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Research Article

IFI30 Modulates Immune Microenvironment and Improves Prognosis in Glioblastoma

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ABSTRACT

Currently, immunotherapy has brought new hope as a potentially effective treatment for glioblastoma (GBM). After unsuccessful previous attempts and experiments, the current effective T cell immune strategies have shown promise in improving antigen presentation, antigen recognition and blocking T cell exhaustion in the GBM tumor microenvironment. The main function of γ-interferon-inducible lysosomal thiol reductase (*IFI30*) is to promote antigen processing and presentation and enhance the anti-tumor effect of cytotoxic lymphocyte (CTL). However, the exact function of *IFI30* in GBM development and progression is not yet known. In this study, we explored multiple public databases for differential expression of *IFI30* at the DNA methylation, mRNA transcription, and protein levels in GBM tissues. Further, we detected DNA methylation in clinical GBM recurrence samples to confirm the key methylation sites of *IFI30* in GBM progression. Subsequently, we confirmed the close relationship of *IFI30* with immune infiltration and immune checkpoint. *IFI30* showed good diagnostic and prognostic value in GBM. Therefore, IFI30 could be an ideal diagnostic and prognostic biomarker and therapeutic target for GBM.

1. Introduction

Glioblastoma (GBM) is the most aggressive malignant tumor of the primary central nervous system [1]. Patients with GBM have a poor prognosis, with a median survival of <2 years even after standard treatment [2]. Therefore, new treatment options are urgently needed. After preliminary attempts and trials, immunotherapy is hoped to succeed where other GBM therapies have failed. At present, the main immunotherapeutic strategy is to modulate the immune response against the tumor and its microenvironment. However, the specific inhibitory immune microenvironment and immune escape mechanisms of GBM are highly complex, including the up-regulation of the inhibitory protein programmed death ligand-1 (PD-L1) [3], increased recruitment of immunosuppressive regulatory T cells and cytotoxic lymphocyte (CTL) cell exhaustion [4], etc. Conversely, some studies have found that activated T cells could cross the blood-brain barrier as patrolling memory T cells and regulatory T cells [5], the discovery of glial lymphatic system [6] and dural macrophage subsets that act as antigen-presenting cells [7]. These are all new ideas for GBM immunotherapy. Therefore, it is currently considered that an effective T cell immune

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strategy should improve antigen presentation and recognition and block T cell exhaustion [8].

In recent years, γ-interferon-inducible lysosomal thiol reductase (*IFI30*, *GILT*) has attracted increasing attention due to its role in regulating the tumor immune microenvironment [9, 10]. Its main function is to promote antigen processing and presentation and enhance the anti-tumor effect of CTL [10-12]. For example, *IFI30* in thymic epithelial cells promotes central T cell tolerance to tissue-restricted melanoma-associated autoantigens [13]. Overexpression of *IFI30* inhibited the proliferation, invasion, migration and tumor formation of breast cancer cells in nude mice and increased the sensitivity of breast cancer cells to standard therapy [14]. However, the exact function of *IFI30* in GBM development and progression is not yet known.

Therefore, in this study, we used multiple public databases to explore the expression profile of *IFI30*, its prognostic significance, methylation profile, and relationship with immune microenvironment in GBM. In addition, DNA methylation assays were performed using primary and recurrent pathology samples from three clinical GBM patients to predict the role of *IFI30* gene in disease relapse. Finally, we investigated the potential functions and pathways of *IFI30* co-expressed genes. Our study highlights the significance of *IFI30* in the prognosis and treatment of GBM.

2. Materials and Methods

2.1. *IFI30* mRNA Expression Levels and DNA Methylation Information

The TCGA database was used to analyze the expression of *IFI30* in 33 human cancers, 166 GBM tissues and 1157 normal brain tissues. Subsequently, *IFI30* transcript levels in GBM tissues were validated using the GSE116520 dataset and the UALCAN database (Link 1). The Human Protein Atlas database (Link 2) was used to analyze the protein expression and localization of *IFI30* in normal cerebral cortex and GBM tissues.

The MethSurv database (Link 3) was used to analyze *IFI30* DNA methylation sites and assess the prognostic value of *IFI30* CpG methylation in GBM patients. Survival outcomes included overall survival. *IFI30* promoter methylation levels were compared between GBM and normal brain tissue using the UALCAN database.

2.2. DNA Methylation in Primary and Recurrent GBM Patients

i) Sixteen patients with recurrent GBM were admitted to the Department of Neurosurgery, Union Hospital of Fujian Medical University from 2013 to 2015. Of these, three patients were randomly selected for DNA methylation assay in primary and recurrent pathology samples. Their clinical history, imaging findings, laboratory results, surgical reports, follow-up, pathological findings, and treatment regimens were retrospectively analyzed. This study was approved by the Ethics Review Committee of Union Hospital Affiliated to Fujian Medical University (Ethics Number: 2020KJT066).

ii) Illumina 850k solution (Illumina, San Diego, CA) was used for DNA methylation detection. Genomic DNA in the pathological samples was extracted, subjected to sulfite conversion and genomic amplification reagents, and incubated overnight at a constant temperature of 37°C. Then, DNA was fragmented, precipitated, resuspended and hybridized. After hybridization, the chip was washed, extended with a single base, and stained. Scan fluorescence spectroscopy was used to generate raw data.

iii) Detection P for each site were obtained using GenomeStudio 2.0 software. The site and individual quality control requirements were more than 95% for detection *P* of less than 0.05. Original signal values were subjected to bias correction and normalization, and differential methylation was analyzed using the empirical bayes statistics in the limma package in R. Meanwhile, the FDR-corrected *p* value (adjusted *P*val) was calculated to address the multiple hypothesis testing problem. The selection criteria for difference sites was adjusted $P\text{val} \leq 0.05$.

2.3. Correlation Analysis of *IFI30* with GBM Subtypes and Prognosis

The correlation between *IFI30* and GBM subtypes was analyzed using the CGGA database. The prognostic value of *IFI30* was subsequently investigated using the TCGA database. Statistics and plotting were performed using the ggplot2 (V3.3.3) package, Kaplan-Meier plots were created and log-rank tests performed using the survival package. We used pROC, timeROC, and the survival package to create diagnostic ROC curves, time-dependent curves for diagnosis, and nomogram model analysis, respectively.

2.4. Genetic Mutant in GBM Patients

The genomic profile of *IFI30* was analyzed using the TGCA-PanCancer Atlas dataset in the cBioPortal database. Kaplan-Meier plots were created and log-rank tests were performed to determine the significance of the difference between the mutant and wild-type *IFI30*.

2.5. Correlation of *IFI30* with Immune Cell Infiltration and Immune Checkpoints

The association of *IFI30* expression with immune cell infiltration and immune checkpoints in GBM was analyzed using TIMER2.0.

2.6. Gene Ontology (GO) Analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) Analysis

The GEPIA2 database was used to analyze co-expressed genes of *IFI30*. The top 25 genes were selected and imported into the Genemania database to create a protein-protein interaction networks (PPI) of *IFI30*. The top 10 functional partner genes were obtained for GO term enrichment and KEGG pathway analysis.

2.7. GSEA Enrichment Analysis

GBM patients in the TGCA database were divided into high and low expression groups according to the median level of *IFI30* for GSEA enrichment analysis (ggplot2, V3.3.3). In the KEGG pathway analysis,

results of enrichment were considered significant based on net enrichment scores (NES), gene ratios and *p*-values. Enrichment was considered significant with norm $p < 0.05$ and FDR $q < 0.25$.

2.8. Statistical Methods

R software (V3.6.3) was used for all statistical analyses. Differences between groups were compared using Wilcoxon rank-sum test or t-test. Correlations among variables were determined using Pearson or Spearman tests. $P < 0.05$ was considered statistically significant.

3. Result

3.1. *IFI30* Expression in GBM Tissues was Higher than in Normal Tissues

To explore the possible role of *IFI30*, we analyzed its expression in 33 human cancers. Compared with corresponding normal tissues, *IFI30* mRNA was significantly up-regulated in 26 cancer types, including GBM, bladder urothelial carcinoma (BLCA), and breast invasive carcinoma (BRCA) (Figure 1A). However, *IFI30* was significantly down-regulated in four cancers, including lung adenocarcinoma (LUAD), lung squamous cell carcinoma (LUSC), and thymic carcinoma (THYM). In addition, mesothelioma (MESO) and uveal melanoma (UVM) could not be compared due to the lack of normal tissue controls.

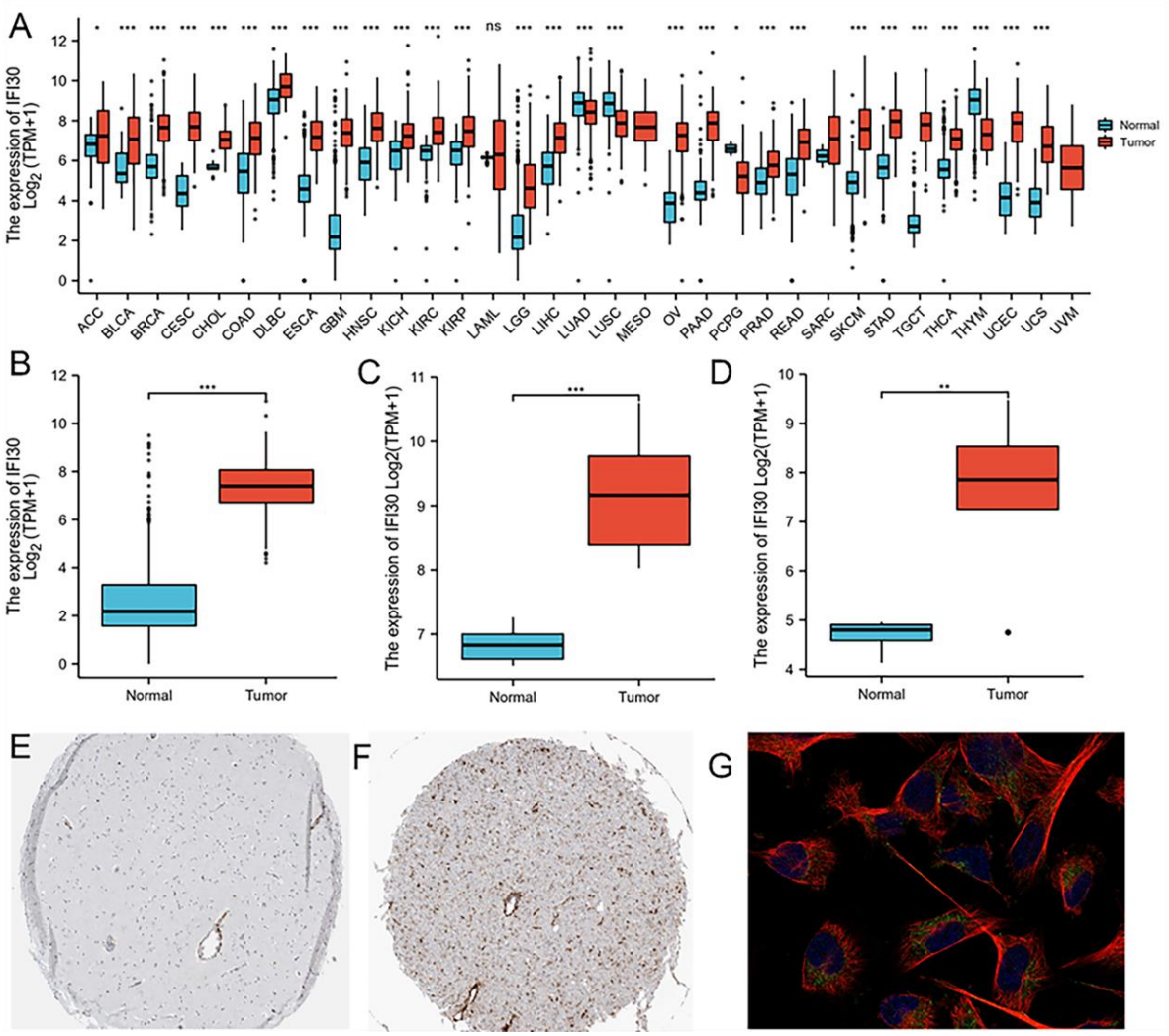

FIGURE 1: *IFI30* expression in GBM tissues and normal tissues. **A)** Expression levels of *IFI30* mRNA in 33 cancer tissues and normal tissues. **B-D)** Based on TCGA database, GSE116520 dataset and UALCAN database, *IFI30* mRNA expression level in GBM was higher than in normal cerebral cortex. *p < 0.05; **p < 0.005; ***p <0.001. **E-G)** Representative immunohistochemical and immunofluorescence images of *IFI30* in HPA database. The expression of *IFI30* in GBM tissue was higher than in normal cerebral cortex. Immunofluorescence assay revealed that *IFI30* was mainly localized in the cytosol.

Using the TCGA database, cancer samples were grouped according to *IFI30* expression levels and showed no significant differences (Table 1). Significant up-regulation of *IFI30* in GBM was observed in a comparative study based on the TCGA database (Figure 1B). Subsequently, differences in *IFI30* transcript levels were further validated using the GSE116520 dataset (Figure 1C) and the UALCAN database (Figure 1D), and similar results were obtained.

TABLE 1: Baseline clinical characteristics of GBM patients in the TCGA database.

Characteristic	Low expression of *IFI30*	High expression of *IFI30*	p
n	84	84	
Gender, n (%)			0.196
Female	34 (20.2%)	25 (14.9%)	
Male	50 (29.8%)	59 (35.1%)	
Race, n (%)			0.391
Asian	4 (2.4%)	1 (0.6%)	
Black or African American	6 (3.6%)	5 (3%)	
White	72 (43.4%)	78 (47%)	
Age, n (%)			0.537
<=60	46 (27.4%)	41 (24.4%)	
>60	38 (22.6%)	43 (25.6%)	
Karnofsky performance score, n (%)			0.092
<80	25 (19.5%)	11 (8.6%)	
>=80	47 (36.7%)	45 (35.2%)	
DSS event, n (%)			0.116
Alive	21 (13.5%)	13 (8.4%)	
Dead	54 (34.8%)	67 (43.2%)	
IDH status, n (%)			0.380
WT	73 (45.3%)	76 (47.2%)	
Mut	8 (5%)	4 (2.5%)	
Age, mean ± SD	58.9 ± 13.54	59.54 ± 13.58	0.763

After determining the transcriptional expression of *IFI30* in GBM, we queried HPA database for representative immunohistochemical and immunofluorescence chemical images of *IFI30*, suggesting that *IFI30* expression in GBM tissue was higher than in normal cerebral cortex (Figures 1E & 1F). This result was consistent with our previous results regarding differential *IFI30* mRNA expression. Meanwhile, immunofluorescence chemistry data suggested that *IFI30* was mainly localized to the cytosol (Figure 1G).

3.2. *IFI30* Methylation in GBM Patients

The prognostic value of each CpG of *IFI30* DNA methylation was investigated using the MethSurv database. Eleven methylated CpG sites were found, with cg00000029 and cg01783195 having the highest degree of DNA methylation (Figure 2A). Eight CpG sites were associated with prognosis: cg01485548, cg01533387, cg04096365, cg07533630, cg15825970, cg17004101, cg26152923, and cg27142905 ($p < 0.05$) (Table 2). Patients with low *IFI30* methylation at these CpG sites had worse overall survival (OS) than those with high *IFI30* methylation. Subsequently, we found a significantly lower global methylation level of the *IFI30* promoter in GBM tissues from the UALCAN database (Figure 2B).

TABLE 2: Effect of *IFI30* methylation level on the prognosis of GBM.

CpG	HR	P-value
TSS200-Island-cg00998146	0.744	0.26
TSS1500-N_Shore-cg01485548	0.642	0.036
1stExon-Island-cg01533387	0.658	0.046
TSS200-N_Shore-cg04096365	0.622	0.024
TSS1500-N_Shore-cg07533630	0.571	0.0097
Body-S_Shore-cg11431981	0.819	0.43
Body-Island-cg11777782	0.822	0.39
TSS200-N_Shore-cg13549667	0.811	0.38
3'UTR-S_Shelf-cg15577634	1.181	0.43
TSS1500-N_Shore-cg15825970	0.508	0.0018
Body-Island-cg17004101	0.623	0.039
TSS1500-N_Shore-cg26152923	0.574	0.0091
1stExon;5'UTR-Island-cg27142905	0.612	0.029

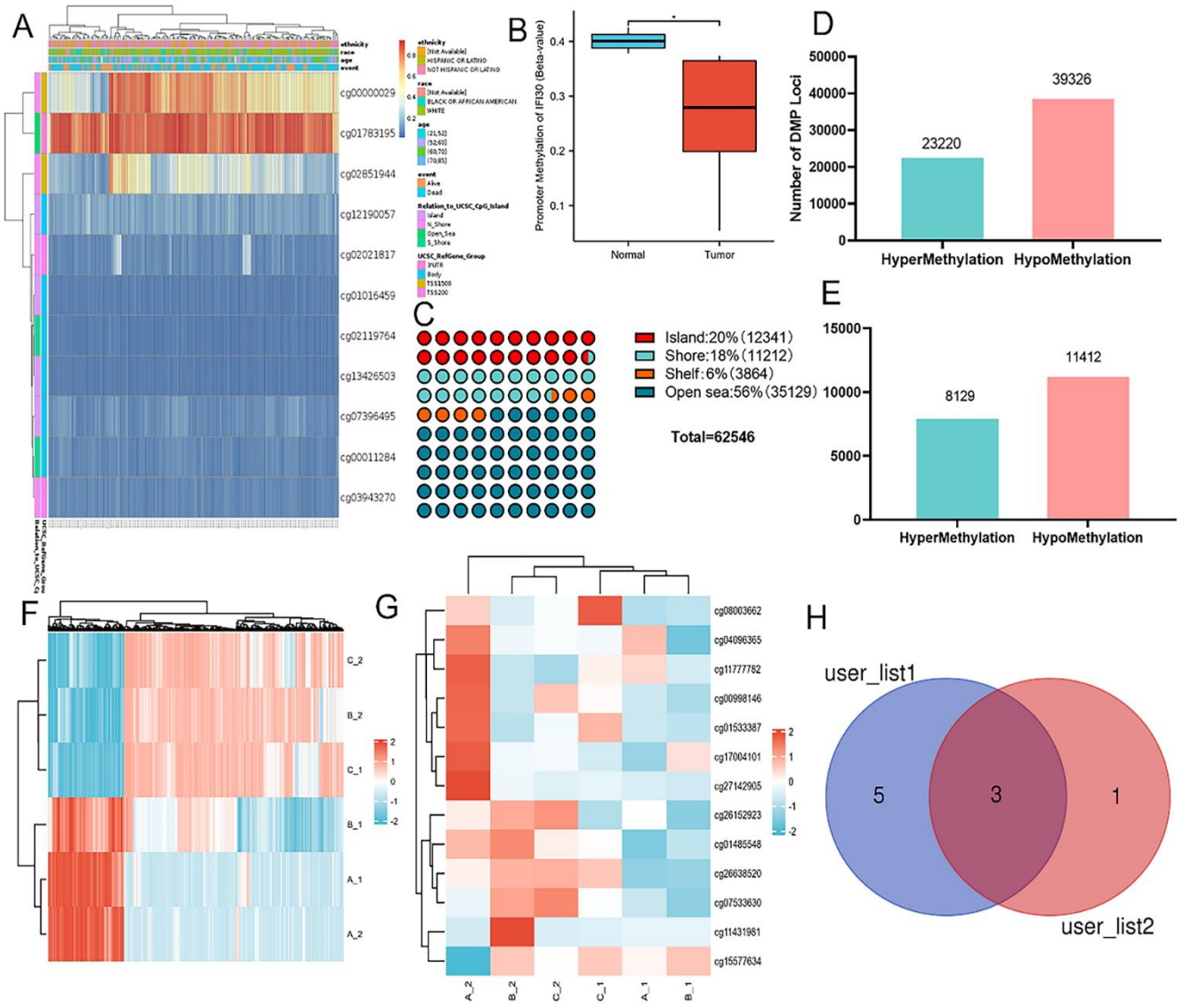

FIGURE 2: *IFI30* methylation in GBM patients. **A)** Visualization between methylation levels and *IFI30* expression levels. **B)** The UALCAN database found that *IFI30* promoter methylation levels were significantly decreased in GBM tissues. DNA methylation of three GBM patients with primary and recurrent samples: **C)** Distribution of differentially methylated genes in total. **D)** Distribution of different methylation levels at differential sites. **E)** Distribution of different methylation levels of differentially expressed genes. **F)** Heat map of differentially methylated genes in treatment-naïve and relapsed samples. **G)** The distribution of methylation at different sites of *IFI30*. Red and blue represent hypermethylation and hypomethylation, respectively. **H)** Cross validation of *IFI30* DNA differential methylation sites by Methsurv database screening (user-LIST1) and illumina 850k identification (user-List2).

To further explore the role of *IFI30* in the mechanism of GBM recurrence, we used Illumina 850K methylation chip to detect DNA methylation in primary and recurrent specimens of three GBM patients. Of the 605,192 probes that passed quality control, 62,546 (10.4%) were differentially methylated between treatment-naïve and relapsed samples (FDR $q < 0.05$, Figure 2C). In total, 37.12% (23,220/62,546) of these differentially methylated cytosines (DMCs) were hypomethylated (Figure 2D). Among the genes corresponding to all probes, 8,129 (41.6%) were found to be differentially methylated (FDR $q < 0.05$, Figure 2E). Subsequently, cluster analysis was performed for CpG loci that met the screening criteria for differential loci. Interestingly, the DNA methylation signature of case A with longer progression-free survival (PFS) in recurrent samples was similar to that of primary specimens, whereas the DNA methylation signature of case C with shorter PFS in primary samples was similar to that of recurrent specimens (Table 3, Figure 2F). Further analysis of the methylation of *IFI30* gene revealed that among the 13 sites corresponding to *IFI30*, four sites were differentially methylated, namely, cg01485548, cg26152923, cg26638520, cg07533630 (FDR $q < 0.05$, (Figure 2G). Cross-validation of *IFI30* differential loci associated with GBM prognosis revealed that cg26152923, cg07533630, and cg01485548 were key prognostic loci (Figure 2H). In conclusion, based on differences in methylation levels of the *IFI30* promoter and the expression profile of *IFI30*, we speculate that *IFI30* may play a key role in the tumorigenesis and recurrence of GBM.

TABLE 3: Baseline clinical characteristics of three patients with GBM**.**

	Age (Years)	Sex	Histopathology (primary)	Removed Degree of Glioma	Standard RTl with concurrent TMZ	Adjuvant TMZ	PFS (months)	Histopathology (recurrent)	OS (months)
Case A	71	male	Glioblastoma	All	Yes	Yes	24	Glioblastoma	32
Case B	49	male	Glioblastoma	All	Yes	Yes	10	Glioblastoma	18
Case C	64	female	Glioblastoma	All	Yes	Yes	2	Glioblastoma	5

3.3. *IFI30* Expression was Related to Pathology and Prognosis in GBM Patients

After comprehensive analysis of the expression pattern of *IFI30*, we used CGGA database to further study the relationship between the expression of *IFI30* and tumor subtype, WHO grade and recurrence status in GBM. First, it could be observed that *IFI30* mRNA was up-regulated in MES subtype of primary and recurrent GBM, which was significantly different from CL and PN subtypes (Figures 3A & 3C). In both primary and recurrent GBM tissues, *IFI30* mRNA expression levels were significantly correlated with WHO grade (Figures 3B & 3D). Further, we found a significant correlation between expression level of *IFI30* mRNA in GBM and recurrence status. Compared with primary tumors, the expression level of *IFI30* mRNA was higher in recurrent tumors (Figure 3E). These findings were almost consistent with our previous results regarding *IFI30* expression.

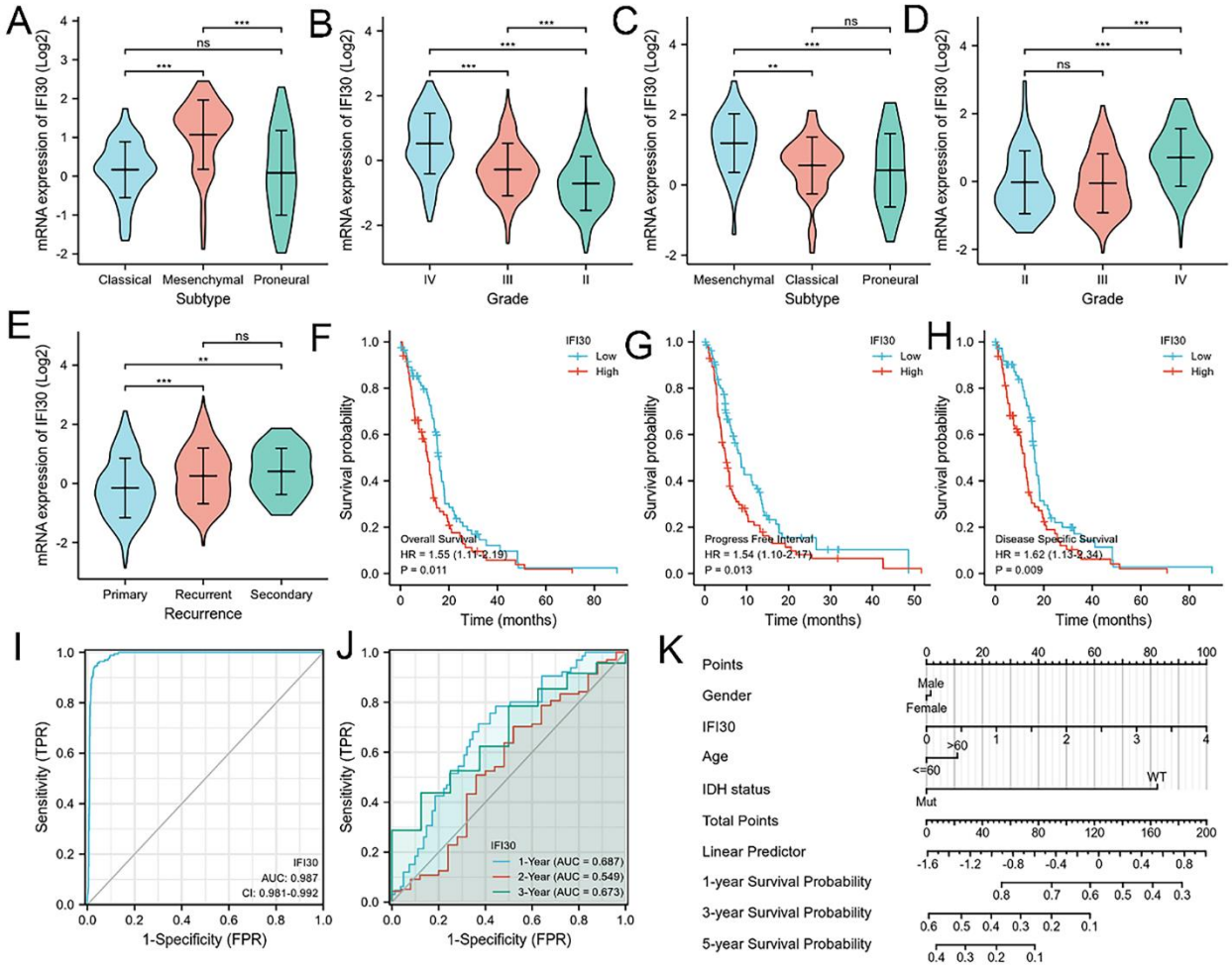

FIGURE 3: The expression of *IFI30* in GBM patients was related to pathology and prognosis. **A)** *IFI30* mRNA expression levels were significantly correlated with GBM subtypes **(A, C)**, WHO grades **(B, D)** and **E)** recurrent status. **F-H)** Comparison of OS, DSS and PFS survival curves of patients with high (red) and low (blue) expression of *IFI30* in GBM using the TCGA database. **p* < 0.05; ***p* < 0.005; ****p* < 0.001; ns, no statistical difference. **I)** *IFI30* expression could be used to differentiate the diagnostic ROC curve of tumor and normal tissue. **J)** Time-dependent survival ROC curve analysis predicted 1-, 3-, and 5-year survival. **K)** Nomogram model: combining clinical factors and *IFI30* levels to predict 1-, 3-, and 5-year survival probabilities in GBM patients.

To investigate the prognostic value of *IFI30* in GBM, we applied the TCGA database to analyze the correlation between differentially expressed *IFI30* and clinical outcomes. GBM patients with higher *IFI30* mRNA expression showed lower OS, worse disease-specific survival (DSS) and PFS compared with those with lower *IFI30* mRNA expression level according to Kaplan-Meier survival curve (Figures 3F-3H). Therefore, *IFI30* mRNA overexpression was associated with poorer prognosis and may be a valuable predictive biomarker.

From the diagnostic ROC curve, *IFI30* mRNA expression could accurately identify tumors from normal tissues (AUC = 0.987) (Figure 3I). *IFI30* time-dependent survival ROC curves were created to predict 1-, 3-, and 5-year survival. AUC showed that *IFI30* was suitable for predicting GBM outcomes (Figure 3J). Subsequently, we integrated clinicopathological factors (including age, gender, and IDH status) and *IFI30* mRNA expression levels, and established a nomogram model, which can be used to predict the 1-, 3- and 5-year survival probability of clinical patients (Figure 3K). Model global statistical test situation: C-index: 0.621 (95%CI 0.594-0.648).

3.4. Gene Alteration and Functional Analysis of *IFI30* in GBM Patients

Genetic mutations in *IFI30* in GBM were explored using the TGCA-PanCancer Atlas dataset in cBioPortal (n = 592 GBM patients). The *IFI30* gene was altered in five samples (0.8%) (Figure 4A). We found that *IFI30* gene mutation had no significant effect on PFS (p = 0.665) and OS (p = 0.214) of GBM patients (Figures 4B & 4C).

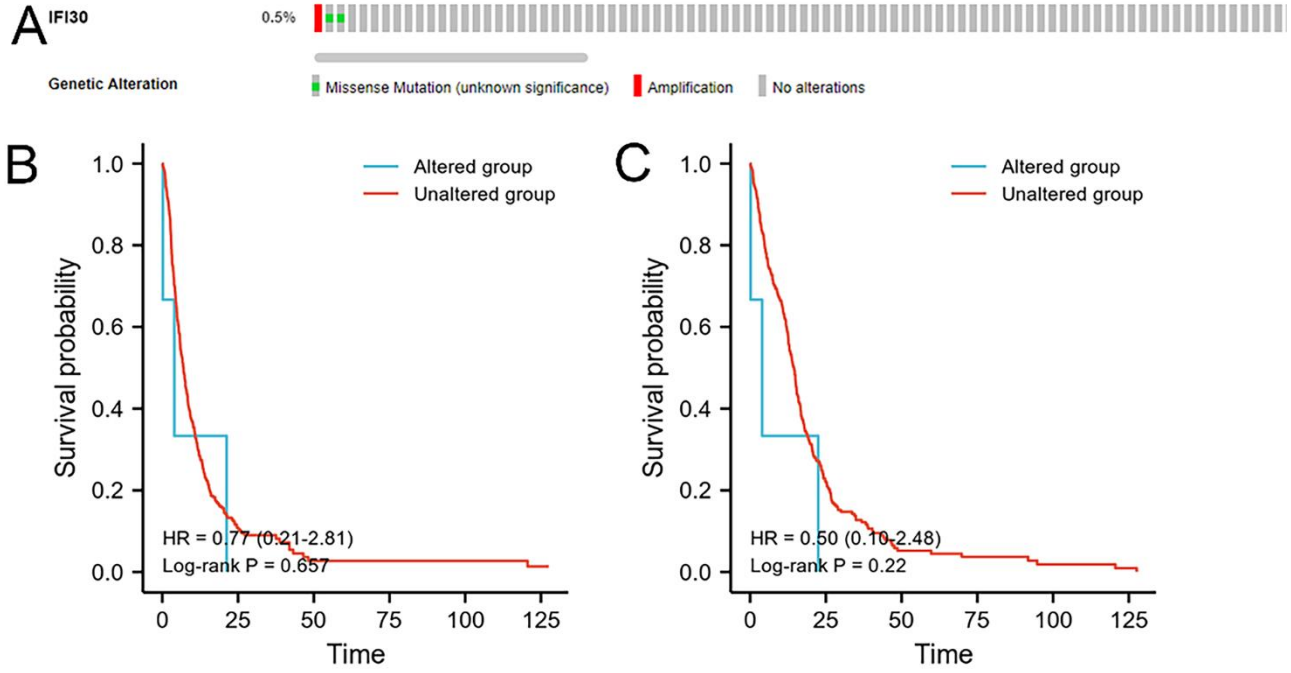

FIGURE 4: Exploration of genetic mutations in *IFI30* in GBM patients in the cBioPortal database. **A)** OncoPrint visual summary of *IFI30* gene changes. **B, C)** Kaplan-Meier plots comparing PFS and OS in patients with *IFI30* mutations.

3.5. *IFI30* Expression was Associated with Immune Cell Infiltration and Immune Checkpoints

The relationship between *IFI30* expression and immune cell infiltration adjusted for purity was investigated using TIMER 2.0. The results showed that *IFI30* expression level in GBM was positively correlated with $CD8^+$ T cells, $CD4^+$ T cells, treg, neutrophils, macrophages, cancer-associated fibers, DCs, MDSCs and other immune cells, but negatively correlated with tumor purity (Figures 5A-5C). These results demonstrated that *IFI30* was positively associated with immune cell infiltration. Subsequently, we assessed the association of *IFI30* with immune checkpoints in the TIMER database. The results suggested that *IFI30* in GBM was significantly positively correlated with PD-1, CTLA-4, CD274, and HAVCR2 (Figure 5D).

3.6. *IFI30* Affects Tumor Immune Microenvironment Through Antigen Presentation

To explore the functions of *IFI30* and co-expressed genes, 20 co-expressed genes were obtained using the GEPIA2 database, with PPC values ranging from 0.87 to 0.90. A PPI network of *IFI30* was constructed using the Genemania database (Figure 6A). The top 10 functional partner genes (PCC>0.89) were selected as highly correlated. These genes were *HK3*, *CTSS*, *MS4A6A*, *SIGLEC7*, *C1QC*, *TYROBP*, *FTLP3*, *LAIR1*, *CTSL*, and *SLC7A7*. The results showed that *CTSS*, *CTSL* and *C1QC* were highly expressed in antigen processing and presentation (Figures 6B-6C). Subsequently, we performed gene correlation analysis using the TGCA database, which showed that *CTSS*, *CTSL*, *C1QC*, and *IFI30* transcript levels were positively correlated (Figure 6D). GO enrichment analysis included three main functions of biological process, cellular component, and molecular function (Table 4) ($p < 0.05$). KEGG analysis mainly included "antigen processing and presentation", "lysosome", "apoptosis". KEGG enrichment items

showed that the high expression of *IFI30* was mainly associated with treg development, Toll-like receptor signaling pathway, T cell receptor signaling pathway, PPAR signaling pathway, NOD signaling pathway, NK cell-mediated cytotoxicity, JAK/STAT signaling pathway, chemokine signaling pathway and antigen processing and presentation. GSEA analysis was performed to identify functional enrichment with high and low expression of *IFI30* (Figures 6E-6G). Low expression of *IFI30* was associated with disruption of postsynaptic signaling by CNV, synaptic vesicle pathway, GABA receptor signaling, neurotransmitter release cycle, neurofilament and neurogenic proteins, and protein interactions at synapses.

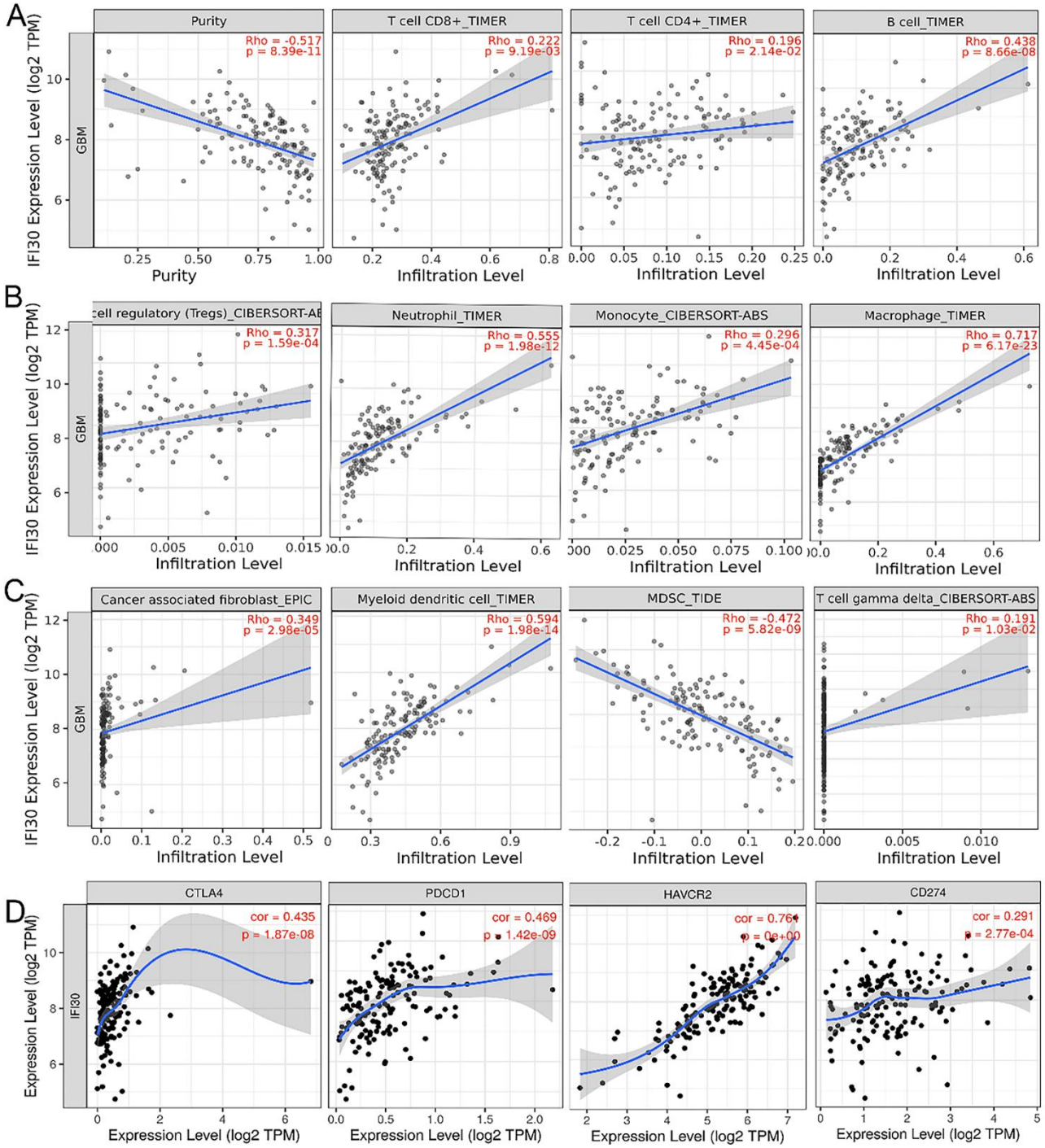

FIGURE 5: The expression of *IFI30* in GBM was related to immune cell infiltration and immune checkpoints. **A-C)** The expression of *IFI30* was positively correlated with immune cells. **D)** The expression of *IFI30* was positively correlated with the levels of PD-1, CTLA-4, CD274 and havcr2.

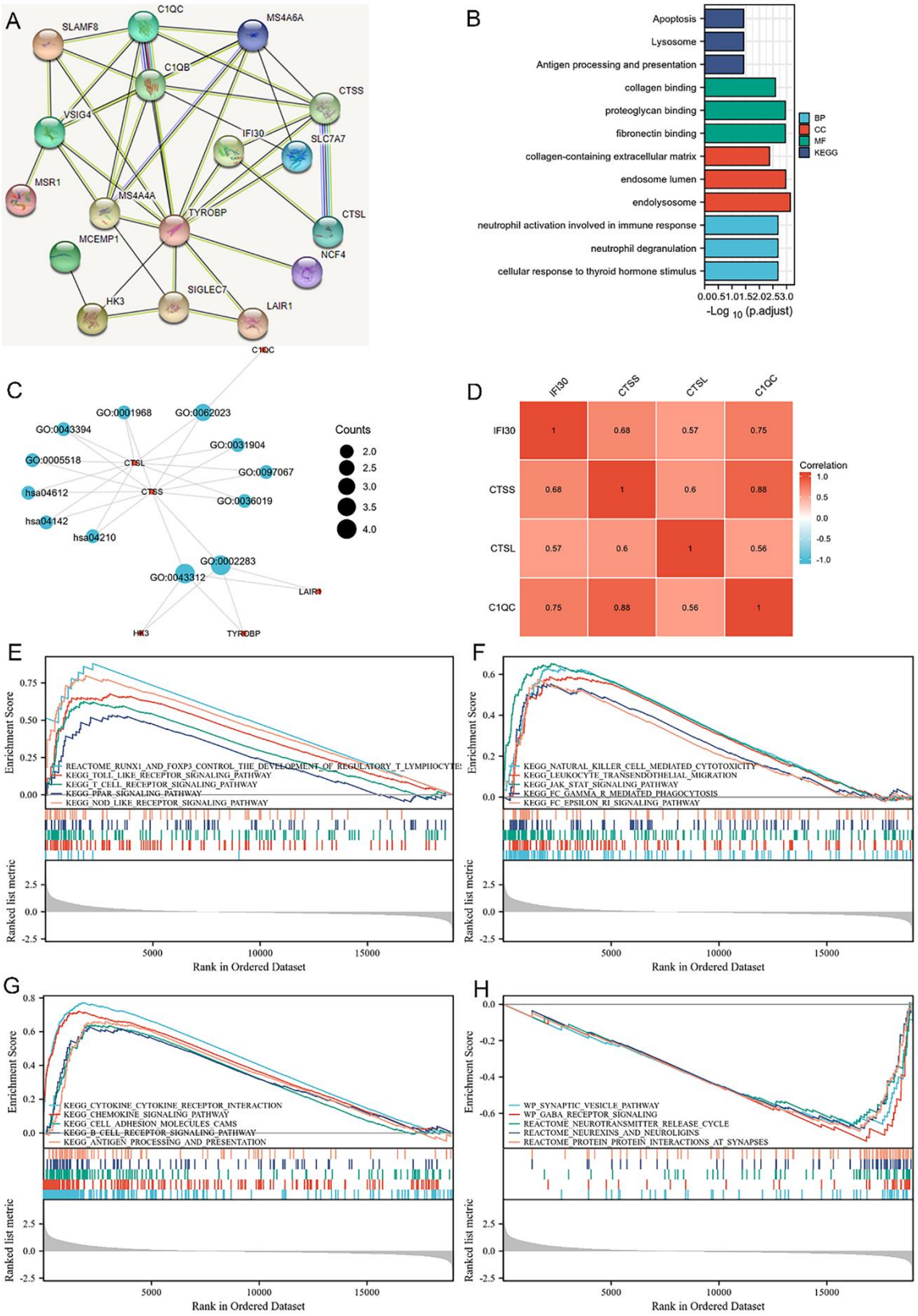

FIGURE 6: *IFI30* functional annotation and predicted signaling pathways. **A)** *IFI30*-interacting proteins in GBM are visualized in bubble plots. **B, C)** GO terms and KEGG pathway enrichment analysis. **D)** The transcript levels of *CTSS*, *CTSL*, *C1QC* and *IFI30* were positively correlated. **E-G)** GSEA enrichment analysis of *IFI30* high expression group enrichment pathway. **H)** GSEA enrichment analysis of pathways in the *IFI30* low expression group.

TABLE 4: GO and KEGG enrichment analyses of *IFI30* and functional partner genes in GBM.

ONTOLOGY	ID	Description	pvalue
BP	GO:0097067	cellular response to thyroid hormone stimulus	1.92e-05
BP	GO:0043312	neutrophil degranulation	2.90e-05
BP	GO:0002283	neutrophil activation involved in immune response	2.97e-05
BP	GO:0042119	neutrophil activation	3.22e-05
BP	GO:0002446	neutrophil mediated immunity	3.24e-05
CC	GO:0036019	endolysosome	3.50e-05
CC	GO:0031904	endosome lumen	1.03e-04
CC	GO:0062023	collagen-containing extracellular matrix	6.64e-04
CC	GO:0043202	lysosomal lumen	8.09e-04
CC	GO:1904813	ficolin-1-rich granule lumen	0.001
MF	GO:0001968	fibronectin binding	6.24e-05
MF	GO:0043394	proteoglycan binding	1.12e-04
MF	GO:0005518	collagen binding	3.90e-04
MF	GO:0004197	cysteine-type endopeptidase activity	0.001
MF	GO:0008234	cysteine-type peptidase activity	0.003
KEGG	hsa04612	Antigen processing and presentation	0.001
KEGG	hsa04142	Lysosome	0.004
KEGG	hsa04210	Apoptosis	0.004
KEGG	hsa04145	Phagosome	0.005

4. Discussion

Although the *IFI30* gene is expressed in various organs, its expression has been shown to be increased in various cancer tissues [9, 15, 16]. In this study, we found that *IFI30* was markedly up-regulated in most human cancers. In addition, we confirmed that *IFI30* was overexpressed in GBM through analyses performed on the TCGA, GEO, and UALCAN databases. Validation in the MethSurv database and clinical samples revealed that the *IFI30* promoter methylation was decreased in GBM and its local locus was significantly associated with GBM recurrence. Additionally, higher *IFI30* expression was associated with poorer prognosis. *IFI30* expression showed a good ability to differentiate tumor from normal tissue and predicted 1-, 3-, and 5-year survival, suggesting that it could be used as a valuable diagnostic and prognostic biomarker for GBM. Further, our results showed that the expression of *IFI30* mRNA was significantly correlated with GBM subtype and WHO grade. Both MES and high WHO grade were markers of poor prognosis for glioma, which is consistent with findings reported in previous studies [17, 18].

IFI30 was highly expressed in GBM and its function was closely associated with its mutation and epigenetics. In this study, *IFI30* gene mutation was only 0.5%, and was not associated with PFS and OS. However, epigenetic changes in DNA methylation may affect its function [19, 20]. On this basis, we studied the DNA methylation of three patients. Although the global methylation level of *IFI30* promoter in GBM decreased, high methylation level was detected at local sites. Eight of these CpG sites were hypermethylated and associated with poor OS, with cg00000029 and cg01783195 having the highest DNA methylation levels. Subsequently, we used clinical samples to explore the relationship between GBM recurrence and *IFI30* methylation sites. Cross validation with the former showed that cg26152923, cg07533630 and cg01485548 were key sites potentially affecting GBM recurrence and prognosis. These three key sites have not been reported in the literature, and it was also the first time to evaluate IFI30 DNA methylation.

IFI30 might lead to recurrence of GBM and affect prognosis. Previous studies have found that it could regulate the tumor immune microenvironment, resulting in changes that could affect the tumor prognosis [21, 22]. Interestingly, our study showed that *IFI30* was positively associated with immune cells such as TAM. Among them, *IFI30* had the highest positive correlation with TAM (r = 0.717), which plays an important role in tumor growth, invasion, angiogenesis and metastasis, and is negatively correlated with the prognosis of GBM [23, 24]. In addition, we found that *IFI30* was positively correlated with treg (r = 0.317). Tregs, the major suppressive immune cell population in GBM, inhibits the antitumor activity of CTL and may mediate resistance to immune checkpoint blockers [12, 25, 26]. These results suggest that *IFI30* may reflect the state of the GBM immune microenvironment and regulate immune functions. As immune checkpoint inhibition is considered another important prognostic factor in glioma, we evaluated the relationship between *IFI30* and immune checkpoints.

The results showed that *IFI30* was positively correlated with PD-1, CTLA-4, CD274 and HAVCR2 expression in GBM, which is consistent with the results of previous studies [16]. In particular, HAVCR2 had the highest correlation with *IFI30* (r=0.761) and acted through a different signaling pathway from PD-1 and CTLA-4 [16, 27, 28]. This suggested that targeting *IFI30* could increase the efficacy of immune checkpoint inhibitors in GBM through multiple pathways. The results of the present study are similar to those of previous studies [9, 16]. Therefore, targeting *IFI30* could improve the suppressive immune microenvironment and alleviate T cell exhaustion. To further study the specific mechanism of *IFI30* affecting the immune microenvironment of GBM, we showed that *IFI30* and its functional partner genes *CTSS*, *CTSL* and *C1QC* were upregulated in the process of antigen processing and presentation and apoptosis through KEGG pathway enrichment analysis.

GO analysis showed that *IFI30* and its interacting genes were not only involved in responses to temperature stimuli, but also in immune responses that regulate tumorigenesis and tumor progression, such as neutrophil degranulation, neutrophil activation. Recent studies have found that neutrophil degranulation is associated with tumor progression [29, 30]. These results are consistent with findings reported in previous studies [10, 31]. Subsequent GSEA enrichment analysis showed that high IFI30 expression was mainly associated with treg development, Toll-like receptor signaling pathway, T cell receptor signaling pathway, PPAR signaling pathway, NOD signaling pathway, NK cell-mediated cytotoxicity, JAK/STAT signaling pathway, chemokine signaling pathway, and antigen processing and presentation. These signal pathways are significantly related to the growth, progression and recurrence of glioma [32-36]. This suggests that *IFI30* plays an important role in the immune regulation of GBM and is an ideal target for tumor immunotherapy.

5. Conclusion

In this study, we found differential expression of *IFI30* in GBM at mRNA and protein levels as well as DNA methylation level, demonstrating a close relationship among *IFI30*, immune infiltration, and immune checkpoints. In addition, our results showed that *IFI30* had a good diagnostic and prognostic value in GBM. Therefore, *IFI30* is an ideal potential diagnostic, prognostic biomarker and therapeutic target for GBM.

Author Contributions

Jianhuang Huang: Conception, design of the study, conducting experiments, data validation and wrote the draft of the manuscript. Guiting You: Conducting experiments, formal analysis, project administration, wrote the draft of the manuscript. Yijing Lin: Resources, visualization, funding acquisition. Yunpeng Lin: Resources, methodology, data curation. Shishi Wu: Data analysis and investigation. Fan Chen: Visualization and data analysis. Caihou Lin: Conception, funding acquisition, writing-review and editing. Jianwu Chen: Conception, design of the study, writing-review and editing. All authors contributed to approving the submitted version.

Competing Interests

None.

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Ethical Approval

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Ethics Review Committee of Union Hospital Affiliated to Fujian Medical University (Ethics Number: 2020KJT066).

Availability of Data and Materials

The TCGA database: (Link 4); GSE116520: (Link 5); The UALCAN database: (Link 1); The Human Protein Atlas database: (Link 2); The MethSurv database: (Link 3); The GEPIA2 database: (Link 6); The CGGA database: (Link 7); The cBioPortal database: (Link 8).

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