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Ferroptosis-related gene MTF-1 as a novel prognostic biomarker in low-grade glioma and its correlation with immune infiltration

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ABSTRACT

Background: Metal-responsive transcription factor-1 performs a necessary position in a range of cancers. It is unknown, though, how the prognosis of patients with low-grade gliomas is related to immune infiltration.
Method: The Cancer Genome Atlas database was used in this investigation to evaluate MTF-1 transcription in low-grade glioma and healthy brain tissues, and immunohistochemistry was used to confirm MTF-1 levels. By using functional enrichment analysis and R software, the putative biological roles and signaling pathways connected to MTF-1 in LGG as well as its prognostic significance were investigated. Further research was done on the connection involving MTF-1 and tumor mutational burden in LGG. Finally, the research evaluated how MTF-1 and immune cell infiltration are related.
Results: We noticed that the WHO grade, 1p/19q codeletion, and older age were all substantially linked with MTF-1 overexpression in low-grade gliomas. OS and disease-specific survival were significantly lowered as a result of MTF-1 transcription. MTF-1 was recognized as an independent OS prognostic predictor with a poor prognosis by multifactorial Cox analysis. Functional enrichment analysis revealed that the primary enrichment pathways were chemical carcinogenesis—receptor activation and the generation of miRNAs implicated in gene suppression by miRNA. Additionally, there was a negative correlation between MTF-1 overexpression and the degree of immune cell infiltration in neutrophils and DC.
Conclusion: MTF-1 may be a novel prognostic biomarker.

1. Introduction

With rapidly rising morbidity and death rates, cancer has emerged as a severe public health issue on a global scale and with very

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complex mechanisms for its development, invasion and metastasis [1]. According to research by the International Agency for Research on Cancer (IARC), there would be 10.3 million cancer-related deaths and 19.3 million new cases of cancer globally in 2020 [2]. Aggressive primary central nervous system (CNS) tumors called diffuse malignant gliomas make up around 30 % of all tumors and 81 % of all brain malignancies, with more than 600,000 deaths annually in the United States due to this disease [3,4]. Based on histological characteristics, the World Health Organization (WHO) divided gliomas into low grade gliomas (LGG, I/II) and high grade gliomas (HGG, III/IV) in 2021 [5]. LGG is a central nervous system malignant tumor caused by abnormal growth of neuroglia, mostly made up of oligodendrocytes and astrocytes, develops more slowly than high-grade malignancies, and has a median lifespan of 4.7–9.8 years [6,7]. Surgery, immunotherapy, specific therapy, and tumor treatment fields (TTF) are now available therapeutic options for LGG. Despite major advancements in therapy, glioma patients' overall survival rates are still dismal [8]. Therefore, it is of the utmost importance to find new prognostic biomarkers and therapeutic targets for glioma, as well as uncover the molecular processes involved in carcinogenesis.

Ferroptosis was identified as a kind of iron-dependent controlled death involving distinct morphological characteristics and molecular pathways in the last ten years [9,10]. It was first discovered that elastin, which specifically causes cell death in cells of tumors containing mutant RAS, causes ferroptosis [11]. Normally, the metal-responsive transcription factor MTF-1 detects and reacts to intracellular metal concentrations [12,13]. Few papers have looked into the potential connection between MTF-1 and iron, while previous investigations centered on the interaction between MTF-1 and zinc in mammals. For instance, overexpression of MTF-1 in flies under iron exposure conditions prolongs their lifespan [14]. In Drosophila, treatment with cadmium, copper, or zinc in an MTF-1-dependent manner stimulates the induction of ferritin [15]. The expression of iron transporter proteins or iron-regulated proteins is regulated by MTF-1 [16,17]. Previous studies have shown the particular transcription factor MTF-1 is necessary for the steady induction of metallothionein-1 and 2 genes by zinc and cadmium [18], While MT reduces oxidative stress, affects metal homeostasis, binds and isolates metals important in the homeostasis of zinc, iron, and copper [14,19]. There is mounting evidence that the iron metabolism of cells from glioma promotes the growth of the malignancy. High blood ferritin levels are related to a bad prognosis and may be caused by the inflammatory condition in GBM patients [20]. High levels of transferrin receptors (TfR) in gliomas stimulate the growth of the tumor through mediating intracellular iron buildup and reactive oxygen species (ROS) production. It also encourages neuronal population decline caused by NMDA receptors [21]. In addition, ferritin light chain (FTL) is overexpressed in gliomas [22]. MTF-1 has a significant impact on how ferroptosis is controlled. But little research has been done on the connection between MTF-1 and LGG. In order to uncover the possible biological roles of MTF-1 and LGG prognosis, the link between MTF-1 and LGG prognosis was investigated by bioinformatics analysis and pertinent basic experiments.

2. Materials and methods

2.1. Brain tissue specimen

The 60 glioma specimens and 10 normal human brain tissue specimens used in this test had been bought from the Department of Neurosurgery, The First Affiliated Hospital of Anhui Medical University. All glioma specimens had been fundamental tumors and had been now not handled with radiotherapy or chemotherapy earlier than surgery. All glioma tissues had been resected for the first time and the prognosis used to be verified with the aid of postoperative pathological examination. Sixty glioma specimens have been graded under the usage of WHO (2021 version) neuroepithelial tumor criteria, together with 20 grade II, 20 grade III and 20 grade IV. Ten normal brain tissues have been taken from brain tissue eliminated all through decompressive brain tissue resection in sufferers with traumatic brain injury. All specimens have been frozen in liquid nitrogen at once after surgical excision and saved for use. Informed consent was once got from sufferers for the acquisition and use of all specimens, and find out about it used to be authorized via the ethics committee of the First Affiliated Hospital of Anhui Medical University (ethics number: P2020-12-07).

2.2. Data gathering and analysis for RNA sequencing

RNAseq data in the TPM file for TCGA and GTEx handled consistently by the Toil method were obtained from UCSC XENA (https://xenabrowser.net/datapages) to examine MTF-1 expression in pan-cancer [23]. For the study of MTF-1 expression in tumor tissues, samples from the TCGA database [24] were chosen, and for the analysis of normal tissue samples, a combination of the TCGA and genotypic tissue expression (GTEx) datasets were used.

2.3. Brain histopathology staining and immunohistochemistry (IHC)

Glioma specimens were prepared into 5-μm paraffin sections, and HE staining was performed using conventional paraffin-embedded sections. In order to clean the specimens, alcohol was employed after they had been soaked in xylene (China Pharmaceutical Group Co., Ltd., Beijing, China). Slides were then restored using 1 EDTA (Bixuan Biotechnology Co., Ltd., Shanghai, China), and then blocked using serum and 3 % H2O2. The slides were subsequently coated with either main or secondary antibodies overnight at 4 °C, followed by DAB and hematoxylin staining (Baso DiagnosticsInc., Zhuhai, China). After that, the neutral resin was used to seal the slides (China Pharmaceutical Group Ltd., Beijing, China). Staining of normal tissue and different grades of gliomas by immunohistochemistry. Proplus 6.0 software (Media Cybernetics, Inc., Rockville, MD, USA) and the value of integrated optical density (IOD) was assessed.

2.4. Enrichment analysis of MTF-1-related genes

The network of protein-molecules interactions in MTF-1 was examined using the STRING site [25]. The correlation study among MTF-1 and other mRNAs in gliomas with a low grade was performed using information obtained from TCGA. The top 100 genes that had the strongest positive correlation with MTF-1 were chosen for enrichment analysis in order to examine the biological role of MTF-1. Microbiology Letter (www.bioinformatics.com.cn) used enrichment for GO and KEGG to depict the top 100 most orthologous-related genes.

2.5. Prognostic analysis of survival

Using R software (version 3.6.3), survival plots of MTF-1 in low-grade gliomas were generated. These plots included overall survival (OS), disease-specific survival (DSS), and progress-free interval (PFI) survival. The segmentation threshold used to separate the population into groups with high and low expression levels was set at 50 %. For assessing MTF-1's utility in predicting patients' prognoses who have low-grade gliomas, it was analyzed and visualised using R software (version 3.6.3) [26].

2.6. The connection involving MTF-1 gene expression level and tumor mutational burden (TMB)

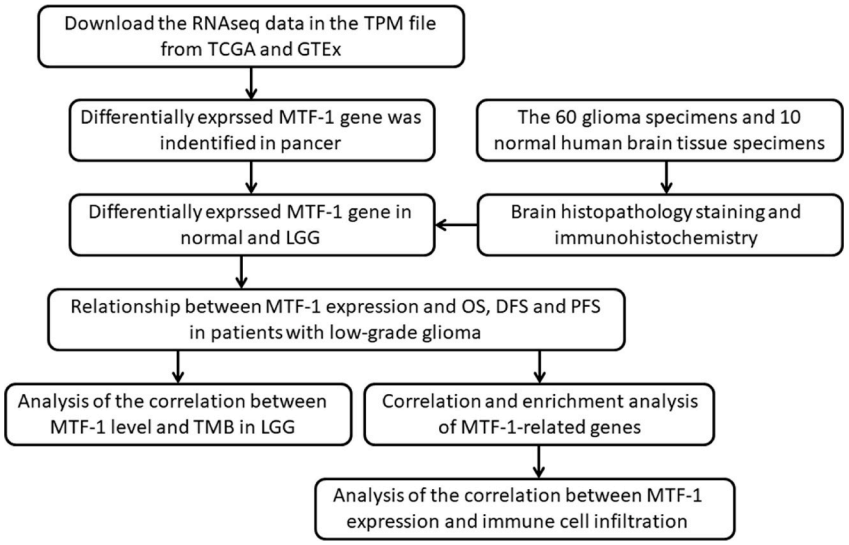
TMB is the total number of base substitutions, deletions, and coding mistakes found in somatic genes per million bases. We acquired biological mutation datasets via the UCSC Xena webpage. The " biological mutation " datasets were selected among the four data file subtypes. Further investigation was done on the connection with TMB and MTF-1 transcript levels within low-grade gliomas. The correlation between numerical variables lacking normal distribution was described using Spearman's assessment of correlation. Statistical significance was defined as a p-value <0.05.

2.7. Immune cell infiltration analysis

A thorough analysis of the tumor-immune in low-grade glioma interactions was conducted using R software (version 3.6.3) [27] and the Tumor Immunology Estimation Resource (TIMER) database (http://TIMER.cistrome.org/) [28]. Using a gene transcription profile file, the impact of MTF-1 transcription on the infiltration of immune cells was examined in this study. Wilcoxon signed rank sum and Spearman correlational tests were used to calculating p-values in order to examine the relationship involving MTF-1 expression and the number of immune cells that infiltrate tumors.

3. Results

3.1. We analyzed MTF-1 in low-grade gliomas by the following flowchart



Flowchart of MTF-1 in low-grade gliomas with immune cell infiltration analysis.

3.2. Abnormal expression levels of MTF-1 in pan-cancer

MTF-1 expression in 33 tumors was analyzed using data downloaded from TCGA and GTEx. Fig. 1A demonstrates that MTF-1 was substantially decreased in the majority of malignancies, such as ACC, BLCA, DLBC, HNSC, KICH, KIRP, LUAD, LUSC, OV, SKCM, TGCT, THCA, THYM, UCEC and UCS. However, MTF-1 was overexpressed in BRCA, CHOL, ESCA, GBM, LAML LGG, LIHC, PAAD, and STAD. Further analysis of the histogram of MTF-1 expression in low-grade glioma versus normal brain tissue was meaningful (Fig. 1B), and immunohistochemical sections of normal tissue versus low-grade glioma tumor tissue were found in the Human Protein Atlas (Fig. 1C). Additionally, we tested the viability of using MTF-1 levels of expression to differentiate both low-grade glioma tissue and healthy brain tissue using the receiver operating characteristic (ROC) line. The variable MTF-1's capacity to predict outcomes with reasonable accuracy as indicated by the area under the ROC curve, which was 0.790 (Fig. 1D).

Furthermore, significant differences in the histopathological morphology of glioma grades II , III and IV with their paraneoplastic histology were found by HE staining. Immunohistochemical staining showed positive results at grades II and III and weakly positive at grade IV, indicating that MTF-1 has promise as a novel prognostic marker for LGG (Fig. 2). Then by semi-quantitative analysis of immunohistochemistry (Fig. 3A–E) of MTF-1 in normal brain tissues and different grades of glioma, the results (Fig. 3F) showed that compared with normal tissues, MTF-1 had significant significance in grade II and III gliomas, while no significant significance was seen in grade I and VI gliomas.

3.3. Elevation of MTF-1 and its clinical relevance in low-grade gliomas

528 individuals with low-grade gliomas had their clinical and gene expression data extracted from the database maintained by the TCGA. Using a 50 % exclusion criteria, patients were split into two groups: the higher MTF-1 transcript team (n = 264) versus the lower MTF-1 transcript team (n = 264). Analytical work was done to determine the correlation involving MTF-1 protein levels and the clinicopathological features of the sufferers. We discovered that there was a strong correlation between MTF-1 expression and WHO grade (P 0.001), IDH status (P 0.001), 1p/19q codeletion (P 0.001), Primary treatment result (P = 0.001), and Histological type (P 0.001). In Table 1, the Wilcoxon rank sum test revealed a correlation between MTF-1 expression and age (P = 0.01).

Logistic regression analysis was used to further examine the link involving MTF-1 transcript levels and clinicopathological features of low-grade gliomas. In Table 2, overall outcomes revealed a strong correlation between MTF-1 transcript ratios and WHO grade (P 0.001), 1p/19q codeletion (P 0.001), primary therapy (P = 0.021), IDH status (P 0.001), histological type (P 0.001), and age (P = 0.037).

3.4. The relationship between MTF-1 level and prognosis of tumor suffers

The overall survival (OS) for individuals with gliomas of low grade was examined using unilateral and multifactorial Cox methods , and the outcomes are displayed in Table 3. WHO grade (P 0.001), 1p/19q codeletion (P 0.001), IDH status (P 0.001), primary therapy outcome (P 0.001), and age (P 0.001) were linked with low-grade glioma in the univariate Cox analysis of MTF-1. However, in multivariate Cox models, it was found that IDH status (P < 0.001), primary therapy outcome (P < 0.001) and age (P

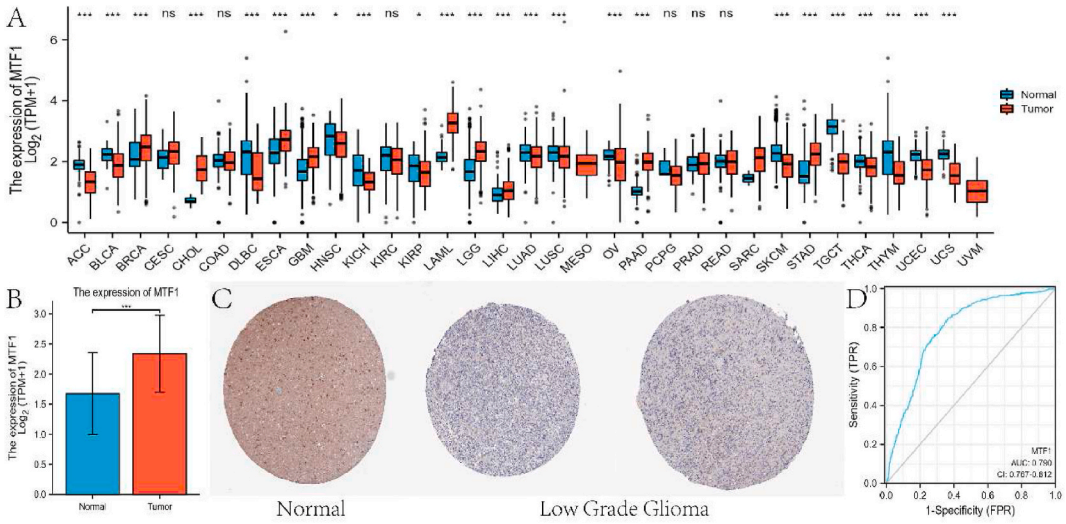


Fig. 1. Abnormal expression of MTF-1 in pancancer. (A) Expression of MTF-1 in normal and tumor tissues in TCGA and GTEx databases; (B) differential expression of MTF-1 in normal and low-grade glioma tissues; (C) sections of normal and low-grade glioma tissues obtained from the Human Protein Atlas. (D) ROC curves of MTF-1 in low-grade glioma: x-axis represents the false positive rate and y-axis represents the true positive rate.

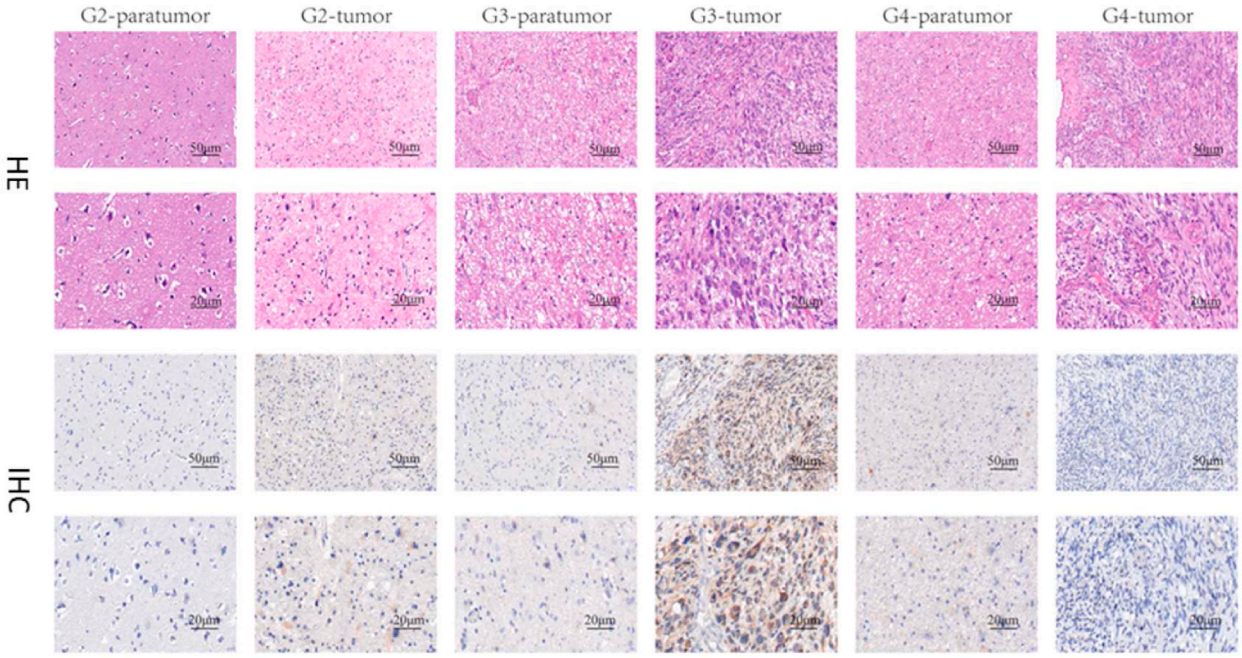


Fig. 2. Histological staining and immunochemical staining of brain tissues. Including HE staining and immunochemical staining of glioma grade II , III and IV and their paraneoplastic tissues.

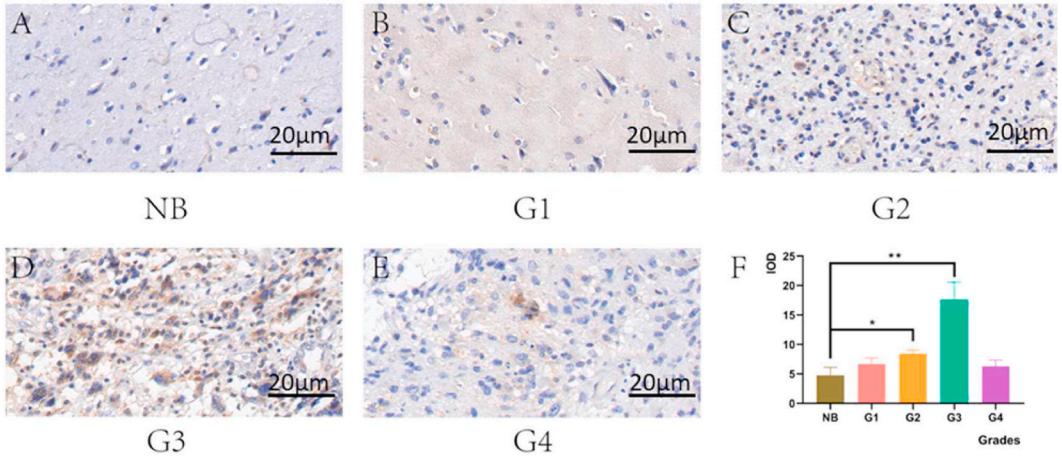


Fig. 3. Immunohistochemistry and semi-quantitative analysis results of MTF-1 in normal brain tissues and different grades of gliomas. (A–E) Immunohistochemistry of MTF-1 in normal brain tissues and G1, G2, G3, and G4; (F) Semi-quantitative analysis results.

= 0.004) were still associated with poorer prognosis. Furthermore, an analysis was done on the relationship between MTF-1 expression and OS, DSS, and PFI survival in patients with low-grade gliomas. Patients with increased MTF-1 mRNA expression had a worse prognosis, as seen by the KM graph (Fig. 4A–C). MTF-1 could thus be a viable indicator for predicting the outcome of LGG individuals.

3.5. Correlation and enrichment analysis of MTF-1-related genes

Considering the interaction of physical binding proteins, the 50 proteins most associated with MTF binding were obtained by STRING in this study (Fig. 5A). In order to further investigate the function of MTF-1, The expression of MTF-1-related genes was discovered using information acquired via the database maintained by TCGA for correlation analysis of pathways. GO and KEGG enrichment analysis using the "clusterProfile" R package yielded the top 100 genes most closely related to MTF-1. The majority of the BP genes were linked to the generation of miRNAs involved in miRNA-mediated gene silencing, according to GO enrichment analysis, most genes in CC were associated with the transcription repressor complex, and most genes in MF were associated with Ras GTPase

Table 1
Correlation analysis of MTF-1 expression with clinicopathological features of low-grade gliomas based on TCGA database.

Characteristic	Low expression of MTF-1	High expression of MTF-1	p
n	264	264	
WHO grade, n (%)			<0.001
G2	132 (28.3 %)	92 (19.7 %)	
G3	101 (21.6 %)	142 (30.4 %)	
IDH status, n (%)			<0.001
WT	29 (5.5 %)	68 (13 %)	
Mut	234 (44.6 %)	194 (37 %)	
1p/19q codeletion, n (%)			<0.001
codel	153 (29 %)	18 (3.4 %)	
non-codel	111 (21 %)	246 (46.6 %)	
Primary therapy outcome, n (%)			0.001
PD	34 (7.4 %)	76 (16.6 %)	
SD	75 (16.4 %)	71 (15.5 %)	
PR	35 (7.6 %)	29 (6.3 %)	
CR	73 (15.9 %)	65 (14.2 %)	
Histological type, n (%)			<0.001
Astrocytoma	61 (11.6 %)	134 (25.4 %)	
Oligoastrocytoma	58 (11 %)	76 (14.4 %)	
Oligodendroglioma	145 (27.5 %)	54 (10.2 %)	
Age, n (%)			0.045
≤40	120 (22.7 %)	144 (27.3 %)	
>40	144 (27.3 %)	120 (22.7 %)	
Age, median (IQR)	43 (34, 54)	38.5 (31, 51)	0.010

Table 2
Logistic regression model approach results showing MTF 1 expression in relation to clinicopathological features.

Characteristics	Total(N)	Odds Ratio (OR)	P value
WHO grade (G3 vs. G2)	467	2.017 (1.398–2.923)	<0.001
1p/19q codeletion (non-codel vs. codel)	528	18.838 (11.275–33.201)	<0.001
Primary therapy outcome (PR&CR vs. PD&SD)	458	0.645 (0.445–0.935)	0.021
IDH status (Mut vs. WT)	525	0.354 (0.217–0.563)	<0.001
Histological type (Oligoastrocytoma&Oligodendroglioma vs. Astrocytoma)	528	0.292 (0.200–0.422)	<0.001
Age (>40 vs.≤40)	528	0.694 (0.492–0.978)	0.037

Table 3
Univariate and multivariate Cox analysis of prognostic factors in patients with low-grade glioma.

Characteristics	Total(N)	Univariate analysis		Multivariate analysis	
		Hazard ratio (95 % CI)	P value	Hazard ratio (95 % CI)	P value
WHO grade	466				
G2	223	Reference			
G3	243	3.059 (2.046–4.573)	**<0.001**	1.583 (0.875–2.864)	0.129
1p/19q codeletion	527				
codel	170	Reference			
non-codel	357	2.493 (1.590–3.910)	**<0.001**	1.517 (0.350–6.586)	0.578
Histological type	329				
Astrocytoma	195	Reference			
Oligoastrocytoma	134	0.667 (0.425–1.047)	0.078	1.374 (0.803–2.352)	0.246
IDH status	524				
WT	97	Reference			
Mut	427	0.186 (0.130–0.265)	**<0.001**	0.201 (0.113–0.359)	**<0.001**
Primary therapy outcome	457				
PR&CR	201	Reference			
PD&SD	256	4.963 (2.782–8.851)	**<0.001**	4.636 (2.173–9.890)	**<0.001**
Age	527				
≤40	264	Reference			
>40	263	2.889 (2.009–4.155)	**<0.001**	2.235 (1.289–3.875)	**0.004**

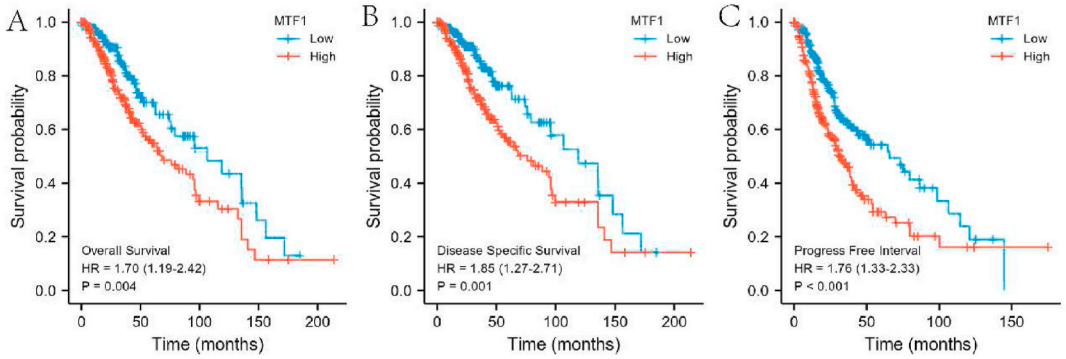


Fig. 4. Relationship between MTF-1 expression and OS, DFS and PFS in patients with low-grade glioma. (A) Relationship between MTF-1 expression and OS. (B) Relationship between MTF-1 expression and DFS. (C) Relationship between MTF-1 expression and PFS.

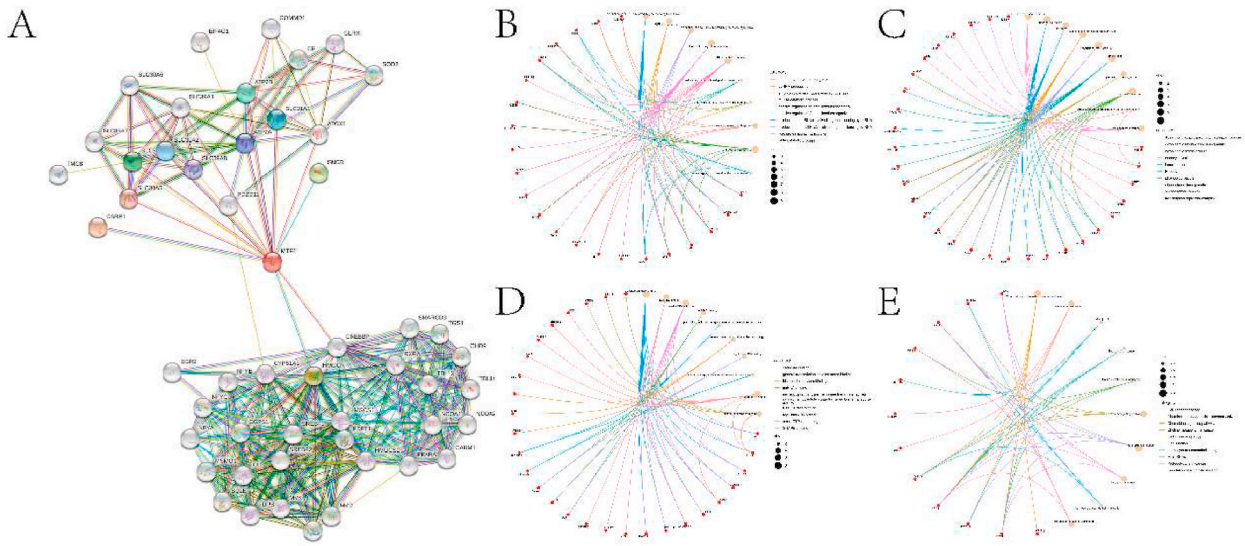


Fig. 5. Correlation and enrichment analysis of the top 100 positively related genes of MTF-1. (A) MTF-1 binding protein obtained by STRING. (B–D) GO enrichment of the top 100 genes positively correlated with MTF-1 (including BP, CC and MF). (E) Significant KEGG enrichment pathways for the top 100 most positively related genes to MTF-1.

binding (Fig. 5B–D). KEGG data suggest that chemical carcinogenesis - receptor activation perhaps connected to MTF-1's oncogenic mechanism (Fig. 5E).

3.6. Analysis of the correlation between MTF-1 level and TMB in LGG

R software was used to investigate the association of MTF-1 levels and TMB in low-grade gliomas. The outcomes demonstrated that TMB in LGG and MTF-1 transcription had a favorable correlation ($p < 0.05$ and Correlation>0.1) (Fig. 6A). Thus, the association between MTF-1 gene expression and TMB depended on the kind of cancer.

3.7. Association of MTF-1 level and immune cell infiltration

The correlation involving MTF-1 levels of expression and immune cell infiltration was investigated to learn more about the role of MTF-1. The ssGSEA and Spearman's r functions in the R package were used to examine any potential relationships involving MTF-1 expression levels and 24 immune cells. Investigations revealed a substantial correlation among MTF-1 expression and aDC, CD8 T cells, DCS, Eosinophils, macrophages, mast cells, neutrophils, NK CD56 bright cells, pDC, T cells, and T helper cells as well as Tcm, Tem, Tgd Th2 cells, Th17 cells, and Treg cells (Fig. 6B).

We looked at the connection involving MTF-1 levels and immunological infiltration as a result of this. The levels of infiltrating immune cells, including aDCs, DCS, Eosinophils, macrophages, mast cells, neutrophils, NK CD56 bright cells, pDC, T cells, T helper cells, Tcm, Tem, Tgd, Th2 cells, Th17 cells, and Treg cells, were found to be significantly different when MTF-1 levels were classified

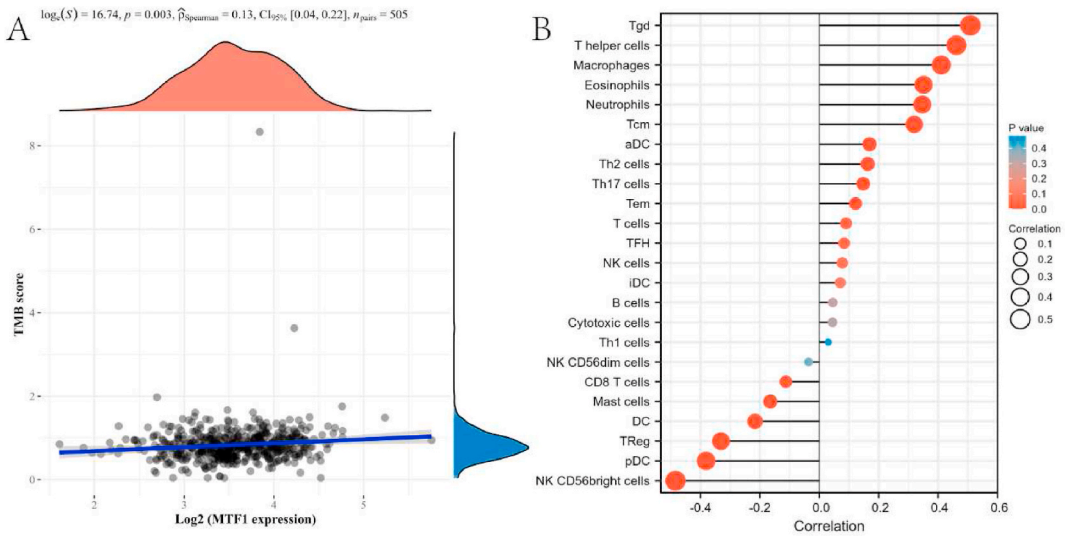


Fig. 6. (A) Correlation between MTF-1 expression and TMB in cancer; (B) lollipop plot of MTF-1 expression levels in 24 immune cells.
Additional investigations indicated that the level of transcription of MTF-1 was related to aDC (r = 0.169, P < 0.001), Eosinophils (r = 0.351, P < 0.001), macrophages (r = 0.411, P < 0.001), neutrophils (r = 0.347, P < 0.001), T helper cells (r = 0.462, P < 0.001), T cells (r = 0.090, P = 0.039), Tcm (r = 0.320, P < 0.001), Tem (r = 0.122, P = 0.005), Tgd (r = 0.509, P < 0.001), Th2 cells (r = 0.163, P < 0.001), and Th17 cells (r = 0.148, P < 0.001) were positively correlated. Conversely, MTF-1 expressing themselves was negatively correlated with pDCs (r = −0.381, P 0.001), Treg cells (r = −0.331, P 0.001), mast cells (r = −0.165, P 0.001), NK CD56 bright cells (r = −0.484, P 0.001), and CD8 T cells (r = −0.112, P = 0.010) (Fig. 7A-Q).

into two separate groups, whereas CD8 T cells were not (Fig. 8A-Q).

Finally, TIMER (http://TIMER.cistrome.org/) evaluated the effect of infiltration of immune cells on medical survival results in patients with low-grade gliomas. The findings showed that low-grade glioma sufferers' prognoses were negatively correlated with increased levels of DC and neutrophil cells (P < 0.05) (Fig. 9A and B).

4. Discussion

Glioma is a prevalent primary tumor that is more difficult to treat and possesses a poor prognosis [29–32]. 35.7 % of patients who get standard surgery along with radiation and chemotherapy recover from their procedures, and the 5-year average survival rate is 4.7 %. Low-grade gliomas account for approximately one-fifth of all gliomas and should receive our attention. It has been demonstrated that the growth and incidence of gliomas are strongly influenced by the genetics of the tumor cells [33]. Wang et al. showed that most gene expression in tumor cells is influenced by the tumor cell microenvironment [34]. Immune cells and stromal cells make up the tumor microenvironment's two primary parts, which are widely considered of higher clinical value for prognostic assessment of tumor diagnosis [35,36]. Finding more precise indicators is, therefore, vital to track the development of diseases and diagnose them early. Current clinical prognostic markers commonly used in glioma disease include isocitrate dehydrogenase (IDH) mutations, combined chromosome 1p/19q deletion status (co-deletion), methylation of the promoter region of O 6 -methylguanine-DNA methyltransferase (MGMT), mutations in the TERT promoter, EGFR amplification and EGFR vIII rearrangement, and mutations in the PTEN gene, TP53 gene mutation, CDKN2A [37–39]. In addition to this, some of the novel prognostic markers for gliomas have been investigated in the last two years, including CASZ1, autophagy-related genes, EVA1C, has-miR-196a-5p and Piezo1 [40–44]. In contrast, MTF-1 is a newly proposed gene that plays a key role in pathways such as iron death and copper death, while MTF-1 is more closely associated with immune cell infiltration. MTF-1 has been recognized as an indicator of outcome and overexpressed in several cancer types, according to earlier investigations [45,46], virtually no research has been done on the connection involving MTF-1 transcription and LGG. In the current work, we aimed to investigate MTF-1's possible processes in LGG and the viability of using it as a diagnostic indicator.

MTF-1 was found to be upregulated in multiple cancer types by pan-cancer analysis. Further analysis indicated that lower OS, DFS, and PFS in LGG patients were linked to increased MTF-1 overexpression. The correlation involving MTF-1 transcription and clinicopathological characteristics of LGG was examined using logistic regression. Results demonstrated a significant link between MTF-1 and histological type. Additionally, MTF-1 was demonstrated to be a distinct predictor of patient outcomes in univariate and multivariate Cox analyses. All of the aforementioned findings and ROC analysis point to MTF-1 as a potential predictive indicator for people with low-grade gliomas.

Prior work has demonstrated that the tumor cell microenvironment (TME) has a significant role in the majority of the genes expressed in tumor cells [34,47]. The task has shown that DC is essential for tumor T-cell tolerance, immunological editing, and cancer immunosurveillance [48,49]. Recent studies have shown that there are drugs available to treat some cancers (including colorectal cancer [50], pancreatic cancer [51], etc) through DCs. Xu et al. showed that neutrophils cells are overexpressed in gliomas [52], which

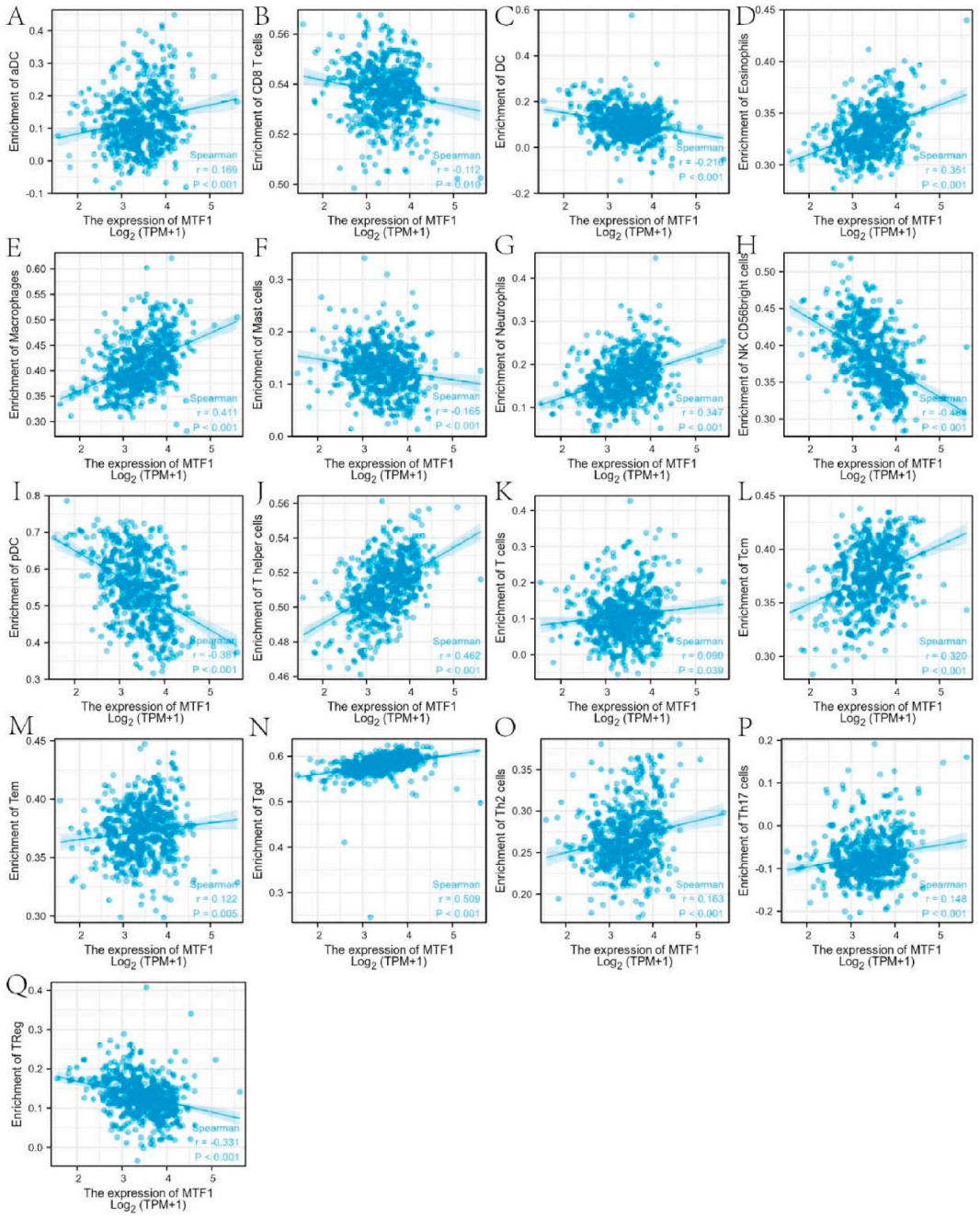


Fig. 7. Correlation between MTF-1 expression and immune cell infiltration of (A)aDCs, (B)CD 8 Tcells, (C)DCS, (D)Eosinophils, (E)macrophages, (F) mast cells, (G)neutrophils, (H)NK CD56 bright cells, (I)pDC, (J)T cells, (K)T helper cells, (L)Tcm, (M)Tem, (N)Tgd, (O)Th2 cells, (P)Th17 cells, and (Q)Treg cells.

is the same result as our analysis. Neutrophil cells are currently known to have a crucial function in the treatment of breast and lung cancer [53,54]. Current immunotherapy for gliomas has made great progress. Qu et al. found that OLFML3 overexpression is associated with malignant behavior and poor prognosis of WHOII/III gliomas, which is promising to be a target gene for immunotherapy [55]; Zhu et al. advanced the current development of immunotherapy by enhancing anti-tumor immunity through tumor-targeting nano-adjuvant synergistic with light-mediated immunotherapy [56]; and what's more, they utilized molecular probe combined with the images of gliomas to guide the diagnosis and treatment [57]. The studies we conducted revealed that MTF-1 transcription was adversely linked with DCS and neutrophil cells in low-grade gliomas. This suggests to us that MTF-1 may act in tumors through the immune microenvironment.

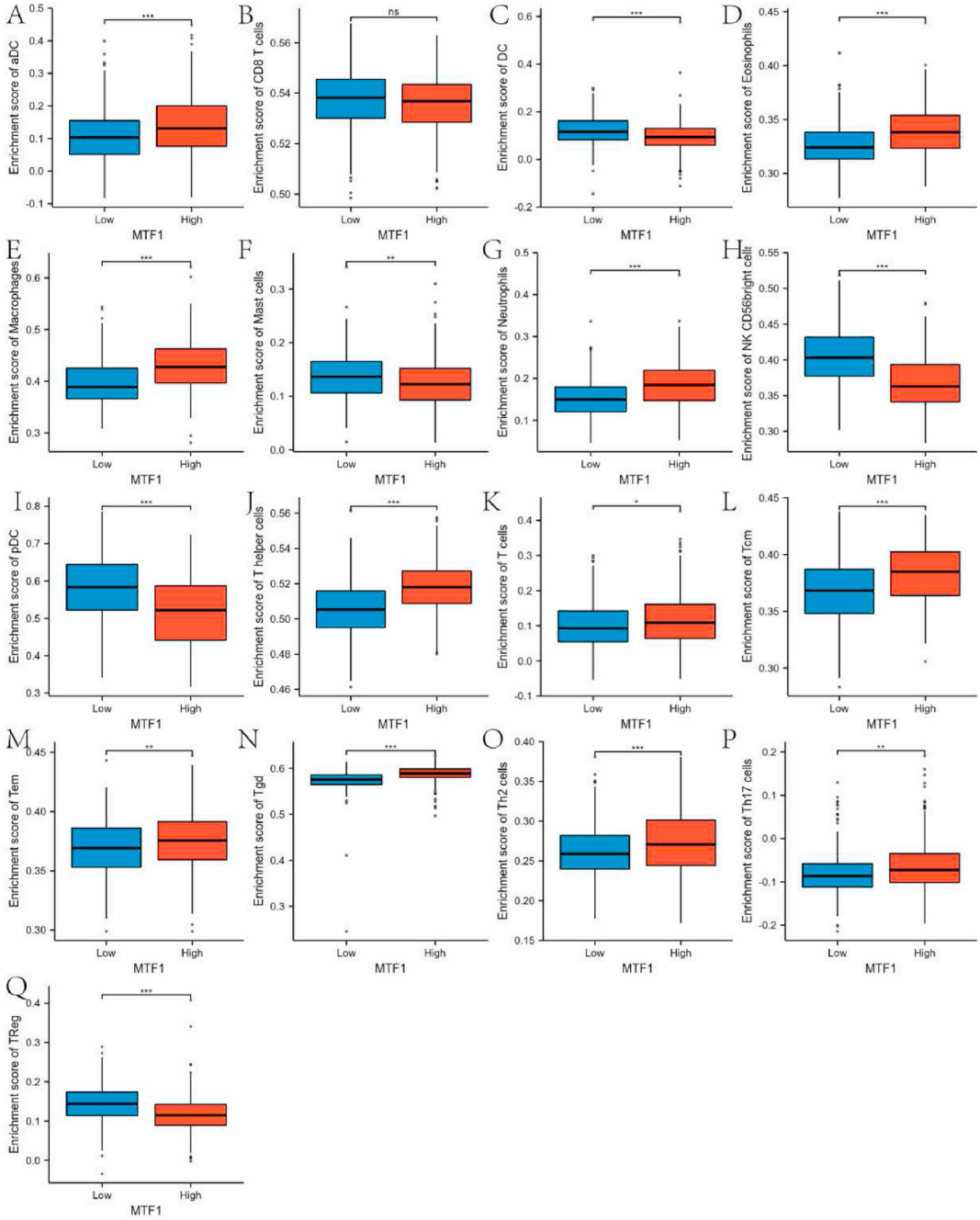


Fig. 8. Comparison of MTF-1 high expression group with low expression group in immuno-infiltrated cells of (A)aDCs, (B)CD 8 Tcells, (C)DCS, (D) Eosinophils, (E)macrophages, (F)mast cells, (G)neutrophils, (H)NK CD56 bright cells, (I)pDC, (J)T cells, (K)T helper cells, (L)Tcm, (M)Tem, (N)Tgd, (O)Th2 cells, (P)Th17 cells, and (Q)Treg cells.

There remain a few drawbacks to our current work, despite the fact that it sheds fresh light on the connection among both MTF-1 expression and the prognosis of sufferers with low-grade gliomas. First off, because all data utilized for the study's bioinformatics analyses were retrieved directly from public sources, we failed to collect key crucial medical evidence, like the patients' chemotherapy regimens; second, the sample size of the control group differed significantly from that of the tumor group, so much farther study relies on to an equitable split of large samples is required; third, To clarify the biochemical processes and possible procedures of MTF-1 in LGG, more research and rigorous experimental validation are needed for both in vitro as well as in vivo.

The study's findings concluded that MTF-1 transcription was elevated in LGG and substantially correlated with worse survival rates. Furthermore, MTF-1 may also affect the degree of infiltration by immune cells, contributing to the onset and progression of LGG.

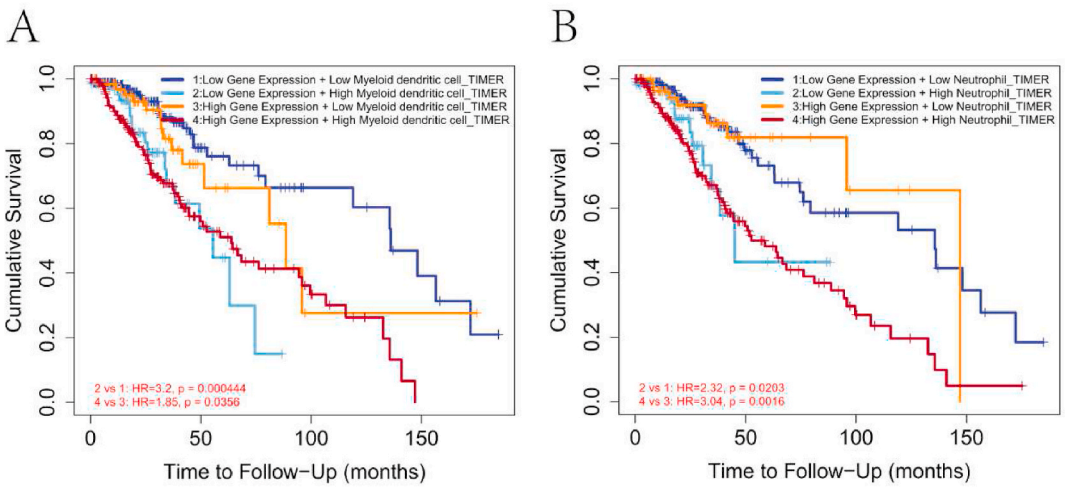


Fig. 9. Prognostic impact of immune cell infiltration in patients with low-grade glioma. (A) Clinical survival outcome of patients with high DC cell LGG. (B) Clinical survival outcome of patients with high neutrophils cell LGG.

Thus, our work discovers a viable predictive biomarker and exposes the function of MTF-1 in LGG.

5. Contributions

(I) Conception and design: H Cheng and X Dai; (II) Administrative support: X Dai (III); Provision of study materials or patients: H Li and P Gao (IV); Collection and assembly of data: L Shu and X Chen; (V) Data analysis and interpretation: H Li and P Gao; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

Data availability

Data and download URLs involved in this study had been described in detail in the.
Methods section. All results generated in this study can be obtained by contacting
The corresponding authors on reasonable request.

Declaration of competing interest

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

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