IFI30 as a prognostic biomarker and correlation with immune infiltrates in glioma

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Background: Increased evidence indicates that the tumour microenvironment (TME) plays an essential role in the development, treatment and prognosis of glioma. High expression of interferon-gamma-inducible protein 30 (*IFI30*) is associated with the malignant phenotype, but the effect of *IFI30* on the tumour immune microenvironment and its potential role in the carcinogenesis of glioma remain unknown.

Methods: The RNA sequencing (RNA-seq) data of 33 types of human cancer were obtained from The Cancer Genome Atlas (TCGA) Genomic Data Commons (GDC). R software was used to perform analyses, such as the expression of *IFI30* in pan-cancer, evaluation of *IFI30* as a prognostic biomarker in glioma, the relationship between *IFI30* expression and clinical characteristics, and immune checkpoint. TIMER was used to analyse the correlation of *IFI30* expression level with immune cell infiltration, and also to conduct survival analysis for immune cells and *IFI30* in low grade glioma (LGG). DAVID was used for Gene Ontology (GO) functional annotations and Kyoto Encyclopedia of Genes and Genomes (KEGG) for pathway analysis of the genes similar to *IFI30* in glioma. The differentially expressed genes (DEGs) between the high- and low-*IFI30* expression groups were determined by DESeq2. Gene set enrichment analysis (GSEA) was then conducted to identify *IFI30*-related functional significance based on the hallmark gene set.

Results: Dysregulated expression of *IFI30* was associated with human cancers. High *IFI30* expression was associated with poor overall survival (OS), disease-specific survival (DSS) and progression-free interval (PFI). Univariate and multivariate analyses identified *IFI30* as an independent predictor for glioma. Meanwhile, *IFI30* overexpression significantly correlated with high-grade tumours, poor OS, and immune infiltration. In addition, *IFI30*-associated genes significantly enriched the hallmark tumour progression-related clusters and cancer pathways.

Conclusions: *IFI30* is a prognostic biomarker correlated with immune infiltrates and acts as an oncogene in glioma.

Keywords: Interferon-gamma-inducible protein 30 (*IFI30*); glioma; prognosis; tumour immune infiltration

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Introduction

Glioma is one of the most malignant tumours in the brain, accounting for around 27% of all primary brain tumours and is responsible for approximately 13,000 cancer-related deaths in the USA each year (1,2). Among them, glioblastoma multiforme (GBM) accounts for 40–50% of glioma. The standard treatment is surgery and radiotherapy, which are the most common and extremely fatal brain tumours. Despite advances in surgical resection and chemoradiotherapy, the prognosis of glioma patients remains dismal (3-5). Thus, it is extremely meaningful and urgent to identify more sensitive and specific prognostic biomarkers.

The tumour microenvironment (TME) comprises infiltrating inflammatory cells, blood vessels, tumour cells, tumour stroma and various associated tissue cells (6). Tumours shape the TME by recruiting stromal cells, such as tumour-associated macrophages and regulatory T cells (Tregs), all of which are associated with poor prognosis (7,8). In addition, tumour-derived factors promote the release of nutrients from the TME to promote tumour growth (9). Therefore, increasing evidence indicates that the TME plays an important role in tumour development, treatment and prognosis (10). In addition to cancer cells, the TME also contains many different types of non-cancerous cells, including endothelial cells, immune cells, which protect the brain from inflammation. A number of studies in the past decade have shown that the brain TME is the basic regulator of cancer progression and the therapeutic effect of primary and metastatic brain malignancies. In recent years it has been found that the brain is also connected to the peripheral immune system through the lymphatic vessels in the meninges (11). This finding shed new light on the relationship between the brain's immune microenvironment and neurological diseases, including glioma. Therefore, the focus of glioma research has moved to the tumour immune microenvironment.

Interferon-gamma-inducible protein 30 (*IFI30*), also known as gamma-interferon-inducible lysosomal thiol reductase (GILT), is involved in major histocompatibility complex (MHC) class I-restricted cross-presentation and MHC class II-restricted antigen processing pathways of adaptive immunity and constitutively expressed in most antigen-presenting cells, including B cells, bone marrow-derived dendritic cells, monocytes, and macrophages (12-14). *IFI30* is also expressed at increased levels in various cancers such as breast cancer and melanoma (12,15). In addition, *IFI30* may be linked to disease progression in prostate cancer (16). Although IFI30 is highly expressed in certain APCs, such as

dendritic cells, B cells, and macrophages, and is known as an immune activator that promotes the antigen presentation process, few studies currently estimate the role of IFI30 in tumour cells. Recent studies demonstrated an association between *IFI30* expression and the malignant phenotype of glioma (17). However, the effects of *IFI30* on the tumour immune microenvironment and its potential role in the carcinogenesis of glioma remain unknown. Although reports have studied the important role of IFI30 in glioma, it has not yet been evaluated whether IFI30 can be used as an independent prognostic factor for patients with glioma. This study analysed the prognostic value of IFI30 in glioma and its relationship with tumour immune infiltration.

In this study, we used The Cancer Genome Atlas (TCGA) pan-cancer data to analyse the expression of *IFI30* in tumour tissues and normal tissues. We present the following article in accordance with the REMARK reporting checklist (available at https://dx.doi.org/10.21037/atm-21-5569).

Methods

TCGA pan-cancer and glioma data acquisition

The level three RNA sequencing (RNA-seq) data [fragments per kilobase per million (FPKM)] of 33 types of human cancer, including TCGA-low grade glioma (LGG) dataset, TCGA-glioblastoma multiforme (GBM) dataset and corresponding clinical information, were obtained from TCGA Genomic Data Commons (GDC) (https://portal.gdc.cancer.gov/). RNA-seq data (FPKM values) were then transformed to transcripts per million reads (TPM) and normalised into log2(TPM +1). The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Gene Ontology (GO) annotations and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways analysis

Gene Expression Profiling Interactive Analysis (GEPIA) (http://gepia.cancer-pku.cn/), a web-based tool, was used to obtain the top 120 similar genes of *IFI30* in LGG and GBM tumours. DAVID (https://david.ncifcrf.gov) was used for the GO functional annotations and KEGG for pathways analysis of the top similar genes.

Differential gene expression and gene set enrichment analysis (GSEA) analysis

The differentially expressed genes (DEGs) between high-

and low-*IFI30* expression groups were determined by DESeq2 with |log$_2$[fold change (FC)]| >1 and adjusted P value <0.05 (18). GSEA (https://software.broadinstitute.org/gsea/index.jsp) was then conducted to identify *IFI30*-related functional significance based on the hallmark gene set ("h.all.v7.0.symbols.gmt"). |Normalised enrichment score (NES)| >1, adjusted P value <0.05, and false discovery rate (FDR) <0.05 were considered to be statistically significant.

TIMER database analysis

TIMER (https://cistrome.shinyapps.io/timer/) was used to analyse the correlation of *IFI30* expression level with immune cell infiltration level in LGG. P value <0.05 was considered statistically significant. TIMER was also used to conduct survival analysis for immune cells and *IFI30* in LGG. Log-rank P value <0.05 was regarded as statistically significant.

Additional bioinformatic and statistical analysis

R software (version 4.1.0, https://www.r-project.org/) was used to perform analyses and plot graphs. Patients' characteristics between groups were compared using the chi-square test and Fisher's exact test. The Wilcoxon's rank-sum test was used for unpaired samples, and the Wilcoxon signed-rank test was used for paired samples. Correlations between the levels of *IFI30* and immune checkpoints in glioma were also analysed using the Spearman correlation. *Survival* analysis for *IFI30* was carried out and visualised by the R *survival* package (https://CRAN.R-project.org/package=survival). Kruskal-Wallis test was used to analyse the *IFI30* expression among different clinicopathological features. Visualisation of the results of analyses was performed using the R package *ggplot2* (https://ggplot2.org) (19). P value <0.05 was considered statistically significant.

Results

Inspection of IFI30 mRNA expression data of 33 cancer types in the TCGA database

First, we obtained the differential expression of *IFI30* in cancer and normal tissue samples from the TCGA database, as shown in *Figure 1A*. In bladder urothelial carcinoma (BLCA), breast invasive carcinoma (BRCA), cervical squamous cell carcinoma and endocervical adenocarcinoma

(CECS), cholangiocarcinoma (CHOL), esophageal carcinoma (ESCA), GBM, head and neck squamous cell carcinoma (HNSC), kidney chromophobe (KICH), kidney renal clear cell carcinoma (KIRC), kidney renal papillary cell carcinoma (KIRP), liver hepatocellular carcinoma (LIHC), prostate adenocarcinoma (PAD), stomach adenocarcinoma (STAD), thyroid carcinoma (THCA), and uterine corpus endometrial carcinoma (UCEC), *IFI30* expression was increased in tumour tissues compared with normal tissues. In lung adenocarcinoma (LUAD), lung squamous cell carcinoma (LUSC), and pancreatic adenocarcinoma (PAAD), *IFI30* expression was reduced in tumour tissues compared with normal tissues. There was no difference in the expression of *IFI30* in normal and tumour tissues between colon adenocarcinoma (COAD) and rectum adenocarcinoma (READ).

We also used the TCGA database to analyse the expression of *IFI30* in LGG, GBM, UCEC, ESCA, BRCA, HNSC, LIHC, and STAD cancers and adjacent normal tissues. We observed that the expression of *IFI30* in cancer tissues was higher than in normal tissues (*Figure 1B-1I*).

Evaluation of IFI30 as a prognostic biomarker in glioma

A difference of IFI30 expression in glioma (LGG and GBM) tissue and normal brain tissue was observed. We compared overall survival (OS) between the high *IFI30* expression group and low *IFI30* expression group in TCGA-LGG and TCGA-GBM cohorts. Median OS for the high *IFI30* group was 63.8 (46.7–95.8) months and 115.7 (81.1–148.2) months for the low *IFI30* group in LGG (P<0.001; *Figure 2A*). Median OS for the high *IFI30* group was 11.2 (9–13.1) months, and 16 (14.9–18.1) months for the low *IFI30* group in GBM (P=0.008; *Figure 2B*). Considering the possibility of non-tumour death during follow-up, we analysed the relationship between *IFI30* gene expression and disease-specific survival (DSS) in LGG and GBM. Median DSS for the high *IFI30* group was 74.5 (52.6–95.8) months, and 119 (81.1–156.5) months for the low *IFI30* group in LGG (P<0.001; *Figure 2C*). Median DSS for the high *IFI30* group was 12 (10–13.5) months, and 16.2 (15.2–18.3) months for the low *IFI30* group in GBM (P=0.006; *Figure 2D*). In addition, we analysed the relationship between *IFI30* expression and the progression-free interval (PFI) in LGG and GBM. Median PFI for the high *IFI30* group was 31.7 (24.1–43.5) months and 53.5 (40.2–75.6) months for the low *IFI30* group in LGG (P=0.003; *Figure 2E*). Median PFI for the high *IFI30*
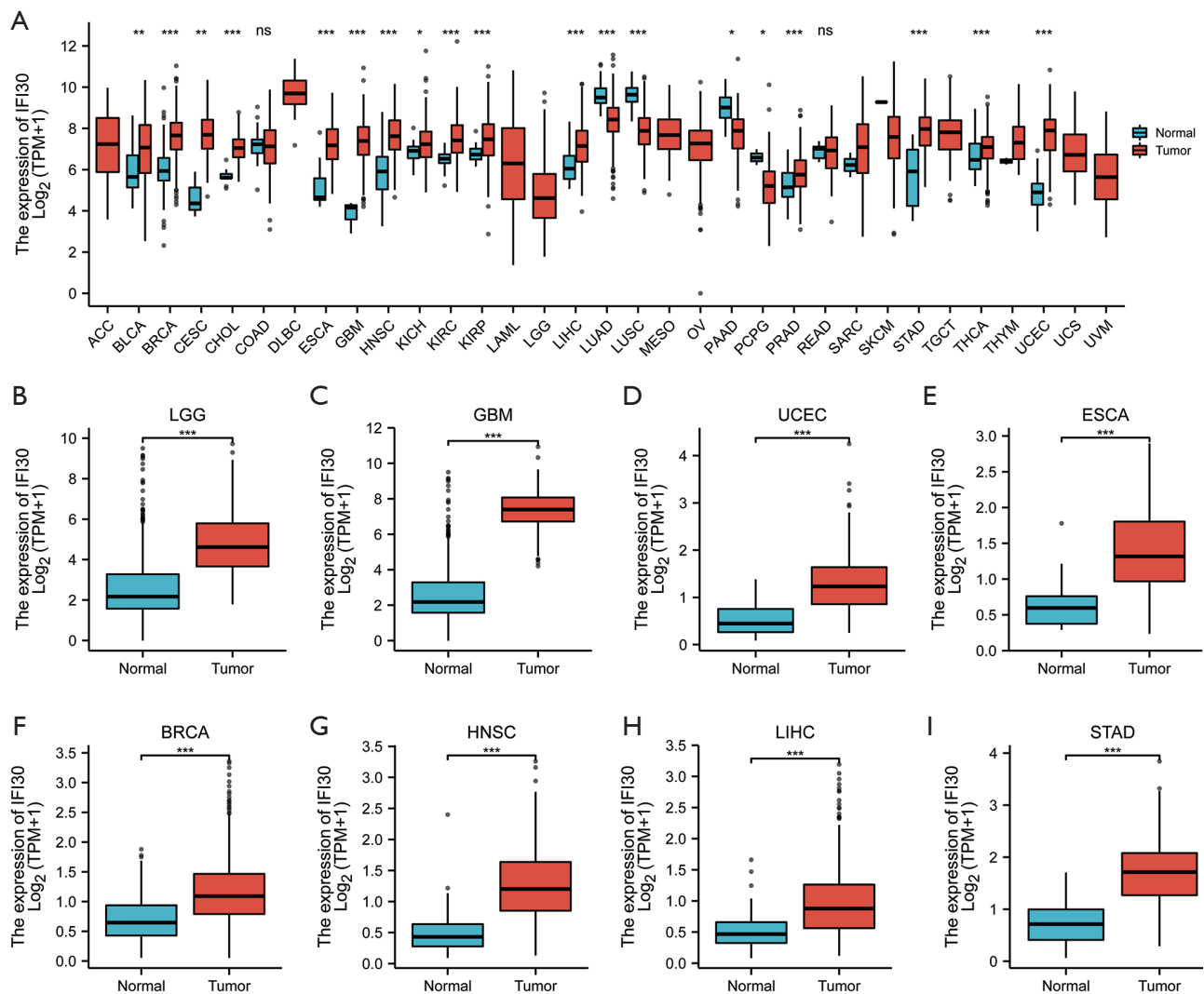
Figure 1 Expression analysis of *IFI30* in pan-cancer data. (A) Expression of *IFI30* in 33 types of human cancer based on TCGA cancer and normal tissue data. (B-I) Representative *IFI30* expression in TCGA-LGG (B), GBM (C), UCEC (D), ESCA (E), BRCA (F), HNSC (G), LIHC (H) and STAD (I) tissues compared with corresponding TCGA normal tissues. *, P value ≤0.05; **, P value ≤0.01; ***, P value ≤0.001; ns, P value >0.05. *IFI30*, interferon-gamma-inducible protein 30; TCGA, The Cancer Genome Atlas; LGG, low grade glioma; GBM, glioblastoma multiforme; UCEC, uterine corpus endometrial carcinoma; ESCA, esophageal carcinoma; BRCA, breast invasive carcinoma; HNSC, head and neck squamous cell carcinoma; LIHC, liver hepatocellular carcinoma; STAD, stomach adenocarcinoma; ACC, adrenocortical carcinoma; BLCA, bladder urothelial carcinoma; CESC, cervical squamous cell carcinoma and endocervical adenocarcinoma; CHOL, cholangiocarcinoma; COAD, colon adenocarcinoma; DLBC, lymphoid neoplasm diffuse large B-cell lymphoma; KICH, kidney chromophobe; KIRC, kidney renal clear cell carcinoma; KIRP, kidney renal papillary cell carcinoma; LAML, acute myeloid leukemia; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; MESO, mesothelioma; OV, ovarian serous cystadenocarcinoma; PAAD, pancreatic adenocarcinoma; PCPG, pheochromocytoma and paraganglioma; PRAD, prostate adenocarcinoma; READ, rectum adenocarcinoma; SARC, sarcoma; SKCM, skin cutaneous melanoma; TGCT, testicular germ cell tumours; THCA, thyroid carcinoma; THYM, thymoma; UCS, uterine carcinosarcoma; UVM, uveal melanoma.
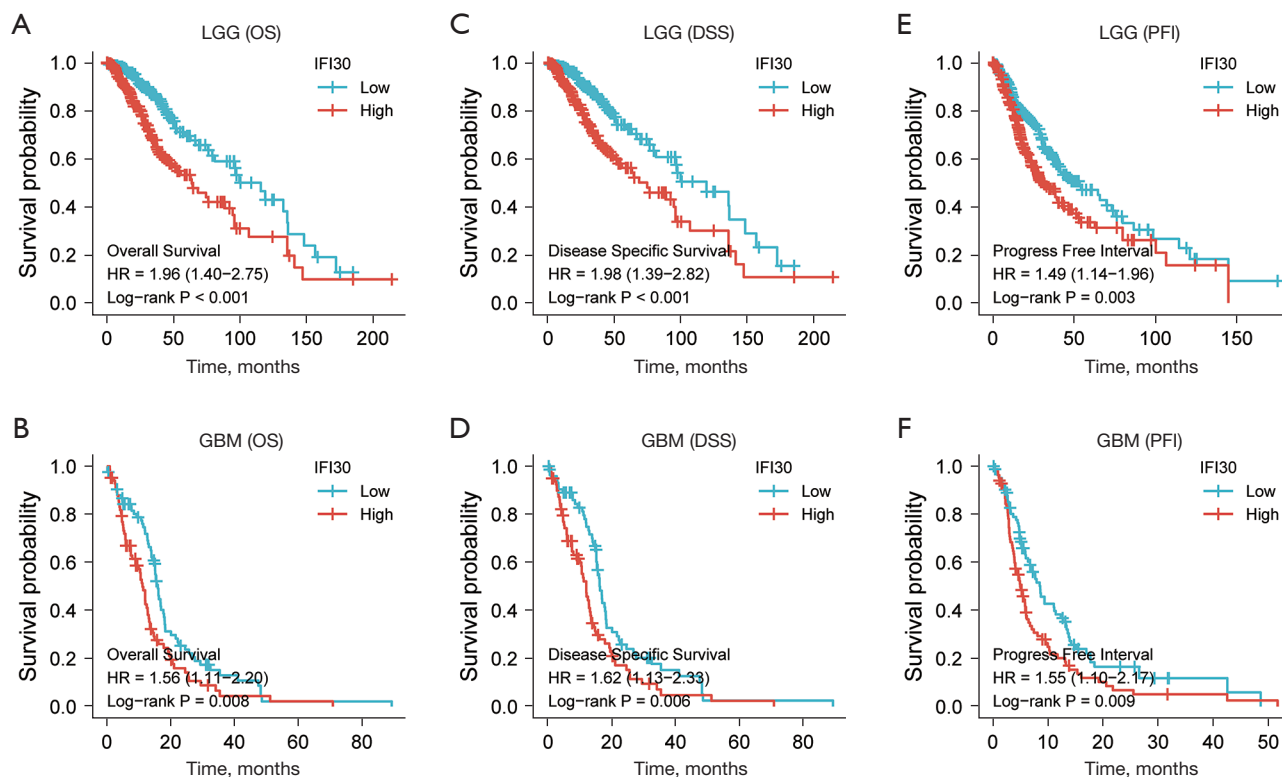
Figure 2 Survival analysis of high and low *IFI30* gene expression in glioma (LGG and GBM). (A,B) The relationship between *IFI30* gene expression in LGG and GBM and OS. (C,D) The relationship between *IFI30* gene expression in LGG and GBM and DSS. (E,F) The relationship between *IFI30* gene expression in LGG and GBM and PFI. *IFI30*, interferon-gamma-inducible protein 30; LGG, low grade glioma; GBM, glioblastoma multiforme; OS, overall survival; DSS, disease-specific survival; PFI, progression-free interval.

group was 5 (4.1–6.2) months and 8.6 (6.8–12.3) months for the low *IFI30* group in GBM (*Figure 2F*).

Univariate Cox regression analysis showed that age, WHO grade and *IFI30* were significantly associated with the prognosis of LGG. Multivariate Cox regression analysis confirmed that *IFI30* was an independent prognostic factor after adjusting for other clinicopathologic factors (*Table 1*). Univariate and multivariate Cox regression analyses showed *IFI30* was also an independent prognostic factor and significantly associated with the prognosis of GBM (*Table 2*).

Relationship between IFI30 expression and clinical characteristics in LGG

Clinical and gene expression data of LGG patients were downloaded from the TCGA database. Patients were divided into high and low expression groups on the basis of median *IFI30* mRNA expression. The relationship

between clinical characteristics and *IFI30* mRNA expression in LGG was analysed and the results revealed that *IFI30* mRNA expression was statistically associated with WHO grade, isocitrate dehydrogenase (IDH) status and 1p/19q codeletion (all P<0.001; *Table 3*).

As shown in *Figure 3A,3B*, there was no difference in the expression level of *IFI30* in LGG patients of different sex or age. The expression level of *IFI30* in IDH wild-type LGG patients was higher than that in IDH wild-type (*Figure 3C*). The expression level of *IFI30* in G3 glioma patients was significantly higher than that in G2 patients, and the expression level of *IFI30* in G4 glioma patients was significantly higher than that in G2 and G3 patients (*Figure 3D,3E*). The expression level of *IFI30* in 1p/19q non-codel LGG patients was higher than that of 1p/19q codel (*Figure 3F*). *IFI30* expression was higher in LGG patients who progressed after initial treatment than in LGG patients who were stable after initial treatment (*Figure 3G*).

Table 1 Univariate and multivariate Cox analyses of clinicopathologic factors and *IFI30* in LGG

Characteristic	Total (n)	Univariate analysis		Multivariate analysis	
		Hazard ratio (95% CI)	P value	Hazard ratio (95% CI)	P value
Sex (female *vs.* male)	527	0.889 (0.633–1.250)	0.499		
Race (Asian & Black or African American *vs.* White)	516	1.178 (0.549–2.529)	0.675		
IFI30 (low *vs.* high)	527	0.505 (0.356–0.714)	<0.001	0.531 (0.358–0.788)	0.002
WHO grade (G3 *vs.* G2)	466	3.059 (2.046–4.573)	<0.001	2.614 (1.725–3.963)	<0.001
Age (>40 *vs.* ≤40) years	527	2.889 (2.009–4.155)	<0.001	3.149 (2.068–4.794)	<0.001

Univariate variables with P<0.05 were chosen for the multivariate analysis. *IFI30*, interferon-gamma-inducible protein 30; LGG, low grade glioma; CI, confidence interval.

Table 2 Univariate and multivariate Cox analyses of clinicopathologic factors and *IFI30* in GBM

Characteristic	Total (n)	Univariate analysis		Multivariate analysis	
		Hazard ratio (95% CI)	P value	Hazard ratio (95% CI)	P value
Sex (male *vs.* female)	168	1.026 (0.719–1.466)	0.887		
Race (White *vs.* Asian & Black or African American)	166	0.864 (0.451–1.653)	0.658		
Age (>60 *vs.* ≤60) years	168	1.365 (0.973–1.915)	0.072		
IFI30 (high *vs.* low)	168	1.581 (1.124–2.224)	0.009	1.581 (1.124–2.224)	0.009

IFI30, interferon-gamma-inducible protein 30; GBM, glioblastoma multiforme; CI, confidence interval.

Relationship between IFI30 expression and ImmuneScore of various tumours

Because of reports indicating that the tumour immune microenvironment plays an essential role in the occurrence and development of tumours, we used the R software package to estimate the ImmuneScore of various tumour samples and observe the *IFI30* gene expression and ImmuneScore in 33 tumours. As shown in *Figure 4*, IFI30 was positively associated with an immune score in all tumours except thymoma (THYM).

Expression correlation of IFI30 and biomarkers of immune cells in LGG

As shown in *Figure 5A*, the arm-level gain copy number of *IFI30* was associated with the level of immune cell infiltration (including B cells, CD4+ T cells, macrophages, neutrophils and dendritic cells) in LGG. The expression level of *IFI30* significantly positively correlated with the level of infiltration of B cells, CD4+ T cells, CD8+ T cells, macrophages, neutrophils and dendritic cells in LGG (*Figure 5B*). Cumulative survival analysis showed high expression of *IFI30*, B cells, CD4+ T cells, CD8+ T cells, macrophages, neutrophils and dendritic cells was associated with poor prognosis in LGG (*Figure 5C*).

Relationship between IFI30 expression and immune checkpoint in glioma

Immune checkpoint molecules expressed on immune cells will inhibit the function of immune cells such that the body cannot produce an effective anti-tumour immune response and there is immune escape of tumour formation. *SIGLEC15*, *TIGIT*, *CD274*, *HAVCR2*, *PDCD1*, *CTLA4*, *LAG3* and *PDCD1LG2* are immune checkpoint-related transcripts. We extracted the expression values of these eight genes and observed the differences in their expression in glioma tissues with high and low *IFI30* expression levels. Expression of *IFI30* correlated positively with all eight immune checkpoints, showing statistical significance in LGG (*Figure 6A*) and GBM (*Figure 6B*).

Table 3 Relationship between *IFI30* expression and clinical characteristics in LGG (n=528)

Characteristic	Low expression of *IFI30*	High expression of *IFI30*	P value
N	264	264	
WHO grade, n (%)			<0.001
G2	143 (30.6)	81 (17.3)	
G3	95 (20.3)	148 (31.7)	
IDH status, n (%)			<0.001
WT	22 (4.2)	75 (14.3)	
Mut	240 (45.7)	188 (35.8)	
1p/19q codeletion, n (%)			<0.001
Codel	115 (21.8)	56 (10.6)	
Non-codel	149 (28.2)	208 (39.4)	
Sex, n (%)			0.600
Female	116 (22.0)	123 (23.3)	
Male	148 (28.0)	141 (26.7)	
Age (years), n (%)			1.000
≤40	132 (25.0)	132 (25.0)	
>40	132 (25.0)	132 (25.0)	
Race, n (%)			0.953
Asian	4 (0.8)	4 (0.8)	
Black or African American	10 (1.9)	12 (2.3)	
White	244 (46.2)	243 (46.0)	

IFI30, interferon-gamma-inducible protein 30; LGG, low grade glioma; IDH, isocitrate dehydrogenase.

Functional enrichment and analyses of IFI30 similar genes in glioma

We used the GEPIA online tool to analyse 120 genes similar to *IFI30* in gliomas (LGG and GBM). Next, these significantly similar genes were chosen for GO and KEGG analysis using DAVID (https://david.ncifcrf.gov/). As shown in *Table 4*, *IFI30* similar genes are involved in many biological processes (BPs), cellular compositions (CCs), molecular functions (MFs), and KEGG pathways, including neutrophil degranulation, neutrophil activation involved in immune responses, neutrophil activation, neutrophil-mediated immunity, regulation of innate immune response, secretory granule membrane, tertiary granules, secretory granule lumen, tertiary granule membrane, cytoplasmic vesicle lumen, IgG binding, cysteine-type endopeptidase activity, immunoglobulin binding, RAGE receptor binding, Toll-like receptor binding, *Staphylococcus aureus* infection,

osteoclast differentiation, phagosome, pertussis and leishmaniasis.

Gene sets enriched in IFI30 expression phenotype

In order to evaluate the function of *IFI30*-associated DEGs in glioma patients, GSEA analysis was applied based on TCGA-LGG cohort. Strikingly, we identified enrichment for several hallmark inflammation signatures, such as inflammatory response and interferon-alpha response (*Figure 7A,7B*). The GSEA showed *IFI30*-associated DEGs significantly enriched in hallmark tumour progression-related clusters, such as angiogenesis (NES =1.984; adjusted P=0.003; FDR =0.002; *Figure 7C*), and epithelial mesenchymal transition (NES =2.195; adjusted P=0.003; FDR =0.002; *Figure 7D*). The *IFI30*-associated DEGs were also enriched in cancer pathways, such as IL6/JAK/STAT3
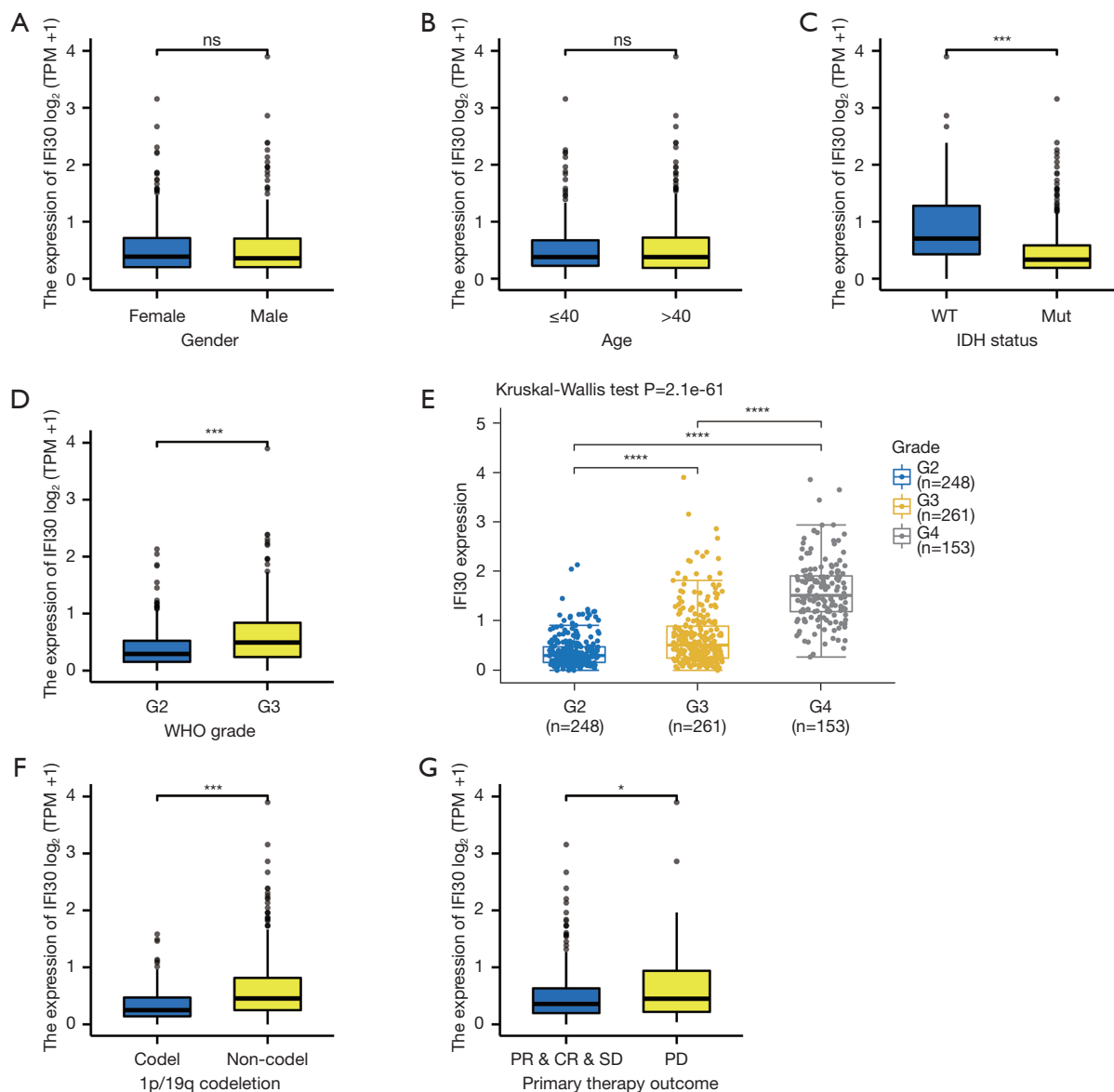
Figure 3 Distribution of the *IFI30* in different clinicopathologic features of glioma. (A-F) Sex, age, IDH status, grade, 1p/19q codeletion and *IFI30* expression level of LGG patients with initial treatment evaluation subgroup. (G) *IFI30* expression levels in gliomas of different grades (LGG and GBM). *, P value ≤0.05; ***, P value ≤0.001; ****, P value ≤0.0001; ns, P value >0.05. *IFI30*, interferon-gamma-inducible protein 30; IDH, isocitrate dehydrogenase; LGG, low grade glioma; GBM, glioblastoma multiforme; TPM, transcripts per million.

signalling (NES =2.205; adjusted P=0.003; FDR =0.002; *Figure 7E*), and TNFA signalling via NF-κB (NES =2.263; adjusted P=0.003; FDR =0.002; *Figure 7F*).

Discussion

Glioma is the most common primary tumour of the central nervous system and has a poor prognosis.

Tumour-infiltrating immune cells (TIIC) exist in the microenvironment of glioma, and their composition and proportion correlate with the prognosis of patients. Increasing evidence has demonstrated that *IFI30* is a prognostic marker and plays key roles in the progression of multiple human cancers, including glioma. However, understanding of the role of *IFI30* in the immune microenvironment of glioma remains inadequate and needs
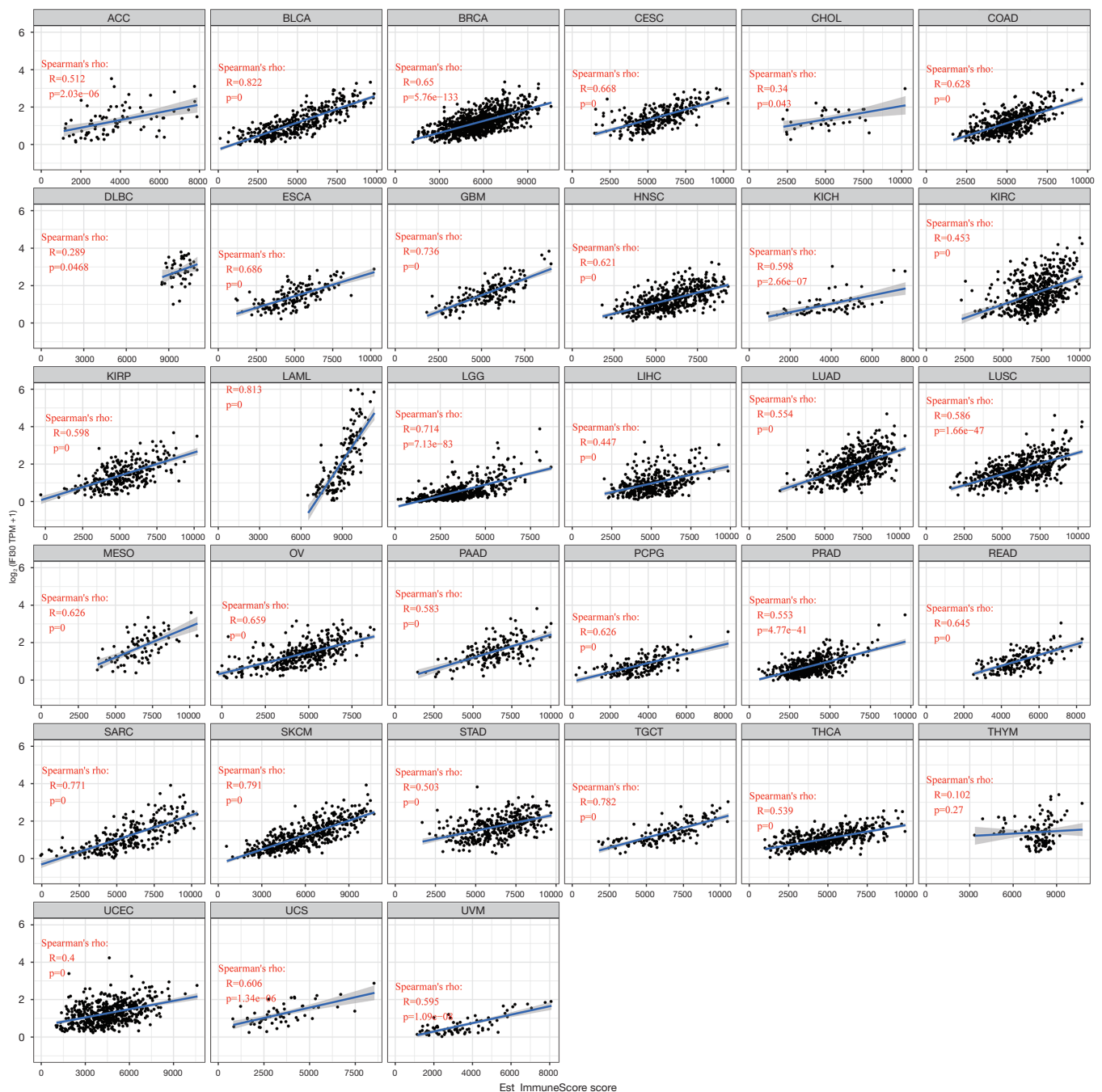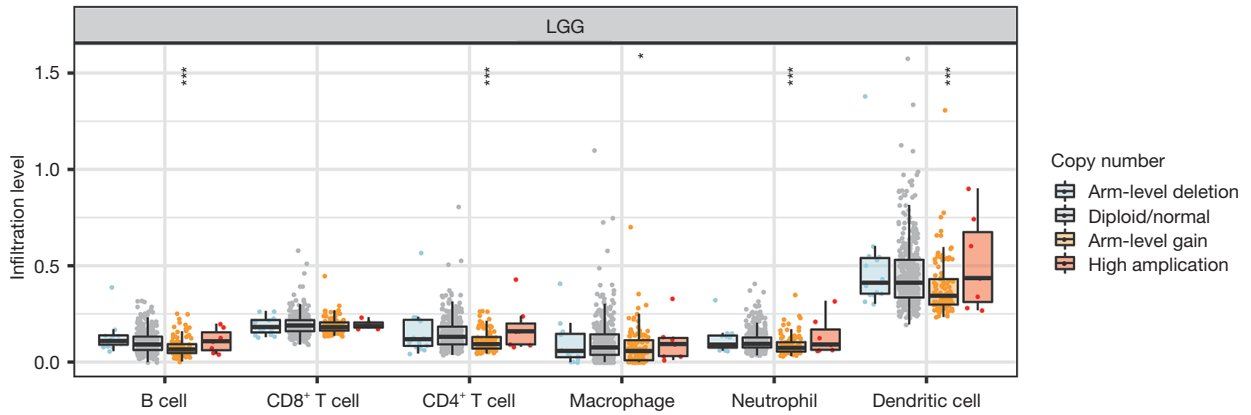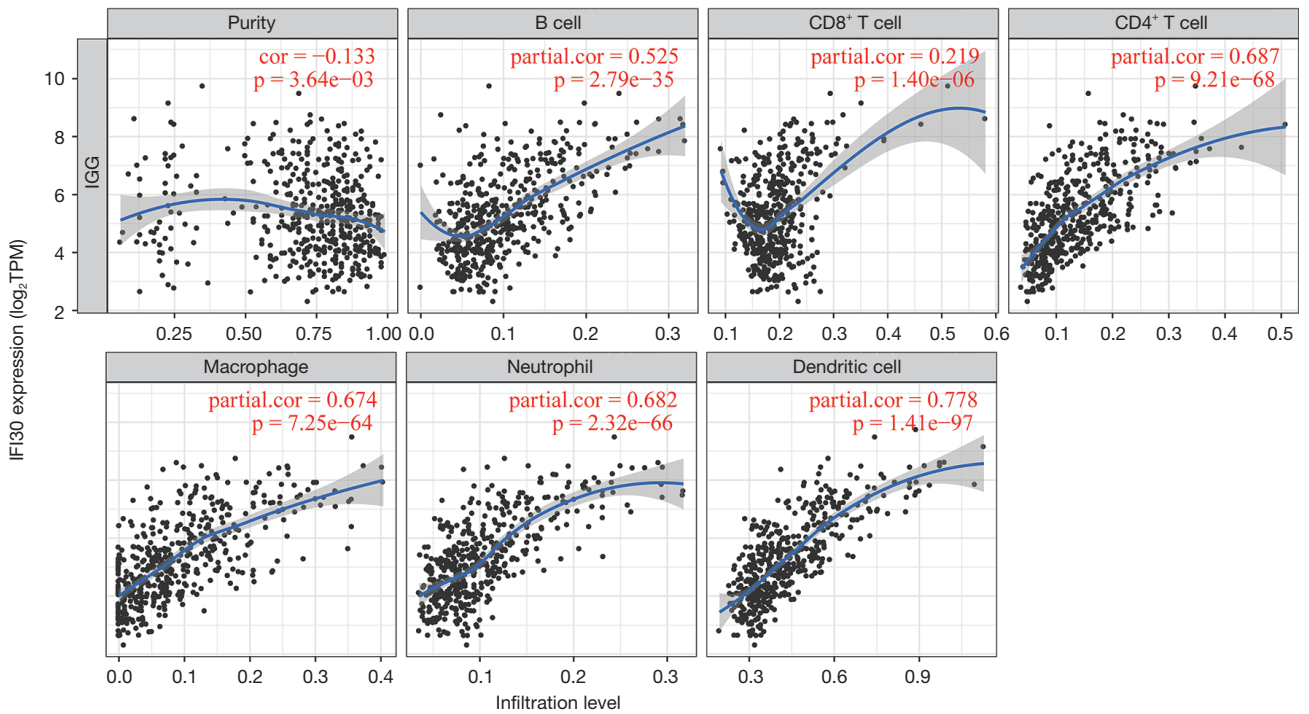
Figure 4 Relationship between *IFI30* expression and ImmuneScore of the 33 various tumour. *IFI30*, interferon-gamma-inducible protein 30; ACC, adrenocortical carcinoma; BLCA, bladder urothelial carcinoma; BRCA, breast invasive carcinoma; CESC, cervical squamous cell carcinoma and endocervical adenocarcinoma; CHOL, cholangiocarcinoma; COAD, colon adenocarcinoma; DLBC, lymphoid neoplasm diffuse large B-cell lymphoma; ESCA, esophageal carcinoma; GBM, glioblastoma multiforme; HNSC, head and neck squamous cell carcinoma; KICH, kidney chromophobe; KIRC, kidney renal clear cell carcinoma; KIRP, kidney renal papillary cell carcinoma; LAML, acute myeloid leukemia; LGG, lower grade glioma; LIHC, liver hepatocellular carcinoma; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; MESO, mesothelioma; OV, ovarian serous cystadenocarcinoma; PAAD, pancreatic adenocarcinoma; PCPG, pheochromocytoma and paraganglioma; PRAD, prostate adenocarcinoma; READ, rectum adenocarcinoma; SARC, sarcoma; SKCM, skin cutaneous melanoma; STAD, stomach adenocarcinoma; TGCT, testicular germ cell tumours; THCA, thyroid carcinoma; THYM, thymoma; UCEC, uterine corpus endometrial carcinoma; UCS, uterine carcinosarcoma; UVM, uveal melanoma.
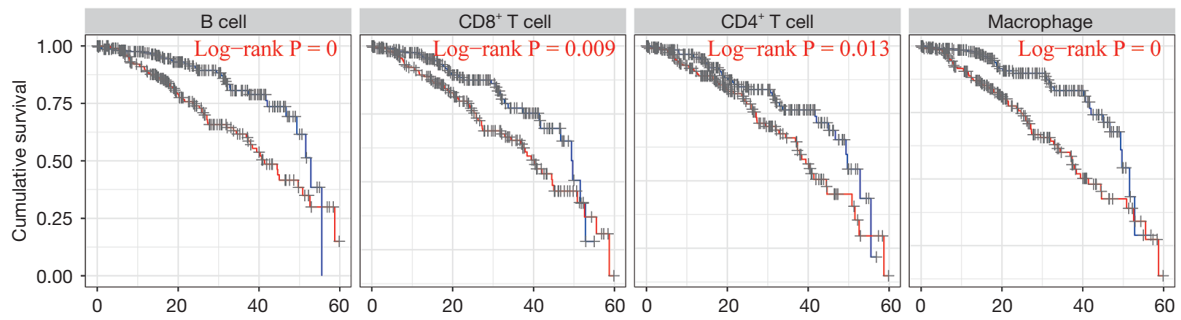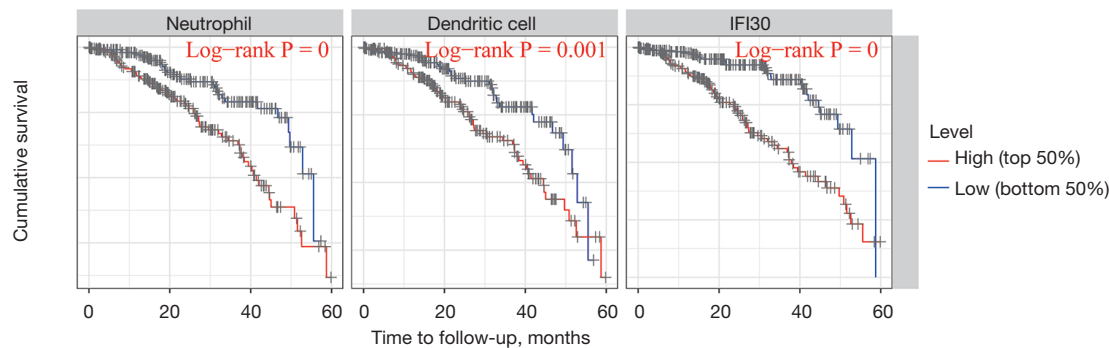
Figure 5 Relationship between immune cell infiltration and *IFI30* level in LGG. (A) Level of infiltration of various immune cells under different copy numbers of *IFI30* in LGG. (B) Correlation of *IFI30* expression level with level of infiltration of B cells, CD8+ T cells, CD4+ T cells, macrophages, neutrophils, or dendritic cells in LGG. (C) Cumulative survival analysis of *IFI30* expression and level of infiltration of B cells, CD8+ T cells, CD4+ T cells, macrophages, neutrophils, and dendritic cells in LGG. *, P<0.05; ***, P<0.001. *IFI30*, interferon-gamma-inducible protein 30; LGG, low grade glioma; TPM, transcripts per million.
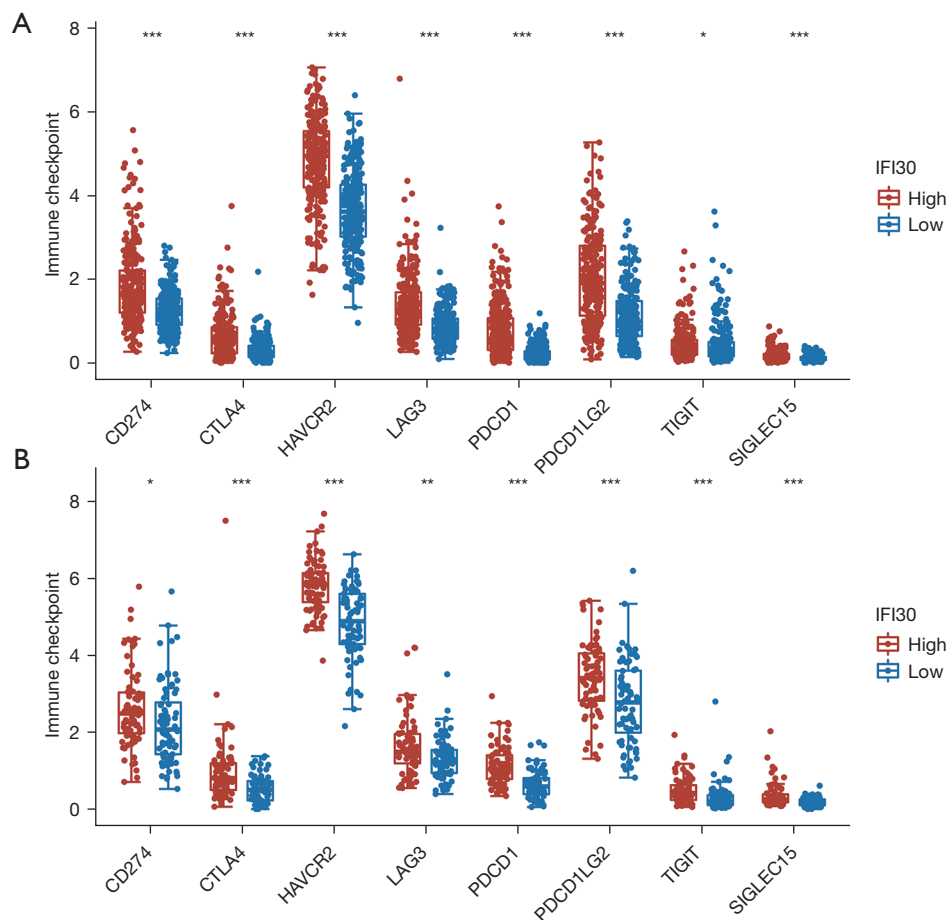
Figure 6 Relationship of immune checkpoint with *IFI30* expression in LGG. Expression of *IFI30* in (A) LGG tissues and (B) GBM tissues. The horizontal axis represents different groups of samples, and the vertical axis represents the gene expression distribution, where different colours represent different groups. The upper left corner represents the significance P value test method. *, P<0.05; **, P<0.01; ***, P<0.001. IFI30, interferon-gamma-inducible protein 30; LGG, low grade glioma; GBM, glioblastoma multiforme.

Table 4 Significantly enriched GO annotations and KEGG pathways of *IFI30* similar genes in glioma

Ontology	Description	GeneRatio	BgRatio	P adjust	P value
BP	Neutrophil degranulation	37/109	485/18,670	2.06e-28	1.51e-28
BP	Neutrophil activation involved in immune response	37/109	488/18,670	2.06e-28	1.51e-28
BP	Neutrophil activation	37/109	498/18,670	2.33e-28	1.70e-28
BP	Neutrophil-mediated immunity	37/109	499/18,670	2.33e-28	1.70e-28
BP	Regulation of innate immune response	23/109	452/18,670	5.05e-13	3.69e-13
CC	Secretory granule membrane	19/113	298/19,717	1.06e-12	7.35e-13
CC	Tertiary granule	15/113	164/19,717	2.30e-12	1.60e-12
CC	Secretory granule lumen	18/113	321/19,717	1.37e-11	9.55e-12
CC	Tertiary granule membrane	11/113	73/19,717	1.37e-11	9.55e-12
CC	Cytoplasmic vesicle lumen	18/113	338/19,717	2.20e-11	1.53e-11
MF	IgG binding	4/105	11/17,697	1.04e-04	8.94e-05
MF	Cysteine-type endopeptidase activity	7/105	116/17,697	8.10e-04	6.94e-04
MF	Immunoglobulin binding	4/105	24/17,697	0.001	9.04e-04
MF	RAGE receptor binding	3/105	11/17,697	0.002	0.002
MF	Toll-like receptor binding	3/105	12/17,697	0.002	0.002
KEGG	*Staphylococcus aureus* infection	12/72	96/8,076	4.58e-09	3.55e-09
KEGG	Osteoclast differentiation	13/72	128/8,076	4.70e-09	3.64e-09
KEGG	Phagosome	12/72	152/8,076	3.37e-07	2.61e-07
KEGG	Pertussis	9/72	76/8,076	6.41e-07	4.96e-07
KEGG	Leishmaniasis	8/72	77/8,076	9.40e-06	7.27e-06

GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; *IFI30*, interferon-gamma-inducible protein 30; BP, biological process; CC, cellular composition; MF, molecular function.

further investigation.

In this study, we used TCGA pan-cancer data to analyse the expression of *IFI30* in both tumour and normal tissues. Survival analysis for *IFI30* indicated that glioma patients with high expression of *IFI30* had poor OS, DSS and PFI. Although previous studies have reported that overexpression of *IFI30* is an adverse prognostic factor for any tumour, including glioma, previous studies only analysed OS. Considering the possibility of non-tumour death during follow-up, we also analysed the relationship between *IFI30* gene expression and DSS in glioma. Therefore, we identified *IFI30* as a marker of poor prognosis of glioma in a more systematic and comprehensive way. *In vitro* experiments showed that Gilt silencing suppresses cell proliferation, colony formation, migration, and tumour growth and induces apoptosis and cell cycle arrest in glioma.

That report, together with our analytic results, show the oncogenic role of *IFI30* in glioma.

Immune infiltrates in the TME play a crucial role in tumour development and could influence the efficacy of chemotherapy, radiotherapy, and immunotherapy and thus the prognosis of tumour patients. A comprehensive analysis of TIIC will shed light on the mechanisms of tumour immune escape and thus provide an opportunity to develop new therapeutic strategies (20). Our work showed *IFI30* was significantly positively correlated with B cells, CD8^{+} T cells, CD4^{+} T cells, macrophages, neutrophils, and dendritic cells in LGG. Cumulative survival analysis showed high expression of B cells, CD4^{+} T cells, CD8^{+} T cells, macrophages, neutrophils and dendritic cells was associated with poor prognosis in LGG. Moreover, *IFI30* was markedly positively associated with the immune score
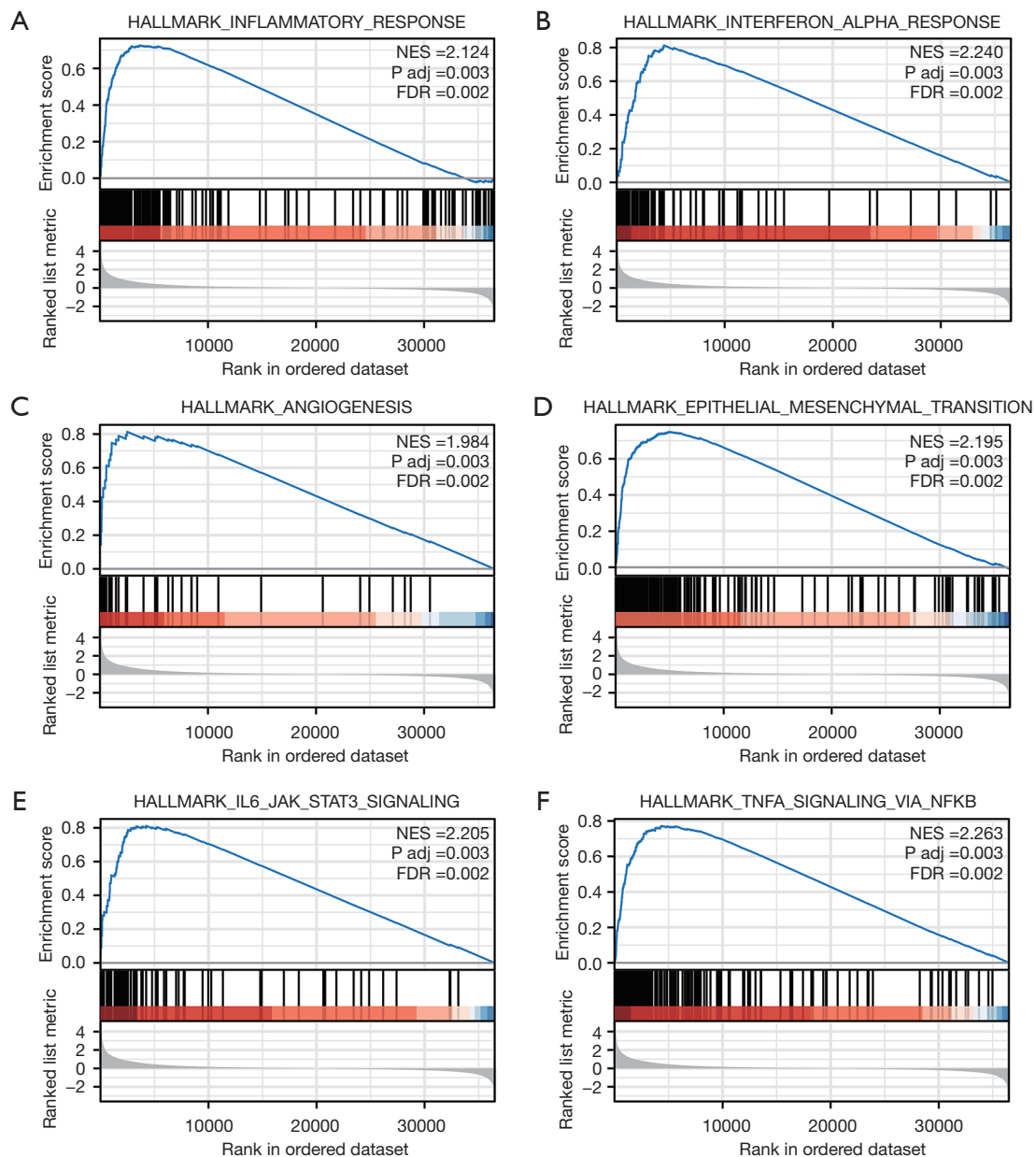
Figure 7 Enrichment plots of GSEA. (A-F) Functional annotation of DEGs in LGG patients with high- and low-*IFI30* expression. GSEA, gene set enrichment analysis; DEGs, differentially expressed genes; LGG, low grade glioma; *IFI30*, interferon-gamma-inducible protein 30; NES, normalised enrichment score; FDR, false discovery rate.

in almost all 33 tumours sampled. These findings indicated that tumour immune infiltration partially accounted for the *IFI30*-mediated oncogenic role in glioma.

Immune checkpoints are negative regulators that inhibit the proliferation and activity of T cells (21,22), and are often used by cancer cells to avoid immune surveillance (23,24). Thus, we also assessed the relationship between

IFI30 and immune checkpoints and found that high expression of *IFI30* was linked to *SIGLEC15*, *TIGIT*, *CD274*, *HAVCR2*, *PDCD1*, *CTLA4*, *LAG3* and *PDCD1LG2* in glioma. To date, this is the first report of an association between *IFI30* and immune checkpoints. Our findings indicated that avoidance of immune surveillance partially accounted for *IFI30*-mediated carcinogenesis in glioma and

targeting *IFI30* took happens the efficacy of immunotherapy in glioma.

Functional enrichment analyses showed that *IFI30* similar genes were involved in neutrophil activation, neutrophil-mediated immunity, IgG binding, cysteine-type endopeptidase activity, immunoglobulin binding, toll-like receptor binding. In addition, GSEA analyses showed that *IFI30*-associated genes were significantly enriched in tumour progression-related pathways, such as angiogenesis, epithelial mesenchymal transition, IL6/JAK/STAT3 signalling, and tumour necrosis factor-alpha signalling via NF-κB. The IL-6/JAK/STAT3 pathway is involved in the pathogenesis of many human malignancies (25). STAT3 has also been shown to regulate the self-renewal potential of glioma cells (26). NF-κB signalling regulates the expression of several molecules participating in various crucial physiological reactions, and has been demonstrated to act as a critical factor in the development or progression of various cancers, as well as glioma (27-29). NF-κB activation is also prevalent in carcinomas, in which NF-κB activation is mainly driven by inflammatory cytokines within the TME (30). These findings indicated that *IFI30* acted as a regulator of key signalling pathways in glioma.

Returning to the question posed at the beginning of this study, it is now possible to state that *IFI30* was highly expressed in multiple types of human cancer and positively correlated with unfavourable prognosis in glioma. Furthermore, *IFI30* might exert its oncogenic role through increasing tumour immune cell infiltration, immune checkpoint expression and regulation of tumour progression-related pathways. However, these results need to be validated by more basic experiments in the future.

Acknowledgments

Footnote

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