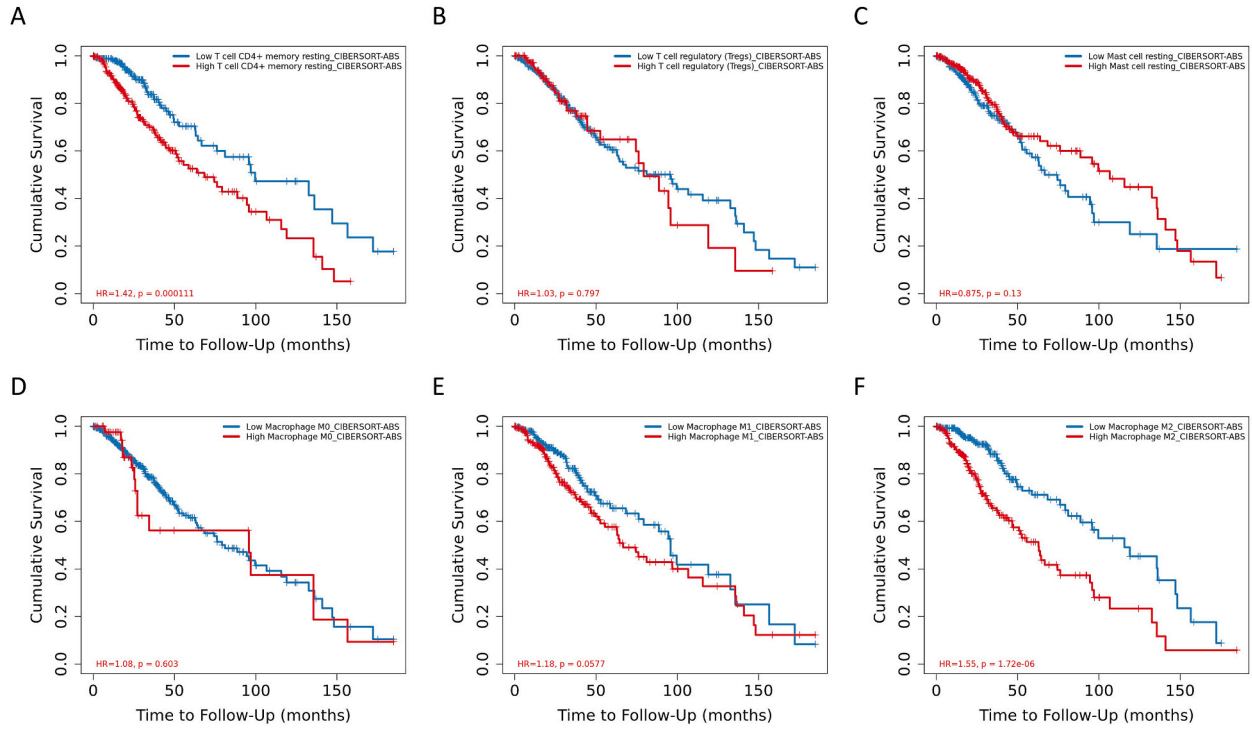


Fig. 6. Kaplan-Meier curves of infiltration level of immune cells and the overall survival of LGG patients. **A.** Resting memory $CD4^+$ T cells. **B.** Tregs. **C.** Resting mast cells. **D.** M0 macrophages. **E.** M1 macrophages. **F.** M2 macrophages.

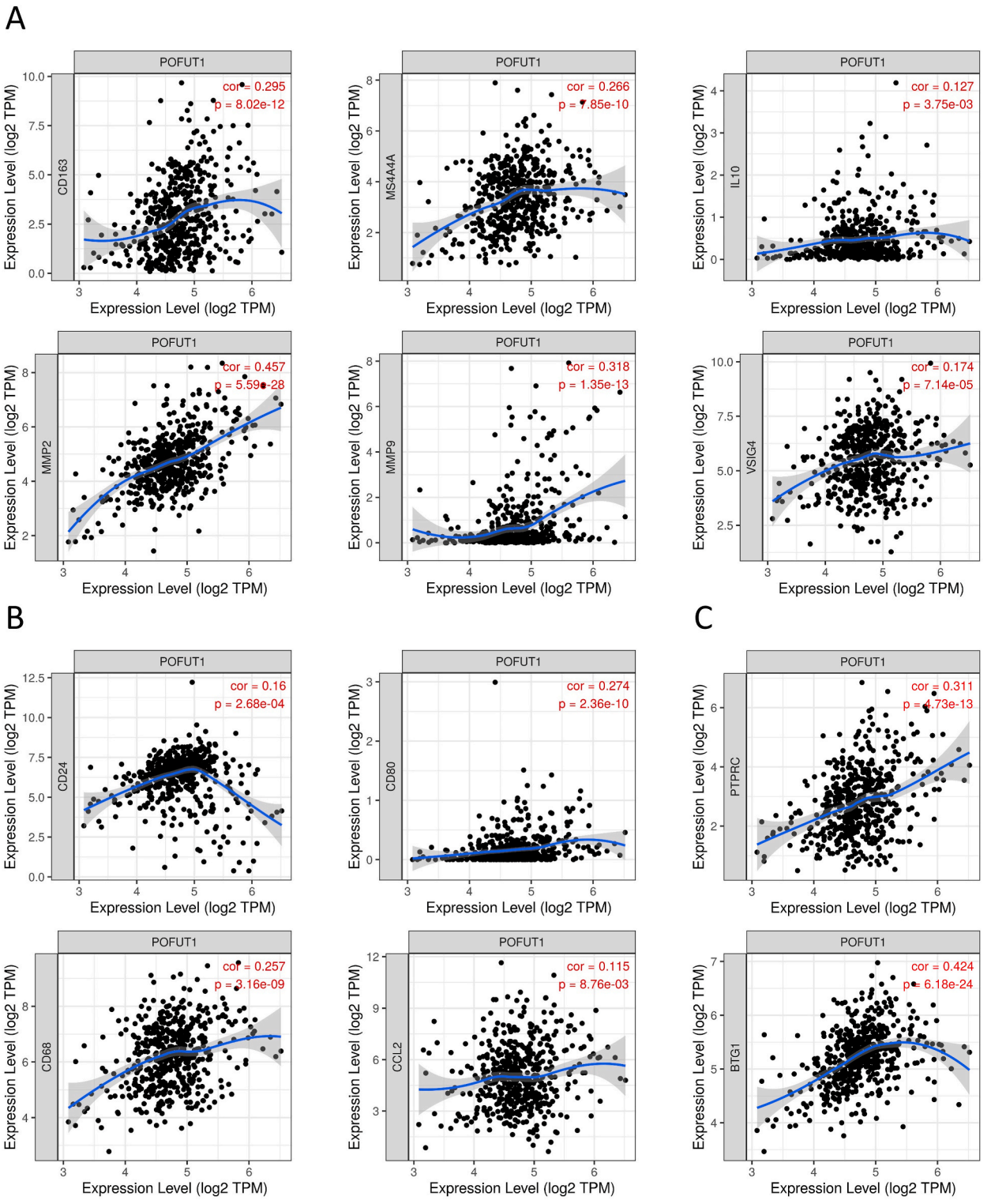


Fig. 7. Correlation between the POFUT1 expression level and the characteristic genes of immune cells. **A.** M2 Macrophages (CD163, MS4A4A, IL10, MMP2, MMP9, VSIGG4). **B.** M2-like TAMs (CD24, CD80, CD68, CCL2). **C.** Resting memory $CD4^{+}$ T cells (PTPRC, BTG1).

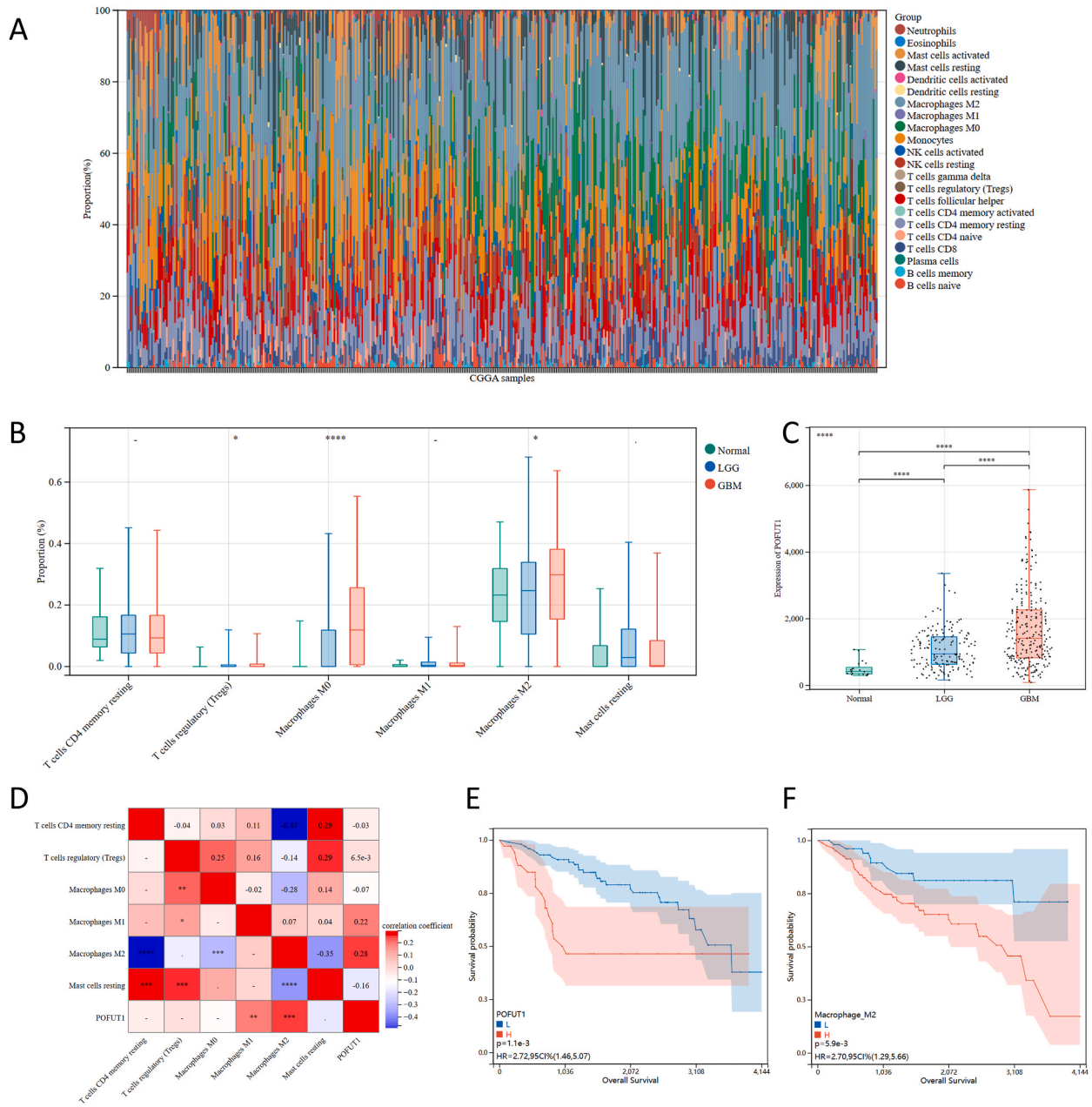


Fig. 8. **A.** Infiltration level of immune cells in samples of external validation dataset. **B.** Infiltration level of the 6 previously screened immune cell types in each group of the validation dataset (-: $p > 0.05$, *: $p < 0.05$, ****: $p < 0.0001$). **C.** POFUT1 expression level in each group of the validation dataset (****: $p < 0.0001$). **D.** Correlation of infiltration level of each cell type and POFUT1 expression level (-: $p > 0.05$, *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$, ****: $p < 0.0001$). **E.** Correlations of overall survival and POFUT1 expression level of the validation dataset. **F.** Correlations of overall survival and infiltration level of M2 macrophages of the validation dataset.

resting memory $CD4^+$ T cell infiltration levels but were correlated with M1 macrophage infiltration levels.

Correlations between overall survival and POFUT1 expression levels (Fig. 8E) as well as M2 macrophage infiltration levels (Fig. 8F) were also analyzed based on LGG samples with complete clinical and survival data from CGGA. In line with previous results, both high POFUT1 expression levels and high M2 macrophage infiltration levels were associated with poor prognosis in LGG patients.

4. Discussion

As tumor research advances, the regulatory effects of differentially expressed genes in tumor tissues on tumorigenesis, development, and their associated mechanisms have gained increasing attention. Numerous molecular markers identified from differentially

expressed genes in tumors have been utilized for early diagnosis, prognosis evaluation, and targeted therapy, offering substantial clinical value [14]. POFUT1 is one of the differentially expressed genes identified in our previous transcriptomic analysis of gastrointestinal tumors. This finding is in line with the results reported in another independent study, further supporting the potential importance of POFUT1 in tumorigenesis and tumor development [15]. Currently, researchers have acknowledged the differences in expression and prognostic significance of POFUT1 in tumor progression. Recent research by Dong has confirmed that POFUT1 promotes the progression of gastric cancer through the Notch/Wnt dual signaling pathways, which are dependent on the parafibromin-NICD1-β-catenin complex [16]. Another study proposed that POFUT1 overexpression promotes a malignant phenotype and mediates perineural invasion in head and neck squamous cell carcinoma (HNSCC), indicating its potential as both a prognostic marker and therapeutic target for HNSCC patients [17]. Actually, aside from the mentioned studies above, POFUT1 has been identified as a biomarker or potential therapeutic target for a variety of tumors, including lung cancer, breast cancer, and glioblastoma [3,13,18]. Our study results revealed that POFUT1 was significantly differentially expressed in up to 20 types of tumors and had a considerable impact on the prognosis of GBMLGG and LGG patients. A previous study confirmed that the silencing mutation of POFUT1 reduces the activity of the NOTCH signaling pathway and inhibits tumor cell development [15]. This finding suggests that the promotion of most tumors by high-level expression of POFUT1 may be attributed to the involvement of the NOTCH signaling pathway. The NOTCH signaling pathway is an evolutionarily conserved pathway involved in regulating a wide range of biological processes, including cell proliferation, apoptosis, migration, self-renewal, and differentiation [19]. In fact, researchers have taken note of the role of the NOTCH signaling pathway in glioma. A recent study reported high levels of expression of NOTCH receptors and ligands in GBM, suggesting abnormal activation of the NOTCH signaling pathway [20]. Moreover, it has been proposed that NOTCH1-mediated upregulation of NOTCH signaling pathway activation contributes to tumorigenesis and chemoresistance in GBM [21]. Glycosylation of NOTCH receptors, particularly O-fucosylation, is an essential step in the function and regulation of the NOTCH signaling pathway, and POFUT1 plays a critical role in this process [22]. A cell line-based study demonstrated that knockdown of POFUT1 led to a decrease in the amount of NOTCH1 on the cell surface [23]. Moreover, in agreement with our functional enrichment analysis results, studies have reported that POFUT1 promotes cell proliferation and invasion, and delays cell apoptosis by activating or participating in PI3K/mTOR/Akt, MAPK/ERK, and NF-kB signaling pathways [24]. Another study suggested that the promoting effect of POFUT1 on cell proliferation is associated with the acceleration of cell cycle progression by facilitating cells to enter the S phase [25].

In addition to the involvement of the NOTCH signaling pathway in tumor development, as demonstrated by previous studies, our findings highlight that POFUT1 is also highly enriched in immune system processes and immune response regulation. This observation warrants further attention, as recent research has reported that the NOTCH signaling pathway itself plays a role in immune regulation and has been established as an independent predictor for evaluating immunotherapy [26]. While there has been limited research directly linking POFUT1 to immune-related processes, our study provides new insights into this relationship. Our immune infiltration analysis revealed that high-level expression of POFUT1 in LGG tissue was positively correlated with increased infiltration levels of six types of immune cells. Notably, the high infiltration level of resting memory $CD4^+$ T cells and M2 macrophages, which exhibited higher correlation coefficients with POFUT1 expression, was also significantly associated with poor prognosis in LGG patients. A lower level of immune infiltration generally suggests that the number of immune cells migrating to the tumor microenvironment is insufficient, leading to inhibition of immune response regulation. This may contribute to the acceleration of the tumor's immune escape process, thereby promoting tumor development [27]. Previous research has demonstrated that the level of immune infiltration in the tumor microenvironment can reflect the body's anti-tumor immune status and the efficacy of immunotherapy. Reduced T lymphocyte infiltration is often closely associated with anti-tumor immunosuppression and tumor immune escape in various cancer types, including lung adenocarcinoma and colorectal cancer [14]. However, some studies have reported contrasting findings, such as a study showing that elevated $CD4^+$ T cell expression levels in breast cancer tissue are typically indicative of a relatively poor prognosis [28]. The preliminary results of our analysis indicate that high expression of POFUT1 in LGG tissue is significantly correlated with poor patient prognosis and a higher level of immune infiltration in tumor tissue. This suggests that, unlike in other cancer types, POFUT1 does not suppress tumor immunity by reducing immune cell infiltration levels in LGG.

In our study, we observed a positive correlation between the infiltration level of M2 macrophages in LGG and POFUT1 expression level, which negatively affected LGG prognosis using both the TCGA dataset and the CGGA validation dataset. This finding is consistent with Stanley and Tanwar's research, which demonstrated the critical role of POFUT1 in lymphopoiesis and myelopoiesis, as well as the defects in both innate and adaptive immune responses among patients with low POFUT1 expression [29]. They further hypothesized that fucosylation of NOTCH receptors, mediated by POFUT1, is essential for immune cell development, and loss of POFUT1 impairs this process, resulting in restricted immune responses. Chennupati's study demonstrated that the loss of NOTCH1 disrupts the generation of T cells involved in the innate immune response [30]. In contrast, Mao's study [31] and Liu's study [32] reported a direct association between increased NOTCH1 levels and CD68+/CD163+ macrophages, as well as IL10-producing macrophages. Both of these macrophage types are considered M2 macrophages or M2-like TAMs. A recent study showed that macrophages activated through the NOTCH signaling pathway increase the secretion of CCL2, leading to an immunosuppressive response [33]. In the context of tumor tissue infiltration, Shi's research on glioblastoma found that macrophages and related cytokines may promote the generation of tumor stem cells and maintain the malignant biological behavior of tumors by binding to a series of receptors of tumor stem cells [34]. This finding aligns with our analysis, which revealed a significant association between a high infiltration level of macrophages and a higher survival hazard ratio. TAMs serve as major components and crucial regulatory factors within the tumor immune microenvironment. Our analyses of both the TCGA dataset and the CGGA validation dataset demonstrated a significant positive correlation between POFUT1 expression level and M2 macrophages or M2-like TAMs, as well as their characteristic genes. M1-like macrophages are known to exhibit anti-tumor properties through their ability to present antigens, engage in Th1-type immune responses, eliminate foreign antigens, and destroy tumor cells [35]. Conversely, M2 macrophages display a relative deficiency in cell-killing activity within the

tumor microenvironment. Characterized by high CD163 expression and the production of anti-inflammatory cytokines such as IL-10 and CCL2, M2 macrophages inhibit inflammatory responses and participate in Th2-type immune responses, thereby promoting cancer progression. Chemotherapeutic agents, including trabectedin, have been employed to diminish CCL2 production, resulting in the clearance of M2 macrophages infiltrating tumor tissue and the activation of M1 macrophages; this process ultimately enhances the anti-tumor immune response of T cells [36]. Furthermore, M2 macrophages can secrete cytokines like epidermal growth factor, which stimulates cancer cell metastasis and recruits additional macrophages to infiltrate primary tumor tissue [37]. M2 macrophages also contribute to tumor invasion and metastasis through the secretion of matrix metalloproteinases (MMPs) such as MMP2 and MMP9, which degrade the extracellular matrix [38]. Moreover, M2 macrophages can indirectly suppress the function of $CD8^+$ T cells by promoting the differentiation of regulatory T (Treg) cells and modulating amino acid metabolism through the indoleamine 2,3-dioxygenase (IDO) pathway, which disrupts T cell metabolic processes [39]. These studies provide a multidimensional understanding of the immunosuppressive mechanisms exerted by M2 macrophages within the tumor immune microenvironment. Furthermore, research has indicated that TAMs participate in angiogenesis during tumor progression. The NOTCH signaling pathway, known to regulate the expression of angiogenesis-related genes, may play a role in the regulation of tumor angiogenesis within TAMs. Based on the functional enrichment analysis of POFUT1 and its related genes, it is suggested that POFUT1's regulatory effect on M2 macrophage infiltration in the low-grade glioma (LGG) immune microenvironment could be a contributing factor to poorer prognosis.

Our study is based on the integrated bioinformatics analysis of a large sample cohort from the TCGA database. The obtained results were also validated using the large sample cohort from CGGA. However, the limitation of this study is that further experimental exploration is needed to investigate the regulatory effects, mechanisms, and potential clinical application value indicated by some of the results.

5. Conclusion

Our study represents the first investigation into the relationship between POFUT1 expression, the tumor immune microenvironment, and patient prognosis using bioinformatics analysis. We found that high POFUT1 expression was significantly associated with increased immune infiltration in LGGs and poorer patient outcomes. Moreover, the strong positive correlation between POFUT1 expression and M2 macrophage or M2-like TAM infiltration levels within the LGG immune microenvironment may be a crucial factor contributing to the poor prognosis of patients. The influence of POFUT1 on the LGG immune microenvironment and patient prognosis suggests its potential as both a prognostic marker and a therapeutic target for this tumor type. However, the specific regulatory mechanisms warrant further investigation through experimental validation.

Data availability

The normalized pan-cancer dataset TCGA Pan-Cancer (PANCAN) can be downloaded from the UCSC database (https://xenabrowser.net/). The external dataset for validation can be downloaded from CGGA database (http://www.cgga.org.cn/).

Ethics approval and consent to participate

No ethics approval is needed for this study.

Consent for publication

NOT APPLICABLE.

Funding

1. The study received grants from Shenzhen Natural Science Foundation (202205303002621).
2. The study is supported by Wu Jieping Medical Foundation-Clinical Research Special Fund (320.6750.2021-06-30).
3. The study is supported by Post-3A Research Funds (22H3AZF05) of Shenzhen Hospital, Southern Medical University.

CRediT authorship contribution statement

Fan Yu: Writing – review & editing, Writing – original draft, Visualization, Formal analysis, Data curation, Conceptualization. **Shuang Lou:** Writing – original draft, Formal analysis, Data curation. **Haihong He:** Visualization, Formal analysis, Data curation. **Yiwen Zhou:** Supervision, Project administration, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

We thank Dr. Lijia Xiao for his suggestion and support on study design and manuscript writing. This study is a part of the non-funded healthcare research project (2023JD227) under the supervision of the Bao'an District Innovation and Entrepreneurship Bureau. We would also like to express our gratitude for their attention and support towards this project.

Abbreviations

CGGA	Chinese Glioma Genome Atlas
FDR	false discovery rate
GBM	glioblastoma
GSEA	Gene Set Enrichment Analysis
KEGG	Kyoto encyclopedia of genes and genomes
LGG	low-grade glioma
POFUT1	protein O-fucosyltransferase 1
TAMs	tumor-associated macrophages
TCGA	The Cancer Genome Atlas

References

[1] C. Loriol, F. Dupuy, R. Rampal, M.A. Dlugosz, R.S. Haltiwanger, A. Maftah, A. Germot, Molecular evolution of protein O-fucosyltransferase genes and splice variants, Glycobiology 16 (8) (2006) 736–747.
[2] M. Schneider, E. Al-Shareffi, R.S. Haltiwanger, Biological functions of fucose in mammals, Glycobiology 27 (7) (2017) 601–618.
[3] G. Wan, L. Tian, Y. Yu, F. Li, X. Wang, C. Li, S. Deng, X. Yu, X. Cai, Z. Zuo, et al., Overexpression of Pofut1 and activated Notch1 may be associated with poor prognosis in breast cancer, Biochem. Biophys. Res. Commun. 491 (1) (2017) 104–111.
[4] Y. Du, D. Li, N. Li, C. Su, C. Yang, C. Lin, M. Chen, R. Wu, X. Li, G. Hu, POFUT1 promotes colorectal cancer development through the activation of Notch1 signaling, Cell Death Dis. 9 (10) (2018) 995.
[5] M.A. Komor, M. de Wit, J. van den Berg, S.R. Martens de Kemp, P.M. Delis-van Diemen, A.S. Bolijn, M. Tijssen, T. Schelfhorst, S.R. Piersma, D. Chiasserini, et al., Molecular characterization of colorectal adenomas reveals POFUT1 as a candidate driver of tumor progression, Int. J. Cancer 146 (7) (2020) 1979–1992.
[6] S. Wahby, J. Jarczyk, A. Fierek, J. Heinkele, C.A. Weis, M. Eckstein, T. Martini, S. Porubsky, M. Hafner, P. Erben, POFUT1 mRNA expression as an independent prognostic parameter in muscle-invasive bladder cancer, Translational oncology 14 (1) (2021) 100900.
[7] Y.L. Tsai, H.H. Chang, Y.C. Chen, Y.C. Chang, Y. Chen, W.C. Tsai, Molecular mechanisms of KDELC2 on glioblastoma tumorigenesis and temozolomide resistance, Biomedicines 8 (9) (2020).
[8] X. Cui, H. Wang, Y. Li, T. Chen, S. Liu, Q. Yan, Epiregulin promotes trophoblast epithelial-mesenchymal transition through poFUT1 and O-fucosylation by poFUT1 on uPA, Cell Prolif. 53 (2) (2020) e12745.
[9] Q. Yan, D. Yao, L.L. Wei, Y. Huang, J. Myers, L. Zhang, W. Xin, J. Shim, Y. Man, B. Petryniak, et al., O-fucose modulates Notch-controlled blood lineage commitment, Am. J. Pathol. 176 (6) (2010) 2921–2934.
[10] M. Weller, W. Wick, K. Aldape, M. Brada, M. Berger, S.M. Pfister, R. Nishikawa, M. Rosenthal, P.Y. Wen, R. Stupp, et al., Glioma, Nat. Rev. Dis. Prim. 1 (2015) 15017.
[11] T.A. Bale, M.K. Rosenblum, The 2021 WHO Classification of Tumors of the Central Nervous System: an update on pediatric low-grade gliomas and glioneuronal tumors, Brain Pathol. 32 (4) (2022) e13060.
[12] S. Xu, L. Tang, X. Li, F. Fan, Z. Liu, Immunotherapy for glioma: current management and future application, Cancer Lett. 476 (2020) 1–12.
[13] Q. Li, J. Wang, X. Ma, M. Wang, L. Zhou, POFUT1 acts as a tumor promoter in glioblastoma by enhancing the activation of Notch signaling, J. Bioenerg. Biomembr. 53 (5) (2021) 621–632.
[14] S. Piawah, A.P. Venook, Targeted therapy for colorectal cancer metastases: a review of current methods of molecularly targeted therapy and the use of tumor biomarkers in the treatment of metastatic colorectal cancer, Cancer 125 (23) (2019) 4139–4147.
[15] M. Deschuyter, F. Pennarubia, E. Pinault, S. Legardinier, A. Maftah, Functional characterization of POFUT1 variants associated with colorectal cancer, Cancers 12 (6) (2020).
[16] S. Dong, Z. Wang, W. Xiong, POFUT1 promotes gastric cancer progression through Notch/Wnt dual signaling pathways dependent on the parafibromin-NICD1-β-catenin complex, J. Chin. Med. Assoc. : J. Chin. Med. Assoc. 86 (9) (2023) 806–817.
[17] N. Barlak, G. Kusdemir, R. Gumus, B. Gundogdu, M.H. Sahin, A. Tatar, M. Ittmann, O.F. Karatas, Overexpression of POFUT1 promotes malignant phenotype and mediates perineural invasion in head and neck squamous cell carcinoma, Cell Biol. Int. 47 (12) (2023) 1950–1963.
[18] Q. Leng, J.H. Tsou, M. Zhan, F. Jiang, Fucosylation genes as circulating biomarkers for lung cancer, J. Cancer Res. Clin. Oncol. 144 (11) (2018) 2109–2115.
[19] B. Zhou, W. Lin, Y. Long, Y. Yang, H. Zhang, K. Wu, Q. Chu, Notch signaling pathway: architecture, disease, and therapeutics, Signal Transduct. Targeted Ther. 7 (1) (2022) 95.
[20] R. Bazzoni, A. Bentivegna, Role of Notch signaling pathway in glioblastoma pathogenesis, Cancers 11 (3) (2019).
[21] Y. Wang, Q. Sun, R. Geng, H. Liu, F. Yuan, Y. Xu, Y. Qi, H. Jiang, Q. Chen, B. Liu, Notch intracellular domain regulates glioblastoma proliferation through the Notch1 signaling pathway, Oncol. Lett. 21 (4) (2021) 303.
[22] K. Matsumoto, K.B. Luther, R.S. Haltiwanger, Diseases related to Notch glycosylation, Mol. Aspect. Med. 79 (2021) 100938.
[23] M. Li, R. Cheng, J. Liang, H. Yan, H. Zhang, L. Yang, C. Li, Q. Jiao, Z. Lu, J. He, et al., Mutations in POFUT1, encoding protein O-fucosyltransferase 1, cause generalized Dowling-Degos disease, Am. J. Hum. Genet. 92 (6) (2013) 895–903.
[24] S. Liu, J. Wang, H.M. Qin, X.M. Yan, X.S. Yang, C. Liu, Q. Yan, LIF upregulates poFUT1 expression and promotes trophoblast cell migration and invasion at the fetal-maternal interface, Cell Death Dis. 5 (8) (2014) e1396.
[25] C. Liu, X. Liang, J. Wang, Q. Zheng, Y. Zhao, M.N. Khan, S. Liu, Q. Yan, Protein O-fucosyltransferase 1 promotes trophoblast cell proliferation through activation of MAPK and PI3K/Akt signaling pathways, Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie 88 (2017) 95–101.
[26] K. Zhang, X. Hong, Z. Song, Y. Xu, C. Li, G. Wang, Y. Zhang, X. Zhao, Z. Zhao, J. Zhao, et al., Identification of deleterious NOTCH mutation as novel predictor to efficacious immunotherapy in NSCLC, Clin. Cancer Res. : an official journal of the American Association for Cancer Research 26 (14) (2020) 3649–3661.
[27] X. Ren, L. Zhang, Y. Zhang, Z. Li, N. Siemers, Z. Zhang, Insights gained from single-cell analysis of immune cells in the tumor microenvironment, Annu. Rev. Immunol. 39 (2021) 583–609.
[28] J.E. Talmadge, Immune cell infiltration of primary and metastatic lesions: mechanisms and clinical impact, Semin. Cancer Biol. 21 (2) (2011) 131–138.

[29] P. Stanley, A. Tanwar, Regulation of myeloid and lymphoid cell development by O-glycans on Notch, Front. Mol. Biosci. 9 (2022) 979724.
[30] V. Chennupati, U. Koch, M. Coutaz, L. Scarpellino, F. Tacchini-Cottier, S.A. Luther, F. Radtke, D. Zehn, H.R. MacDonald, Notch signaling regulates the homeostasis of tissue-restricted innate-like T cells, J. Immunol. 197 (3) (2016) 771–782. Baltimore, Md : 1950.
[31] H. Mao, F. Pan, H. Guo, F. Bu, T. Xin, S. Chen, Y. Guo, Feedback mechanisms between M2 macrophages and Th17 cells in colorectal cancer patients, Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine 37 (9) (2016) 12223–12230.
[32] Q. Liu, C. Yang, S. Wang, D. Shi, C. Wei, J. Song, X. Lin, R. Dou, J. Bai, Z. Xiang, et al., Wnt5a-induced M2 polarization of tumor-associated macrophages via IL-10 promotes colorectal cancer progression, Cell Commun. Signal. : CCS 18 (1) (2020) 51.
[33] Y. Otani, J.Y. Yoo, C.T. Lewis, S. Chao, J. Swanner, T. Shimizu, J.M. Kang, S.A. Murphy, K. Rivera-Caraballo, B. Hong, et al., NOTCH-induced MDSC recruitment after oHSV virotherapy in CNS cancer models modulates antitumor immunotherapy, Clin. Cancer Res. : an official journal of the American Association for Cancer Research 28 (7) (2022) 1460–1473.
[34] Y. Shi, Y.F. Ping, W. Zhou, Z.C. He, C. Chen, B.S. Bian, L. Zhang, L. Chen, X. Lan, X.C. Zhang, et al., Tumour-associated macrophages secrete pleiotrophin to promote PTPRZ1 signalling in glioblastoma stem cells for tumour growth, Nat. Commun. 8 (2017) 15080.
[35] C. Ngambenjawong, H.H. Gustafson, S.H. Pun, Progress in tumor-associated macrophage (TAM)-targeted therapeutics, Adv. Drug Deliv. Rev. 114 (2017) 206–221.
[36] P. Banerjee, R. Zhang, C. Ivan, G. Galletti, K. Clise-Dwyer, F. Barbaglio, L. Scarfò, M. Aracil, C. Klein, W. Wierda, et al., Trabectedin reveals a strategy of immunomodulation in chronic lymphocytic leukemia, Cancer Immunol. Res. 7 (12) (2019) 2036–2051.
[37] H. Wang, M.M.H. Yung, H.Y.S. Ngan, K.K.L. Chan, D.W. Chan, The impact of the tumor microenvironment on macrophage polarization in cancer metastatic progression, Int. J. Mol. Sci. 22 (12) (2021).
[38] Y. Liang, N. Yang, G. Pan, B. Jin, S. Wang, W. Ji, Elevated IL-33 promotes expression of MMP2 and MMP9 via activating STAT3 in alveolar macrophages during LPS-induced acute lung injury, Cell. Mol. Biol. Lett. 23 (2018) 52.
[39] N.R. Anderson, N.G. Minutolo, S. Gill, M. Klichinsky, Macrophage-based approaches for cancer immunotherapy, Cancer Res. 81 (5) (2021) 1201–1208.