Novel interferon-gamma-related 2-gene signature predicts outcome and responsiveness to immune checkpoint blockade in glioma

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Research Article

Keywords: glioma, IFNGR1, IFNGR2, prognostic, tumor relapse, immune checkpoint blockade therapy

Posted Date: October 11th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-936690/v1

Abstract

Background

Glioma represents the most common and aggressive brain malignancy. Interferon-gamma (IFNG)-related gene signatures have been shown of clinical significance in multiple cancers, while its role in glioma remains less addressed.

Methods

A total of 2933 glioma samples ranging from WHO grade II to IV with corresponding demographics were included. Multiple bioinformatics and machine learning algorithms were employed to establish an IFNG-related biomarker to classify survival differences and to elucidate the potential of glioma to respond to immune checkpoint blockade (ICB) therapy.

Results

The ssGSEA score of IFNGR1 and IFNGR2 was defined as the IFNGR score, which well-characterized the IFNG response in the glioma microenvironment. Increased IFNGR score was associated with clinicopathological parameters characterizing glioma malignancy, including WHO grade IV, mesenchymal subtype, and molecular biomarkers, including IDH1 wildtype, 1p19q non-codeletion, and unmethylated MGMT promoter. Consequently, K-M and Cox regression analysis found that the IFNGR score was a robust prognostic biomarker that was associated with tumor relapse for patients in certain subgroups. Moreover, the IFNGR-based group had the potential to predict ICB responsiveness, as effectively as previously validated IFNG-related gene signatures.

Conclusion

Together, we have developed a concise biomarker of clinical significance that may improve the current diagnosis and treatment of glioma.

1. Introduction

Glioma is the most common and lethal primary brain malignancy worldwide, accounting for 31.1% of primary brain tumors in people aged 20-59 years. The overall survival of gliomas varies widely, from 78.1 months for low-grade glioma (WHO grade II) to 14.4 months for glioblastoma (GBM) (WHO grade IV), with 5-year survival rates ranging from 67% for low-grade glioma to 9% for GBM. Despite currently standard treatment, malignant progression and tumor relapse inevitably occur and lead to tolerance to conventional treatments[1–3]. Therefore, novel biomarkers are in urgent need to assist in the accurate diagnosis and improve the currently limited treatments of glioma.

Accumulative evidence has demonstrated the paramount role of the IFNG response in tumors. On the one hand, IFNG is involved in the immune response, promoting antigen presentation and effector T cell activity, and catalyzing immune-mediated tumor clearance. On the other hand, IFNG participants in immune editing and upregulates immune checkpoints to help tumor cells evade immune attacks[4, 5]. Notably, substantial evidence has suggested that gene signatures associated with the IFNG response reliably predict prognosis and responsiveness to immune checkpoint blockade (ICB) therapies of tumor sufferers[6, 7]. As the portal of the IFNG signaling pathway, IFNGR1 and IFNGR2 are of biological and pathophysiological importance. Biologically active IFNGR-IFNG complex is composed of two IFNGR1 and two IFNGR2, with one of each receptor subunit binding to each end of the IFNG homolog, thereby activating the downstream JAK, and STAT families and enabling the biological effects of IFNG[4, 8]. The dysregulation of IFNGR1 may increase cellular sensitivity to IFNG and is therefore associated with excessive inflammatory response[9, 10]. Likewise, IFNGR2 deficient monocytes induce differentiation of CD4+ T cells to Th17 cells, which are associated with a variety of autoimmune diseases[11, 12]. However, the role of IFNGR1 and IFNGR2 in glioma remains to be systematically elucidated.

To fill this gap, we integrated IFNGR1 and IFNGR2 as a 2-gene signature and developed the IFNGR score to probe their clinical significance. We thoroughly explored the association between the IFNGR score and glioma clinicopathological and molecular features, as well as the overall survival (OS) and progression-free interval (PFI) of patients. In addition, we elucidated the potential of the IFNGR score to predict ICB responsiveness. Collectively, our results provide an efficient classifier for determine glioma prognosis and may contribute to optimizing conventional treatment of the desperate disease.

2. Materials And Methods

2.1 Data collection and pre-procession

mRNA-seq data sets of a total of 1693 glioma samples from WHO grade II to grade IV and corresponding demographics were retrieved. Of these, 675 glioma samples derived from the Cancer Genomic Atlas (TCGA) project were retrieved from the USCS Xena (https://xenabrowser.net/datapages/) and used as the exploration data set. The remaining samples (RNA-seq 693, RNA-seq 325, Rembrandt, and microarray CGGA301) were from the Chinese Glioma Genome Atlas (CGGA, http://www.cgga.org.cn/index.jsp) and GlioVis (http://gliovis.bioinfo.cnio.es/) as validation data sets. Samples with no follow-up information or follow-up time of less than 1 day were excluded. Genes with 0 (not detected) expression in more than half of the samples were removed. For mRNA-seq data sets, the gene expression profile was TPM normalized and log-transformed for downstream analysis.

2.2 Development and validation of the gene signature

The IFNGR score was defined as the ssGSEA score of IFNGR1 and IFNGR2 based on the ssGSEA algorithm[13]. Samples were then split into the IFNGR score-high and -low groups by the median value. To demonstrate the ability of the IFNGR score in characterizing the IFNG response in gliomas, we recruited the gene sets developed in previous studies on behalf of the IFNG response, including IFNG.1 (GBP5,

ICAM1, CAMK2D, IRF1, SOCS3, CD44, and CCL2), IFNG.2 (IDO1, CXCL10, CXCL9, HLA-DRA, STAT1, and IFNG), and IFNG.ex (CD3D, IDO1, CIITA, CD3E, CCL5, GZMK, CD2, HLA-DRA, CXCL13, IL2RG, NKG7, HLA-E, CXCR6, LAG3, TAGAP, CXCL10, STAT1, and GZMB). Samples were also split into the IFNG.1 score-high and -low groups, IFNG.2 score-high and -low groups, and IFNG.ex score-high and -low groups following the methods described in the articles[6, 7]. Gene set enrichment analysis (GSEA, v4.1.0) was conducted to calculate the normalized enrichment score (NES) of the IFNG signaling pathway, and biological processes relating to IFNG production in score-high groups[14]. Differentially expressed genes (DEGs) between the IFNGR score-high and -low groups were calculated using the R packages 'limma' and 'edgeR'. Gene Ontology (GO) analysis was further employed to exhibit the function of these DEGs based on the webtool DAVID (https://david.ncifcrf.gov/)[15–18]. Tumor purity was estimated using the ABSOLUTE algorithm[19]. The Kaplan-Meier (K-M) plots were used to exhibit the survival differences and univariate Cox regression analysis was conducted to assess the independent prognostic significance based on the R packages 'survival' and 'survminer'. The receiver operating curves (ROC) and corresponding area under the curve (AUC) were employed to evaluate the time-dependent predictive power. Moreover, the TIDE algorithm was employed to predict the responsiveness of samples to ICB therapies with default parameters, and the predicted results were further tested by a machine learning algorithm, SubMap[20, 21].

2.3 Statistics

All the statistics were performed in R (version 4.0.2) and GSEA software. K-M analysis and log-rank test were used to assess survival differences. ROC curves and corresponding AUCs were used to assess the time-dependent predictive power. Univariate Cox regression analysis was employed to describe the independent prognostic value. $p < 0.05$ was considered statistically significant. For GO and GSEA analysis, a false discovery rate (FDR)-q value < 0.1 was considered significant. For the predicted results of ICB responsiveness, Bonferroni corrected p-value < 0.1 was considered significant. We marked * for $p < 0.05$, ** for $p < 0.01$, *** for $p < 0.001$.

3. Results

3.1 IFNGR1 and IFNGR2 as a novel and concise IFNG response gene signature for glioma

First of all, the ability of IFNGR1 and IFNGR2 to characterize the IFNG response was explored. We calculated the NES scores of the IFNG signaling pathway (Hallmark IFNG response, Reactome IFNG signaling, and WP type II IFNG signaling) as well as IFNG-related BP (GOBP IFNG signaling, IFNG production, and positive regulation of IFNG production) in the IFNGR score-high group based on GSEA algorithm and compared the results of the IFNGR-based group with the previously constructed IFNG-related gene signatures-based groups. As a result, the IFNGR score-high group had the highest average NES score in IFNG-related BP (mean NES = 3.26), while the lowest NES score (mean NES = 1.93) in the IFNG signaling pathway (Fig. 1A). Instead, IFNG.2-based group characterized the IFNG signaling pathway

well (mean NES = 3.44), but scored lower on IFNG-related biological processes (mean NES = 2.16), and the IFNG.1-based group was balanced in characterizing the IFNG signaling pathway (mean NES = 2.97) and related biological processes (mean NES = 3.00). Further, DEGs (IFNGR score-high vs. score-low) were calculated, and GO analysis found that interferon-gamma-mediated signaling pathway (BP) and interferon-gamma signaling (Reactome) were top enriched terms in the IFNGR score-high group (Fig. 1B, C). Notably, inflammatory response, immune response, leukocyte migration (BP), and signaling pathways regulating the activity of immune cells including integrin cell surface interaction (Reactome), immunoregulatory interaction between a lymphoid and a non-lymphoid cell (Reactome), B lymphocyte, and T helper cell surface molecule (Biocarta), and CTL-mediate immune response against target cell (Biocarta) were also highly enriched terms of the IFNGR score-high group, indicating a pro-inflammatory microenvironment which corroborates the function of IFNG. Together, these results suggested that an increased IFNGR score was indicative of an elevated IFNG response.

3.2 The IFNGR score was indicative of a malignant phenotype glioma

The association between the IFNGR score and prevalent clinicopathological features of glioma was demonstrated. In terms of histology, the IFNGR score was lowest in oligodendroglioma and sequentially increased in oligoastrocytoma, astrocytoma, and glioblastoma (Fig. 2A). Likewise, the IFNGR score increased with the WHO tumor grade, with WHO grade IV having the highest IFNGR scores (Fig. 2B). Among the transcriptome subtype of glioma, the IFNGR score was lower in the neural and proneural subtypes which have a relatively improved prognosis, while higher in the classical and mesenchymal subtypes, and highest in the mesenchymal type that has the worst prognosis (Fig. 2C). Moreover, the association of the IFNGR score with molecular pathology biomarkers with clinical significance was interrogated. Samples with IDH1 wildtype tended to have increased IFNGR scores, consistent with the association of the IFNGR score with subgroups of poorer prognosis (Fig. 2D). Patients with 1p19q co-deletion had decreased IFNGR score, possibly because 1p19q co-deletion was associated with oligodendroglial histology of glioma, which had the lowest IFNGR score among the 4 histology-based subtypes (Fig. 2E). Interestingly, samples with unmethylated MGMT promoter had higher IFNGR scores, which, considering that high IFNGR scores were enriched in glioblastoma, may suggest that this group of patients may not benefit from the treatment with alkylating agents (Fig. 2F). In addition, samples with increased IFNGR scores had decreased tumor purity, which may be associated with the putative pro-inflammatory microenvironment of the IFNGR score-high group (Fig. 2G). Together, these results demonstrated a relevance between increased IFNGR score and poor prognosis of gliomas.

3.3 An increased IFNGR score was indicative of poor prognosis and short-term glioma relapse

Next, we explored the clinical significance of the IFNGR score. In the TCGA cohort, an increased IFNGR score strongly suggested a poor prognosis ($p < 0.0001$, median survival of 722 days in the IFNGR score-

high group), along with a significantly decreased progression-free interval (PFI) ($p < 0.0001$, median PFI of 402 days in the IFNGR score-high group) (Fig. 3A). Similar results were yielded in the CGGA325 and CGGA693 cohorts ($p < 0.0001$, the median survival of 423 and 640 days in the IFNGR score-high group, respectively). Besides, univariate Cox regression analysis showed that the IFNGR-based group had independent risk prognostic significance, with an HR of 2.95 (with the IFNGR score-low group being the reference), suggesting a nearly 3-fold increase in mortality of patients in the IFNGR score-high (Fig. 3B). Notably, the IFNGR-based group held up as an independent risk prognostic factor in multiple datasets with HRs ranging from 2.95 (TCGA) to 1.24 (CGGA301) (Fig. 3C). Further, time-dependent ROC analysis exhibited 1- to 5-year AUC values of 76%, 78%, 80%, 80%, and 81% for the IFNGR-based group, second to age, WHO grade, and IDH mutation-based groups, and superior to MGMT promoter (Fig. 3D). Moreover, given the significantly improved PFI in the IFNGR score-low group, the association between the IFNGR score and tumor relapse was further explored. Samples were divided into short-term relapse group (PFI < 6 months, n = 177) and delayed relapse group (PFI > 12 months, n = 389). For patients who suffered from WHO grade III tumor, the IFNGR score was significantly higher in the early relapse group ($p < 0.001$) (Fig. 3E). Similarly, for patients in NE and PN subtypes, increased IFNGR score was indicative of a short-term tumor recurrence ($p < 0.05$) (Fig. 3F). In conclusion, the IFNGR-based group was a robust prognostic biomarker for glioma patients.

3.4 IFNGR-based group predicts the ICB responsiveness of glioma

Previous studies have linked IFNG-related gene signatures to tumor responsiveness to ICB therapy in melanoma and small cell lung cancer[7]. We analyzed the expression profiles based on machine learning algorithms and predicted the responsiveness of samples to ICB, and compared the results of the IFNGR-based group with previously validated IFNG-related gene signatures-based groups. Based on the TCGA cohort, the IFNGR score-high group tended to respond to anti-PD-1 therapy (Bonferroni corrected p-value = 0.016), similar to the previously established IFNG-related gene signatures which have been validated by experiments (Fig. 4A). Similar results were yielded based on the CGGA325 cohort, with the IFNGR score-high group more likely to respond to the anti-PD1 therapy (Bonferroni corrected p-value = 0.001) (Fig. 4B). These results demonstrated the potential of ICB responsiveness of the IFNGR score-high group.

4. Discussion

Gliomas are a group of tumors with widely varying prognoses, and their accurate diagnosis and effective treatment remain a challenge[1, 3]. Here, we have developed the IFNGR score to characterize the prognosis of gliomas based on the expression of IFNGR1 and IFNGR2, two receptors necessary for IFNG signaling. As expected, the IFNGR score well represents the IFNG-related biological processes and extensively correlates with clinicopathological parameters that indicate a poor prognosis of glioma. Besides, we found that the IFNGR score was a robust biomarker of OS and PFI, and had the potential to

screen ICB responders. Together, our work provides valuable information for the diagnosis, prognosis, and classification of gliomas and may help to optimize immunotherapy.

To our knowledge, we have for the first time explored the role of IFNGR1 and IFNGR2 in glioma. IFNGR1 and IFNGR2 comprise the heterodimeric receptor for IFNG and their dysfunction is involved in various pathologies[4]. One clinical study contains 213 patients and 733 controls has shown a correlation between IFNGR1 -56C/T polymorphism and early onset of gastric carcinoma[9]. A possible explanation is that individuals carrying the IFNGR1 -56*T allele produce more IFNGR1, which renders cells more sensitive to IFNG, resulting in a more pro-inflammatory microenvironment upon H pylori infection. Besides, loss of tumor-suppressive transcription factor Elf5 promotes the growth and metastasis of triple-negative breast cancer through stabilizing IFNGR1[10]. On the other hand, the IFNGR2 is involved in the regulation of Th1 and Th17 homeostasis, and the lack of which is associated with mycobacterial disease[11, 12]. In line with these findings, we found that the IFNGR score was positively correlated with the malignant biomarkers of gliomas in terms of histology, WHO grade, and transcriptome subtype. Interestingly, GO analysis corroborates the proinflammatory microenvironment of the IFNGR score-high group, suggesting that the upregulation of IFNGR1 and IFNGR2 was associated with enhanced inflammatory and immune response in gliomas. Nevertheless, an active immune response does not necessarily benefit glioma patients. For instance, the immune cytolytic activity measuring the function of CD8+ T cell, and the IFNG response genes indicating activation of adaptive immune responses were negatively correlated with the overall survival of glioma patients[6, 22], which is in line with our findings. Therefore, manipulation of the immune response for long-term control of glioma growth requires a deeper understanding of the composition of the immune response and the specific tumor microenvironment of gliomas.

IFNG is a double-edged sword immune-modulator and its role in glioma remains controversial. On the one hand, IFNG involves in the differentiation of Th1 cells, maintains the Th1-type immune response, as well as enhances the cytotoxicity of T lymphocytes, making the IFNG gene signatures effective biomarkers of an activated anti-tumor immune response[7, 23–25]. Nevertheless, studies revealed that IFNG promotes tumor immune evasion by upregulating PD-L1 in a JAK-STAT pathway-dependent manner, implying that tumors characterized by increased IFNG response may be sensitive to immune checkpoint blockade therapy[5–7, 26]. Given that IFNGR1 and IFNGR2 are receptors indispensable in the IFNG-mediated activation of the JAK and STAT families[4, 8], it was plausible that the 2-gene signature characterizes the upregulation of the IFNG signaling pathway well, and that patients in the IFNGR score-high group were potential responders to ICB therapy. However, multiple mechanisms are involved in the formation of glioma immunosuppressive microenvironment. For example, TGF-beta is involved in the inhibition of antigen presentation, the function of antigen-presenting cells, and the activation of T cells[27], COX-2 and PGE2 participate in tumor growth and angiogenesis[28], as well as CCL2, recruits immunosuppressive cells such as regulatory T cells and MDSC[29, 30]. Therefore, it is necessary to take into account the role of other immunosuppressive mechanisms while applying ICB in the treatment of gliomas.

5. Conclusions

In conclusion, we have constructed a clinical valuable biomarker for gliomas based on large-size and multi-cohort samples. These findings were based on general bioinformatics analysis and reliable statistical methodologies, but further basic and clinical studies are still needed to verify their validity as well as molecular mechanisms.

6. List Of Abbreviations

IFNG: interferon-gamma; ICB: immune checkpoint blockade therapy; GSEA: gene set enrichment analysis; GBM: glioblastoma; OS: overall survival; PFI: progression free interval; NES: normalized enrichment score; DEG: differentially expressed gene; GO: gene ontology; ROC: receiver operating curve; AUC: area under the curve; FDR: false discovery rate; BP: biological process; CC: cellular component; MF: molecular function; HR: hazard ratio.

7. Declarations

7.1 Ethics approval and consent to participate

Not applicable.

7.2 Consent for publication

Not applicable.

7.3 Availability of data and materials

675 glioma samples from the TCGA project and corresponding clinical information were retrieved from the UCSC Xena database (https://xena.ucsc.edu/). Data sets including mRNA-seq 693, mRNA-seq 325, and microarray 301 were retrieved from the CGGA data base (http://www.cgga.org.cn/). The Rembrandt data set was retrieved from the GlioVis database (http://gliovis.bioinfo.cnio.es/).

7.4 Competing interests

The authors declare no conflict of interest.

7.5 Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

7.6 Authors' contributions

Yongzhe Li and Hang Ji conceived and designed the study. Hang Ji provided analytical technical support and drafted the manuscript. Yongzhe Li participated in the production of charts and pictures. Yongzhe Li and Hang Ji revised the manuscript. Yongzhe li supervised the study. All authors have read and approved the final manuscript.

7.7 Acknowledgements

We thank the TCGA, CGGA, GlioVis, and other databases for their contributions to the progress of scientific research, the developers of the R package for their efforts in data analysis.

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8. References

1. Jiang T, Mao Y, Ma W, Mao Q, You Y, Yang X, et al. CGCG clinical practice guidelines for the management of adult diffuse gliomas. Cancer Lett. 2016;375(2):263–73.
2. Siegelin MD, Schneider E, Westhoff MA, Wirtz CR, Karpel-Massler G. Current state and future perspective of drug repurposing in malignant glioma. Semin Cancer Biol. 2019.
3. Tan AC, Ashley DM, Lopez GY, Malinzak M, Friedman HS, Khasraw M. Management of glioblastoma: State of the art and future directions. CA Cancer J Clin. 2020;70(4):299–312.
4. Alspach E, Lussier DM, Schreiber RD. Interferon gamma and Its Important Roles in Promoting and Inhibiting Spontaneous and Therapeutic Cancer Immunity. Cold Spring Harb Perspect Biol. 2019;11(3).
5. Zhao T, Li Y, Zhang J, Zhang B. PD–L1 expression increased by IFN–γ via JAK2–STAT1 signaling and predicts a poor survival in colorectal cancer. Oncology letters. 2020;20:1127–34.
6. Qian J, Wang C, Wang B, Yang J, Wang Y, Luo F, et al. The IFN-γ/PD-L1 axis between T cells and tumor microenvironment: hints for glioma anti-PD-1/PD-L1 therapy. Journal of Neuroinflammation 2018;15:290.
7. Ayers M, Lunceford J, Nebozhyn M, Murphy E, Loboda A, Kaufman D, et al. IFN-γ–related mRNA profile predicts clinical response to PD-1 blockade. The Journal of clinical investigation. 2017;127:2930–40.
8. Castro F, Cardoso A, Gonçalves R, Serre K, Oliveira M. Interferon-Gamma at the Crossroads of Tumor Immune Surveillance or Evasion. Frontiers in immunology. 2018;9:847.
9. Canedo P, Corso G, Pereira F, Lunet N, Suriano G, Figueiredo C, et al. The interferon gamma receptor 1 (IFNGR1) 56C/T gene polymorphism is associated with increased risk of early gastric carcinoma. Gut. 2008;57:1504–8.

10. Singh S, Kumar S, Srivastava R, Nandi A, Thacker G, Murali H, et al. Loss of ELF5–FBXW7 stabilizes IFNGR1 to promote the growth and metastasis of triple-negative breast cancer through interferon-γ signalling. Nature cell biology. 2020;22:591–602.
11. Holzer U, Reinhardt K, Lang P, Handgretinger R, Fischer N. Influence of a mutation in IFN-c receptor 2 (IFNGR2) in human cells on the generation of Th17 cells in memory T cells. Human immunology. 2013;74:693–700.
12. Kong X, Vogt G, Itan Y, Macura-Biegun A, Szaflarska A, Kowalczyk D, et al. Haploinsufficiency at the human IFNGR2 locus contributes to mycobacterial disease. Human molecular genetics. 2013;22:769–81.
13. Hanzelmann S, Castelo R, Guinney J. GSVA: gene set variation analysis for microarray and RNA-seq data. BMC Bioinformatics. 2013;14:7.
14. Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, et al. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. Proc Natl Acad Sci U S A. 2005;102(43):15545–50.
15. Ritchie ME, Phipson B, Wu D, Hu Y, Law CW, Shi W, et al. limma powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic Acids Res. 2015;43(7):e47.
16. Robinson MD, McCarthy DJ, Smyth GK. edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics. 2010;26(1):139–40.
17. Huang da W, Sherman BT, Lempicki RA. Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists. Nucleic Acids Res. 2009;37(1):1–13.
18. Huang dW, Sherman B, Lempicki R. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nature protocols. 2009;4:44–57.
19. Carter SL, Cibulskis K, Helman E, McKenna A, Shen H, Zack T, et al. Absolute quantification of somatic DNA alterations in human cancer. Nat Biotechnol. 2012;30(5):413–21.
20. Jiang P, Gu S, Pan D, Fu J, Sahu A, Hu X, et al. Signatures of T cell dysfunction and exclusion predict cancer immunotherapy response. Nat Med. 2018;24(10):1550–8.
21. Hoshida Y, Brunet JP, Tamayo P, Golub TR, Mesirov JP. Subclass mapping: identifying common subtypes in independent disease data sets. PLoS One. 2007;2(11):e1195.
22. Wang Z, Wang Z, Li G, Wang Q, Bao Z, Zhang C, et al. Immune Cytolytic Activity Is Associated With Genetic and Clinical Properties of Glioma. Frontiers in immunology. 2019;10:1756.
23. Bradley LM, Dalton DK, Croft M. A direct role for IFN-gamma in regulation of Th1 cell development. J Immunol. 1996;157(4):1350–8.
24. Shankaran V, Ikeda H, Bruce AT, White JM, Swanson PE, Old LJ, et al. IFNgamma and lymphocytes prevent primary tumour development and shape tumour immunogenicity. Nature. 2001;410(6832):1107–11.
25. Shao S, Risch E, Burner D, Lu L, Minev B, Ma W. IFNgamma enhances cytotoxic efficiency of the cytotoxic T lymphocytes against human glioma cells. Int Immunopharmacol. 2017;47:159–65.

26. Moon JW, Kong SK, Kim BS, Kim HJ, Lim H, Noh K, et al. IFNgamma induces PD-L1 overexpression by JAK2/STAT1/IRF-1 signaling in EBV-positive gastric carcinoma. Sci Rep. 2017;7(1):17810.
27. Gorelik L, Flavell RA. Transforming growth factor-beta in T-cell biology. Nat Rev Immunol. 2002;2(1):46–53.
28. Onguru O, Gamsizkan M, Ulutin C, Gunhan O. Cyclooxygenase-2 (Cox-2) expression and angiogenesis in glioblastoma. Neuropathology. 2008;28(1):29–34.
29. Ahn BJ, Pollack IF, Okada H. Immune-checkpoint blockade and active immunotherapy for glioma. Cancers (Basel). 2013;5(4):1379–412.
30. Huang B, Lei Z, Zhao J, Gong W, Liu J, Chen Z, et al. CCL2/CCR2 pathway mediates recruitment of myeloid suppressor cells to cancers. Cancer Lett. 2007;252(1):86–92.

Figures

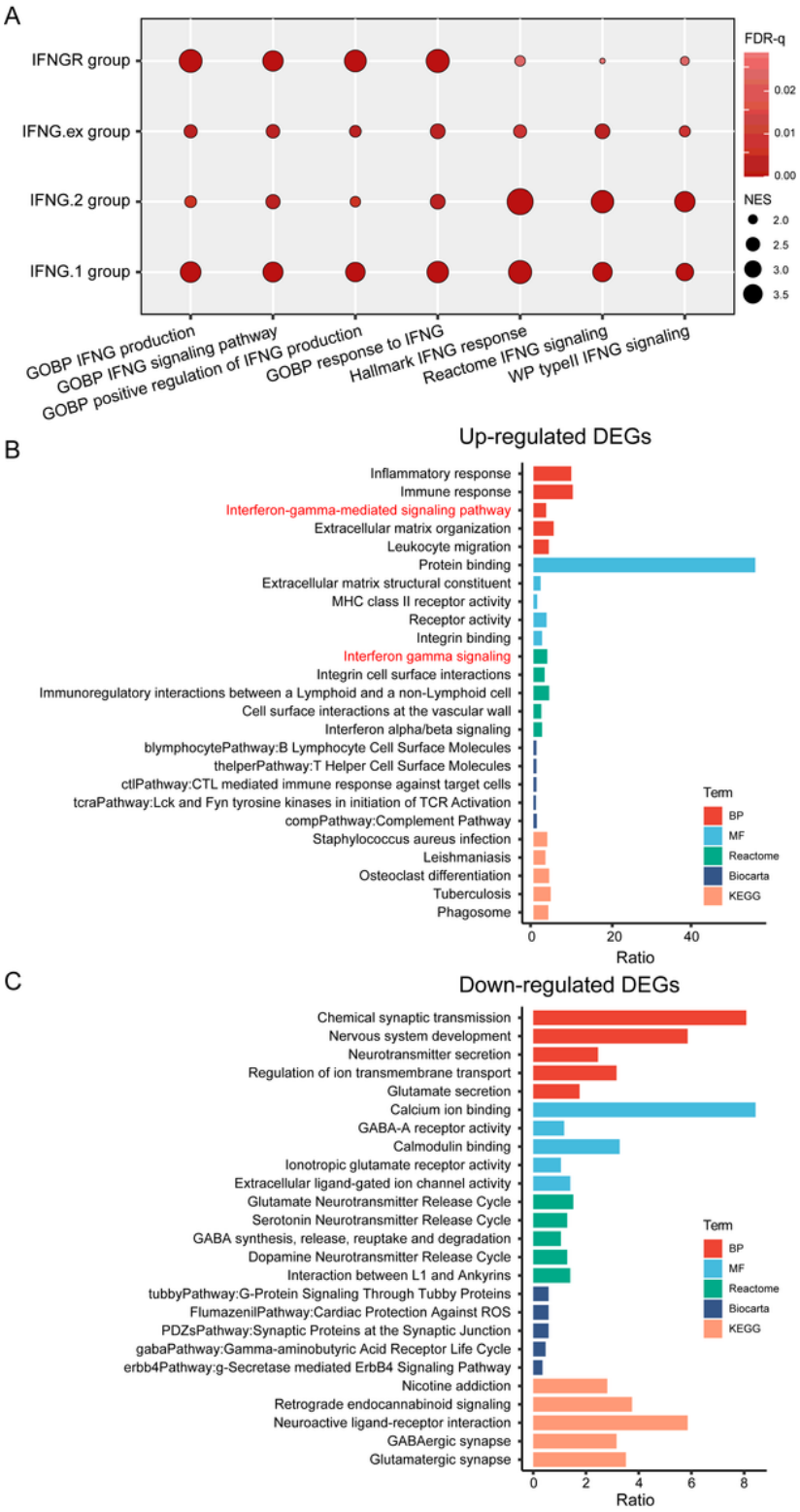

Figure 1

IFNGR1 and IFNGR2 based gene signature in characterizing the IFNG response in glioma. (A) GSEA analysis of the IFNGR, IFNG.1, IFNG.2, and IFNG.ex-based group in characterizing the IFNG signaling pathway and related biological processes in glioma. The size of the bubble was proportional to the NES score and color was proportional to the FDR-q value of each term. GO analysis of the (B) up-regulated

and (C) down-regulated DEGs between the IFNGR score-high and -low groups based on the TCGA cohort. The top 5 terms in each category were exhibited.

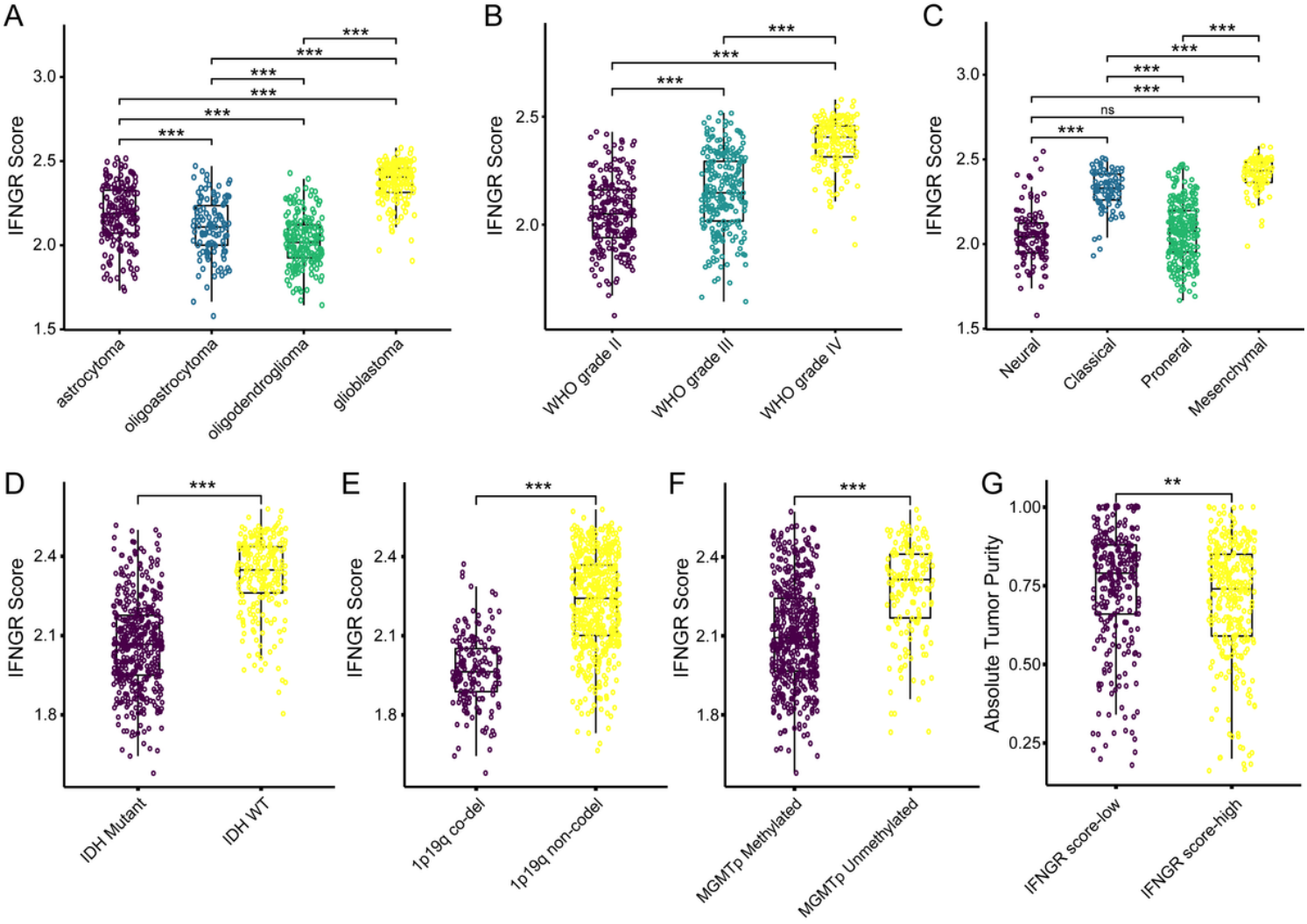

Figure 2

IFNGR1 and IFNGR2 based gene signature in characterizing the IFNG response in glioma. (A) GSEA analysis of the IFNGR, IFNG.1, IFNG.2, and IFNG.ex-based group in characterizing the IFNG signaling pathway and related biological processes in glioma. The size of the bubble was proportional to the NES score and color was proportional to the FDR-q value of each term. GO analysis of the (B) up-regulated and (C) down-regulated DEGs between the IFNGR score-high and -low groups based on the TCGA cohort. The top 5 terms in each category were exhibited.

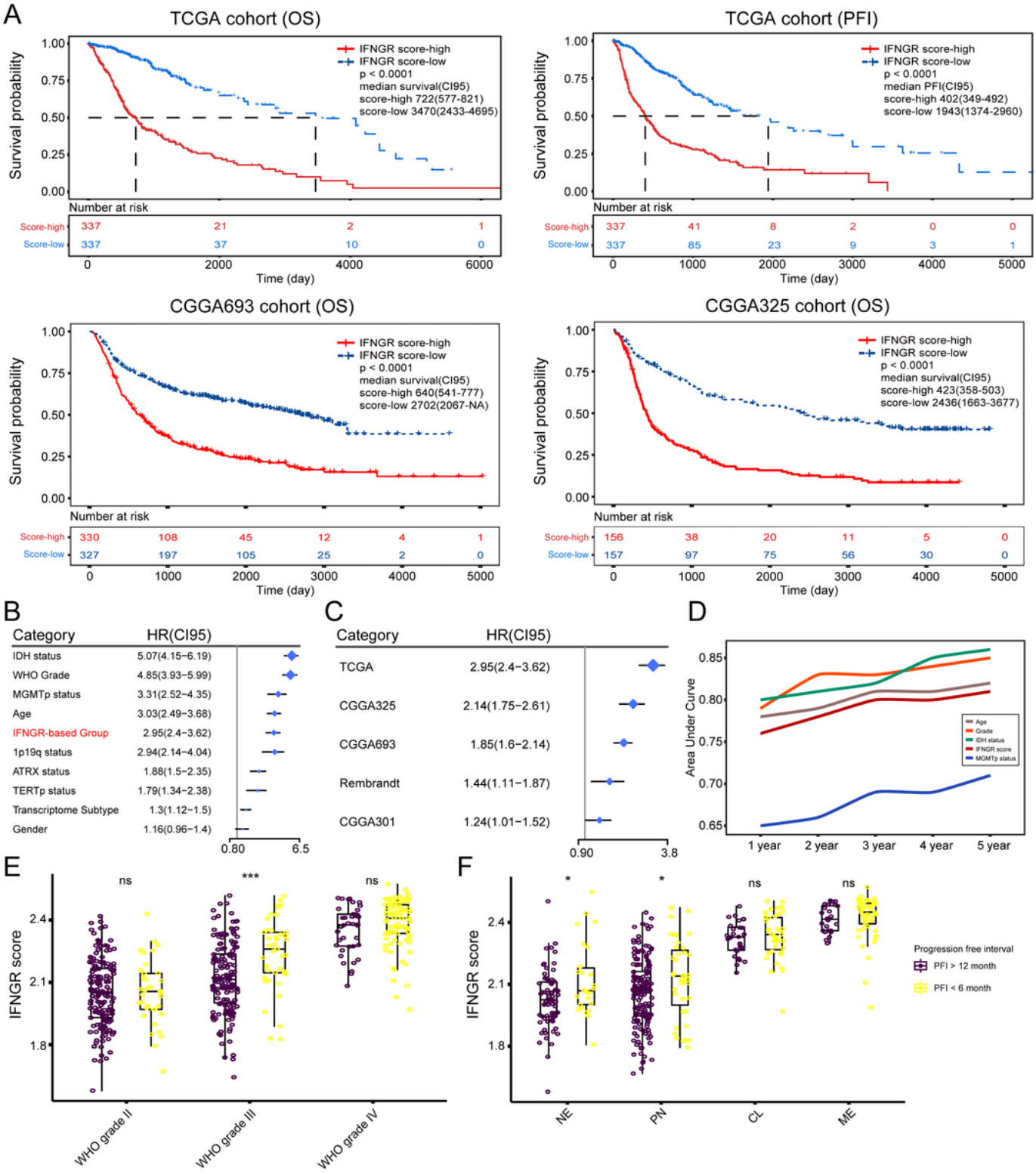

Figure 3

Prognostic significance of the IFNGR-based group. (A) K-M analysis of the IFNGR-based group in terms of OS and PFI based on TCGA, CGGA693, and CGGA325 cohorts. (B) Univariate Cox regression of the independent prognostic significance of the IFNGR-based group and other clinicopathological parameters based on the TCGA cohort. (C) Univariate Cox regression analysis of the independent prognostic significance of the IFNGR-based group based on TCGA, CGGA693, and CGGA325 group, as well as two

external validation data sets (Rembrandt, n = 476, and CGGA301, n = 301). (D) Time-dependent ROC and corresponding AUCs of age, WHO tumor grade, IDH mutation status, IFNGR score, and MGMT promoter methylation status. The association between the IFNGR score and tumor early (PFI < 6 months) and delayed (PFI > 12 months) relapse in different tumor classifications including (E) WHO tumor grade, and (F) transcriptome subtype.

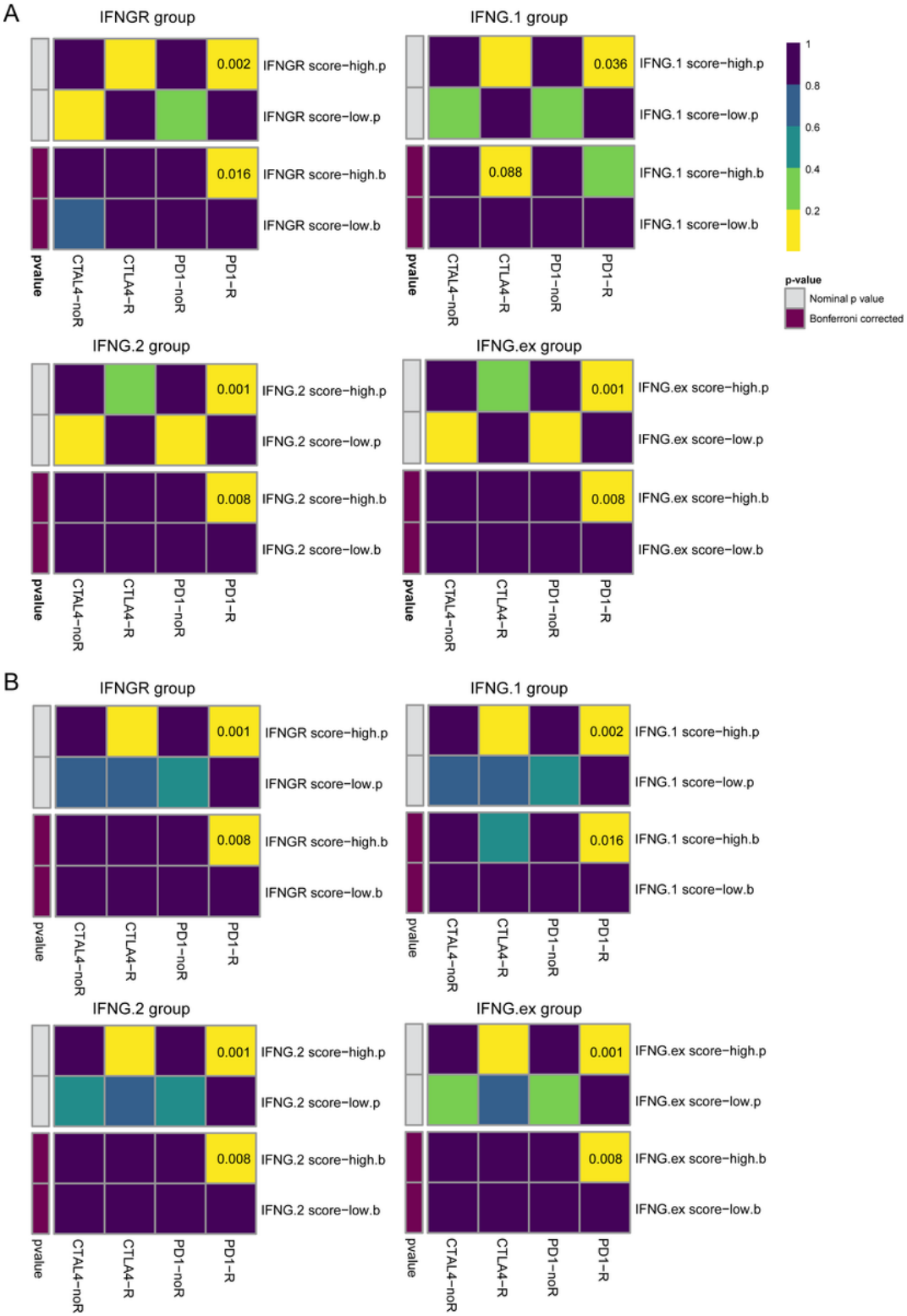

Figure 4

Prognostic significance of the IFNGR-based group. (A) K-M analysis of the IFNGR-based group in terms of OS and PFI based on TCGA, CGGA693, and CGGA325 cohorts. (B) Univariate Cox regression of the independent prognostic significance of the IFNGR-based group and other clinicopathological parameters based on the TCGA cohort. (C) Univariate Cox regression analysis of the independent prognostic significance of the IFNGR-based group based on TCGA, CGGA693, and CGGA325 group, as well as two external validation data sets (Rembrandt, n = 476, and CGGA301, n = 301). (D) Time-dependent ROC and corresponding AUCs of age, WHO tumor grade, IDH mutation status, IFNGR score, and MGMT promoter methylation status. The association between the IFNGR score and tumor early (PFI < 6 months) and delayed (PFI > 12 months) relapse in different tumor classifications including (E) WHO tumor grade, and (F) transcriptome subtype.