The Prognostic Value of EMT in Glioma and its Role in the Glioma Immune Microenvironment

Weihai Ning[1] · Zhiqiang Qiu[1] · Xiangwen Ji[2] · Xiang Wang[1] · Yihua An[1,3] · Shengdian Wang[4] · Hongwei Zhang[1]

Abstract

Diffuse glioma is the deadliest form of brain cancer, and the median survival of grade IV glioma (glioblastoma, GBM) is no more than 2 years even with maximal surgical resection followed by radiotherapy and chemotherapy, which are now the standard of care for GBM. Glioma shares common characteristics with most malignant tumours, such as invasiveness, rapid progression, resistance to various therapies and inevitable recurrence, while it also has its own unique features, such as high aggressiveness and immunotherapy resistance, which can be, respectively, attributed to epithelial-mesenchymal transition (EMT) and the immunosuppressive microenvironment. Here, we calculated the EMT score of glioma using The Cancer Genome Atlas (TCGA), the Chinese Glioma Genome Atlas (CGGA) and the Gene Expression Omnibus (GEO) datasets and validated its prognostic value. Then, we investigated its role in the glioma immune microenvironment, identified the enriched EMT-related immune genes and determined their specific biological functions in glioma. Furthermore, clinical relevance analysis showed the translational value of these EMT-related immune genes. In short, our findings reveal a critical link between EMT and the glioma immune microenvironment and offer important clues for further investigation of the underlying molecular mechanism.

Keywords EMT · Prognostic indicator · Immune microenvironment · Gene expression

Introduction

Diffuse glioma, the most prevalent and life-threatening malignant brain tumour, has always been notorious for its diffuse and infiltrative nature, which yields devastating clinical outcomes (Ferlay et al. 2019). According to histological criteria, adult diffuse glioma is classified into oligodendroglioma, oligoastrocytoma, astrocytoma and glioblastoma and is graded into WHO II to IV stages, respectively (Molinaro et al.

2019). Among them, glioblastoma is the most common (accounting for approximately 55% of gliomas) and also the deadliest glioma (with a dismal median survival of 14–16 months) (Wen and Kesari 2008). Despite decades of research and aggressive treatment, only negligible benefit has been gained in the clinical practice of patient care (Bray et al. 2018). Because of the poor prognosis of glioma after the initial diagnosis, it is urgently needed to better understand the molecular pathways and specific molecules that coordinate the aggressive and malignant nature of brain tumours. EMT, a reversible cellular programme that transitions epithelial cells into mesenchymal cell states, is known to be critical for malignant progression (Dongre and Weinberg 2019). In the context of neoplasia, EMT confers on neoplastic cells increased invasive and metastatic potential and a stronger resistance against elimination by several common treatment methods (Lambert et al. 2017; Shibue and Weinberg 2017). Owing to the extremely aggressive and invasive nature of glioma, it has been gradually appreciated that EMT in glioma likely contributes significantly to gliomagenesis and remodelling of the glioma microenvironment (Lu et al. 2012). When EMT is induced in neoplastic cells, the resulting mesenchymal carcinoma cells can in return respond to other non-neoplastic cells, such as by modifying the activities and representation of those

[1] Department of Neurosurgery, Sanbo Brain Hospital, Capital Medical University, Beijing 100093, China

[2] Department of Biomedical Informatics, Department of Physiology and Pathophysiology, Center for Noncoding RNA Medicine, School of Basic Medical Sciences, Peking University, 38 Xueyuan Rd, Beijing 100191, China

[3] Department of Neurosurgery, Chinese PLA General Hospital, Beijing 100853, China

[4] CAS Key Laboratory of Infection and Immunity, Institute of Biophysics, Chinese Academy of Sciences, Beijing, China

cells in the tumour microenvironment (TME) (Gajewski et al. 2013), specifically, various subtypes of adaptive innate and adaptive immune cells involved in tumourigenesis and progression. Some of these cells are anti-neoplastic cells (natural killer (NK) cells, the cytotoxic CD8^{+} T cells and classically activated M1 macrophages), while others are pro-neoplastic cells (alternately activated M2 macrophages, regulatory T cells (Treg cells) and myeloid-derived suppressor cells (MDSCs)) (Kerkar and Restifo 2012). With the emergence of novel immunotherapies that are revolutionary therapeutic regimens for cancers (Mellman et al. 2011), identifying the underlying regulatory mechanism of these two groups of immune cells that act in distinct manners (immune suppression and immune activation) is of great significance, and EMT may play a crucial role in this process.

Recent clinical immunotherapies have been discovered in a variety of tumours, and immune checkpoint blockade therapy is now the standard of care for treatment of non-small cell lung cancer, melanoma and a growing list of other malignant tumours (Gong et al. 2018). However, this revolutionary therapeutic advance did not benefit glioma patients, even though data from preclinical models favoured immunotherapy as a feasible approach for glioma (McGranahan et al. 2019). Accordingly, the focus of glioma immunology research is moving to the development of strategies that aim at various resistance mechanisms. Currently, studies of glioma have revealed some key resistance mechanisms of the anti-glioma immune response. Examples include the specific immunoprivileged site of glioma (Jackson et al. 2019), the scarce glioma infiltrating lymphocyte population with highly expressed immune checkpoints and the existence of a large number of immunosuppressive cells called tumour-associated macrophages (TAMs) (Hambardzumyan et al. 2016). In general, the anti-glioma immune response resistance mechanisms have been revealed to occur at all phases of anti-tumour immunity, including the prevention of the initiation of the response (intrinsic immunoresistance), deactivation of tumour-infiltrating immune cells (adaptive immunoresistance) and protection of the tumour from elimination by the immune system (acquired immunoresistance) (Bauer et al. 2014; Chongsathidkiet et al. 2018; Grossman et al. 2011; Woroniecka et al. 2018). Although there are a large number of factors that have been shown to cause the immune resistance of glioma, the mechanisms by which EMT contributes to glioma immune resistance are largely unknown, and answering this question may represent a novel research direction.

To explore the relationship between EMT of glioma and the adaptive changes of its immune microenvironment, we uncovered the underlying molecular mechanism. In this study, by applying single sample gene set enrichment analysis (ssGSEA) (Barbie et al. 2009) to TCGA, CGGA and GEO datasets, we calculated the EMT scores, determined their clinical relevance and validated their prognostic value in glioma. Then, we revealed the close relationships between EMT

scores and the glioma immune microenvironment. Using gene expression correlation analysis, we selected EMT-related immune genes and enriched relevant signalling pathways. We found that all top 26 EMT-related immune genes played critical roles in oncobiology. Importantly, we have validated their significance with regard to clinical outcomes of glioma patients.

Materials and Methods

Data Collection TCGA RNAseq data in fragments per kilobase of transcript per million mapped reads (FPKM) of lower-grade glioma (LGG) and GBM, as well as relevant clinical information, were downloaded from the GDC data portal (https://portal.gdc.cancer.gov/). We obtained data on WHO grade, histology type, IDH mutation status and 1p/19q codeletion status of TCGA submitters from the study by Ceccarelli et al. (2016). Gene expression data of thousands of glioma patients were obtained from CGGA (http://www.cgga.org.cn/), including one microarray dataset of 301 patients and two RNAseq datasets of 325 and 693 patients, respectively (Ceccarelli et al. 2016). Additionally, 11 microarray gene expression profiling datasets of glioma (GSE4290, GSE4412, GSE7696, GSE16011, GSE35158, GSE50161, GSE52009, GSE54004, GSE60898, GSE61374, GSE107850) were available at GEO (https://www.ncbi.nlm.nih.gov/gds/). Duplicate genes were excluded except their max values.

Single Sample Gene Set Enrichment Analysis The EMT gene set was extracted from the Gene Ontology (GO) database based on the term 'positive regulation of epithelial to mesenchymal transition' (GO: 0010718). The ESTIMATE immune and stromal gene sets were obtained from the R (v3.5.2) package estimate (Yoshihara et al. 2013) (v1.0.13). The enrichment scores were processed by the python (v3.6.8) package gseapy (v0.9.13).

Immune Cell Abundance Estimate ABsolute Immune Signal (ABIS) (Monaco et al. 2019), Estimate the Proportion of Immune and Cancer cells (EPIC) (Racle et al. 2017) and Immune Cell Abundance Identifier (ImmuCellAI) (Miao et al. 2019) were used to estimate the abundance of immune cells in TCGA RNAseq gene expression data.

GO Enrichment Analysis Spearman's correlation analyses were performed between EMT score and gene expression and between the immune score and gene expression in TCGA (GBM and LGG), CGGA (3 datasets) and 12 GEO glioma datasets. P values were adjusted by the Benjamini and Hochberg methods. Genes correlated with both EMT and the immune score ($|rho| > = 0.3$, FDR < 0.05) in more than 12 of

the 16 datasets were selected. The R package clusterProfiler (Yu et al. 2012) (v3.10.1) was used for GO enrichment analysis.

Statistical Analysis Kaplan–Meier curves with log rank test and Cox proportional hazards regression were generated by R packages survival (v 3.1–8) and survminer (v0.4.6). The significance of the difference between two groups was calculated by the two-sided Wilcoxon rank sum test. Spearman's correlation was applied to evaluate the correlation between two groups of continuous values. All statistical analyses above were performed by R (v3.5.2). P values <0.05 were considered significant (Fig. 1).

Results

Clinical Relevance of EMT Scores

First, we extracted EMT-related genes from the GO dataset, and then the enrichment scores for these genes were calculated. Based on the extent of EMT-related gene expression, we clustered gliomas into two major groups by median value. Second, using TCGA datasets, we showed that high stromal and immune signatures were correlated with high glioma grades (Fig. 2a). To validate this result, CGGA microarray, CGGA RNAseq batch 1, CGGA RNAseq batch 2, GSE4290, GSE7696, GSE50161, GSE52009 and GSE54004 were used, and similar results were found (Fig. 2b–i). Furthermore, compared to normal brain tissues, different grades of glioma, including LGG and GBM, showed higher EMT scores (Fig. 2e–g). Together, these findings suggest that EMT scores increase with the malignant progression of glioma. Considering the underlying significance of EMT, which represents the acquisition of cancer invasiveness and metastasis abilities, we

hypothesised that EMT scores may be a prognostic marker of glioma.

The Prognostic Value of EMT Scores in Glioma

Using TCGA datasets, we revealed that high EMT scores were associated with decreased survival in patients with glioma, while low EMT scores were associated with increased survival in glioma patients (Fig. 3a). Next, we repeated the analysis in other datasets (CGGA microarray, CGGA RNAseq batch 1, CGGA RNAseq batch 2, GSE4412-GPL96) and we obtained consistent results (Fig. 3b–e). The results above were confirmed by univariate and multivariate Cox proportional hazard regression analyses (Fig. 3f). It is worth noting that all survival differences between the high ETM score group and the low ETM score group were significant in various datasets except for the CGGA microarray dataset. Therefore, we believe that EMT scores can serve as a reliable prognostic marker in patients with glioma.

The Role of EMT in the Glioma Immune Microenvironment

Recently, several reports have indicated that neoplastic cells expressing markers associated with EMT show increased resistance to elimination by components of the adaptive and innate immune systems (Akalay et al. 2013; Dongre et al. 2017; Kudo-Saito et al. 2009; Terry et al. 2017). However, whether these EMT-induced immunosuppressive effects also exist and play important roles in glioma remains largely unknown. Therefore, to further explore the relationship between EMT scores and the glioma immune microenvironment in a panoramic way, we not only enriched the ESTIMATE immune scores and stromal scores of gliomas but also calculated the abundance of various immune cells of the glioma microenvironment using three different tools, ABIS, EPIC and ImmuCellAI. Through Spearman correlation analysis, we found that high EMT scores were associated with high immune scores and stromal scores, which suggested that these glioma tissues have abundant non-neoplastic cells (immune cells and stromal cells) in their TME (Fig. 4a). Next, when we analysed the relationship between EMT scores and the immune cell subtypes, two opposite correlation patterns were revealed, namely positive correlation (cell subtypes that include macrophages, cancer-associated fibroblasts, monocytes, etc.) and negative correlation (cell subtypes that include CD8 T cells, CD4 naive T cells, B cells, etc.) (Fig. 4a). The correlation coefficients between EMT scores and immune scores, stromal scores and other main immune cells were fairly high (immune scores (ESTIMATE), rho = 0.74; stromal scores (ESTIMATE), rho = 0.8; macrophages (EPIC), rho = 0.66; macrophages (ImmuCellAI), rho = 0.48; CD8 T cells (EPIC), rho = −0.61; CD4 naive (ImmuCellAI), rho = −0.55)
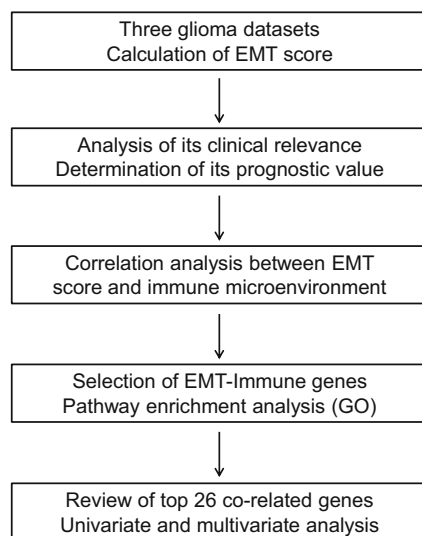
Fig. 1 Workflow of the current study

Fig. 2 Different EMT scores exist between different grades of glioma and normal brain tissues. (a–i) Quantification of EMT scores in non-tumour brain and gliomas of grade II, grade III and grade IV using different datasets, TCGA (a), CGGA microarray (b), CGGA RNAseq batch 1 (c), CGGA RNAseq batch 2 (d), GSE4290 (e), GSE7696 (f), GSE50161 (g), GSE52009 (h) and GSE54004 (i)
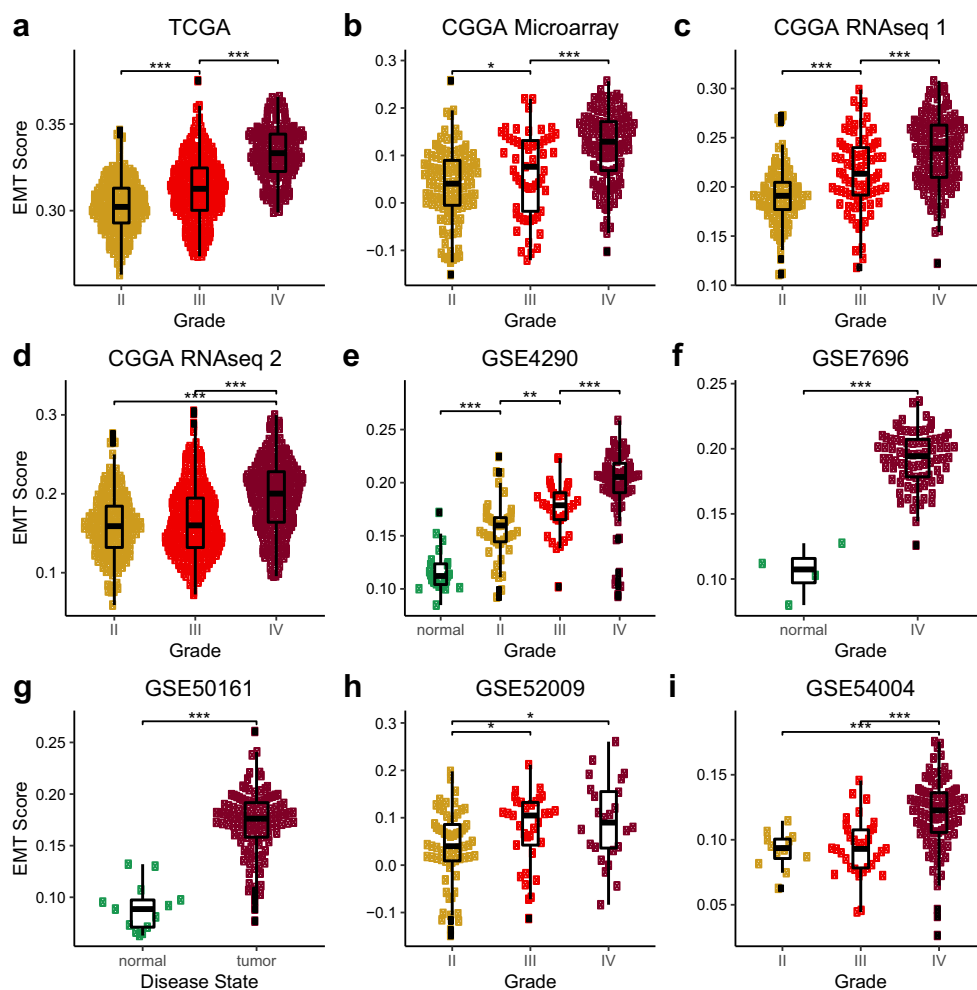

(Fig. 4b-g). It is known that TAMs are closely related to an immunosuppressive TME, while abundant CD8 T cells and CD4 T cells often signify a favourable prognosis (Hambardzumyan and Gutmann 2016; Hilf et al. 2019; Keskin et al. 2019). Together, our results suggest that the EMT of glioma may exert an immunosuppressive effect on the glioma microenvironment.

EMT-Related Immune Genes Enrichment, Analysis and its Clinical Relevance

To further investigate the molecular signatures behind the EMT and immune microenvironment of glioma, we performed Spearman's correlation analyses between EMT scores and gene expression and between immune scores and gene expression in the TCGA, CGGA and GEO datasets. The genes associated with both EMT scores and immune scores in at least 15 of the 16 datasets were considered enriched and are depicted in Fig. 5a. We can see that most of these genes were previously reported to play different roles in glioma tumourigenesis and progression (Table 1). However, few studies have investigated the specific role of these genes in

EMT and immune responses in gliomas. For example, CD44, a transmembrane molecule, is overexpressed in many tumours (Ishimoto et al. 2010; Jijiwa et al. 2011). The role of CD44 has been implicated in various malignant processes, including cell motility, tumour growth and angiogenesis (Naor 2016; Naor et al. 2008). In glioma, studies found that CD44 expression increased tumour cell invasion, proliferation and therapy resistance to decrease survival in glioma patients (Anido et al. 2010; Ariza et al. 1995; Liu et al. 2018; Mooney et al. 2016). However, whether it has dual roles in the EMT of glioma and regulates the glioma immune environment remains unclear. To explore which signalling pathways are involved in the EMT-related immune genes, we conducted GO enrichment analysis of the EMT-related immune genes (Fig. 5b). Specifically, in the biological process module of the GO analysis, the top four pathways that had the highest number of EMT-related immune genes were all related to neutrophils, namely neutrophil mediated immunity, neutrophil activation, neutrophil activation involved in the immune response and neutrophil degranulation, suggesting that the neutrophil signalling pathways may be of significance in regulating both glioma EMT and the immune microenvironment. The other
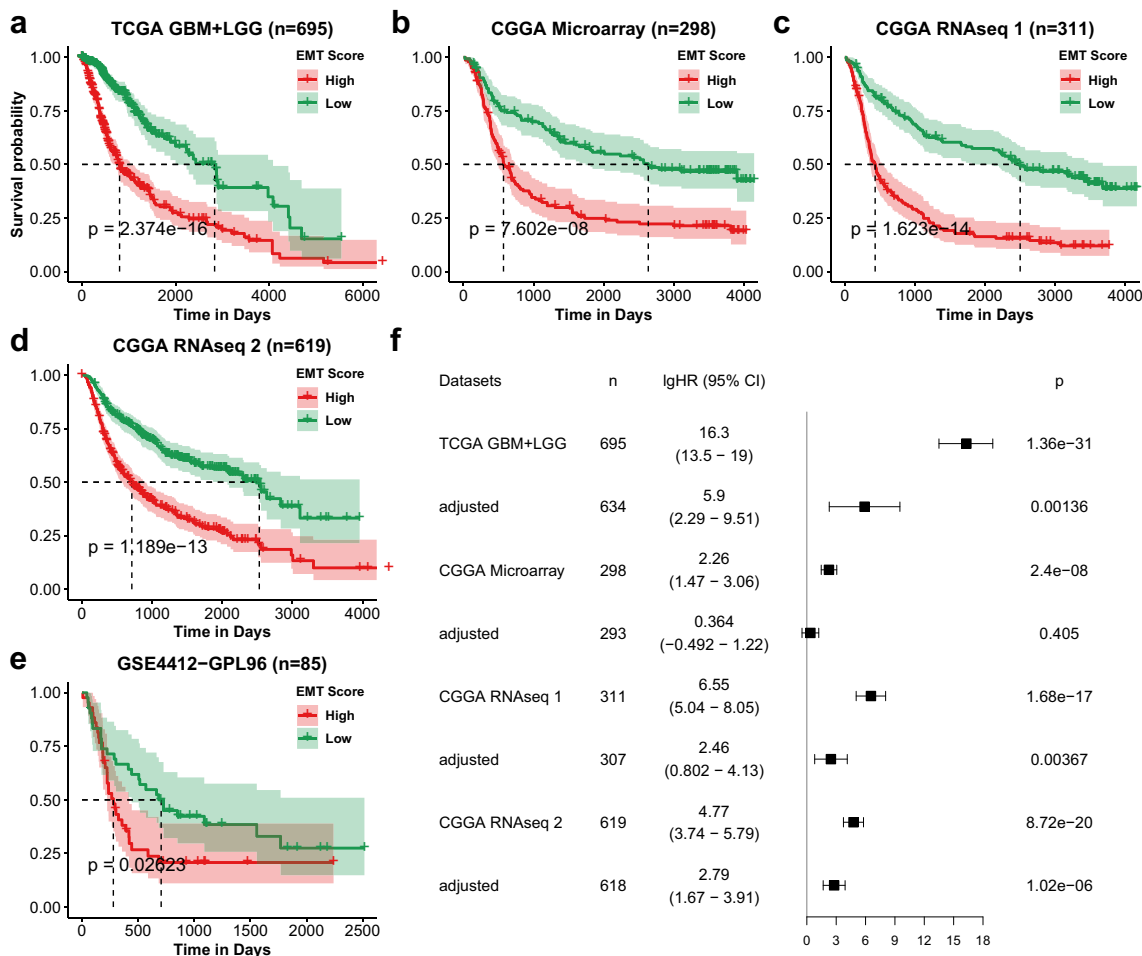
Fig. 3 Kaplan–Meier survival curves and Cox regression of EMT scores in glioma. (**a**–**e**) Correlation between patient survival and EMT scores (high or low) in glioma based on datasets from TCGA, CGGA microarray, CGGA RNAseq batch 1, CGGA RNAseq batch 2 and GSE4412-GPL96. The log-rank test was used for statistical analysis. (**f**) The unadjusted and adjusted (by age, gender, histology and grade) hazard ratio (HR) and *P* value of the Cox regression of (**a**–**e**)

main pathways enriched with EMT-related immune genes included T cell activation, leukocyte migration, regulation of inflammatory response, leukocyte cell–cell adhesion, positive regulation of defence response, positive regulation of cytokine production, extracellular structure organization, extracellular matrix organization, response to molecule of bacterial origin, coagulation, haemostasis, blood coagulation, response to interferon-gamma, cellular response to interferon-gamma, platelet degranulation and the interferon-gamma-mediated signalling pathway. Through gene expression analysis, we detected an increased expression of EMT-related immune genes corresponding to GBM, IDH-WT glioma and 1p/19q non-codeleted glioma (Fig. 5c). Overall, our analysis incorporated the EMT-related immune genes and the relevant signalling pathways and their clinical significance, suggesting the potentially basic and clinical utility of EMT-related immune genes.
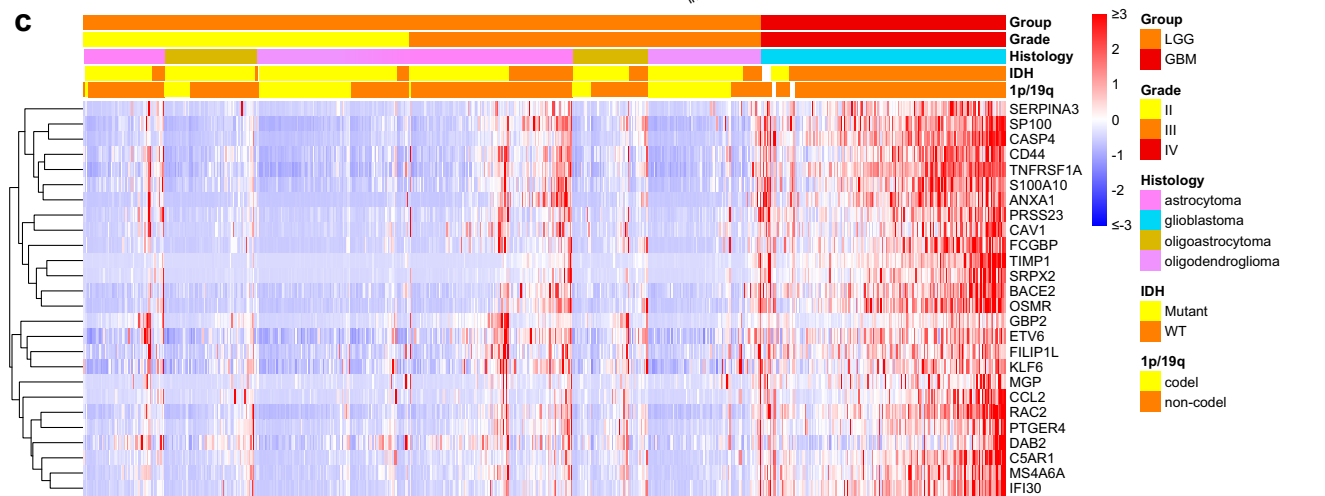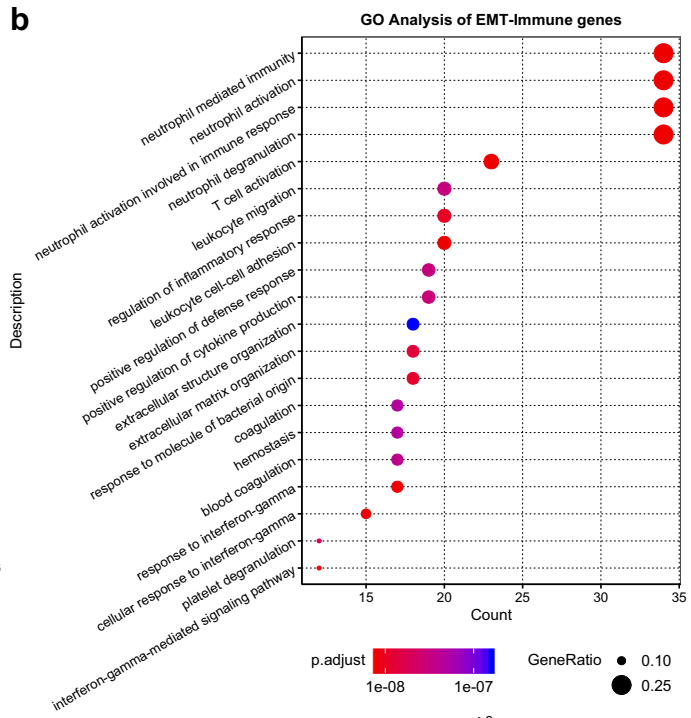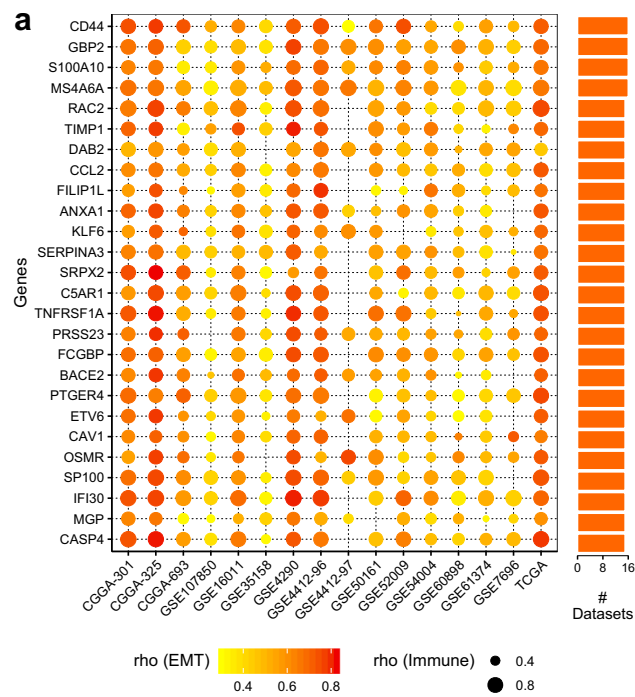
Association of EMT-Related Immune Genes with Clinical Outcomes in Glioma

To characterize the relationship between glioma EMT-related immune genes and clinical outcomes, we built a multivariate model in a forward fashion using relevant variables (age, gender, histology, WHO grade) using TCGA datasets (Table 2). In the univariate analysis, all top 26 EMT-related immune genes were positively associated with a significant increase in mortality of glioma patients, and the top five hazard genes were *OSMR*, *CASP4*, *ANXA1*, *SP100* and *BACE2*. Moreover, using these 26 genes in the multivariate model adjusted by age, gender, histology and WHO grade, 18 EMT-related immune genes were positively associated with poor outcomes of glioma patients, and the top five HR genes were *OSMR*, *BACE2*, *RAC2*, *IFI30* and *TIMP1*. Taken together, these data support the role of EMT-related immune genes in glioma pathogenesis.

◀ **Fig. 4** Association between EMT scores and the glioma immune microenvironment. (**a**) Heatmap of Spearman's correlation between EMT scores and immune scores, stromal scores and the abundance of various immune cells in the TME in GBM (*n* = 155) and LGG (*n* = 510) samples generated using the TCGA dataset. (**b and c**) Spearman correlation analysis between EMT scores and immune scores (ESTIMATE) (**b**) and stromal scores (ESTIMATE) (**c**). (**d and e**) Spearman correlation analysis between EMT scores and macrophages (EPIC) (**d**) and macrophages (ImmuCellAI) (**e**). (**f and g**) Spearman correlation analysis between EMT scores and CD8 T cells (EPIC) (**f**) and CD4 naive cells(ImmuCellAI) (**g**)

Discussion

During malignancy progression, neoplastic cells undergo dynamic and reversible transformations between a spectrum of phenotypic states, the extremes of which are defined by the expression of epithelial and mesenchymal phenotypes (Dongre and Weinberg 2019; Nieto et al. 2016; Tam and Weinberg 2013). In the course of EMT, the interactions between cells and between cells and the extracellular matrix are reshaped, which results in the detachment of epithelial cells

Fig. 5 EMT-related immune genes and their relevant signalling pathways and clinical relevance. (**a**) Correlation between EMT-related immune genes and EMT scores and immune scores. The colour of the dots denotes EMT scores and the area of the dots denotes immune scores. The X-axis is different datasets, and the y-axis is gene names. (**b**) GO signalling pathway enrichment analysis of EMT-related genes identified in more than 12 of the 16 datasets. (**c**) Heat map of the expression of genes (from **a**) in GBM (*n* = 168) and LGG (*n* = 467) samples using the TCGA dataset

Table 1 Background information on the top 26 EMT-related immune genes in cancer biology

CD44	Hou et al., J Neuro-Oncol,2019
GBP2	Miettinen et al., Hum Pathol, 2017
S100A10	Johnson et al., Mol Cell Proteomics, 2012
MS4A6A	Ma et al., J Cancer Res Ther, 2015
RAC2	Zhang et al., J Neurooncol, 2012
TIMP1	Vaitkiene et al., Cells, 2019
DAB2	Bahado-Singh et al., Int J Mol Sci, 2019
CCL2	Flores-Toro et al., P Natl Acad Sci USA, 2019
FILIP1L	Dieterich et al., J Pathol, 2012
ANXA1	Schittenhelm et al., Modern Pathol, 2009
KLF6	Masilamani et al., Oncogene, 2017
SERPINA3	Luo et al., Oncotargets Ther, 2017
SRPX2	Hung et al., Modern Pathol, 2016
C5AR1	Nabizadeh et al., Faseb J, 2019
TNFRSF1A	Wang et al., Ebiomedicine, 2018
PRSS23	Han et al., Pharmacol Res, 2019
FCGBP	Oba-Shinjo et al., Molecular Brain Research, 2005
BACE2	Wang et al., Mol Oncol, 2019
PTGER4	Kambe et al., Cancer Prev Res, 2009
ETV6	Gatalica et al., Modern Pathol, 2018
CAV1	Gu et al., FASEB J, 2019
OSMR	Guo et al., J Cell Physiol, 2019
SP100	Held-Feindt et al., Int J Oncol, 2011
IFI30	Tauber et al., Mol Cancer, 2010
MGP	Fu et al., Mol Neurobiol, 2018
CASP4	Zhong et al., PLoS One, 2012

Bold text indicates genes that were previously studied in glioma

from other cells and their adhesive basement membrane. At the same time, a transcriptional programme is activated to promote mesenchymal fate. Thus, neoplastic cells can successfully eliminate their differentiated epithelial traits, including polarity, cell–cell adhesion and absence of motility, and acquire mesenchymal characteristics, including invasiveness, motility and the stemness of malignancies, which are all associated with high-grade malignancy (Polyak and Weinberg 2009; Thiery et al. 2009). Our studies on the role of EMT in gliomagenesis and progression validated these findings, suggesting that EMT may be a shared characteristic of malignancies. Here, we found that EMT scores increased with the malignant progression of gliomas in different datasets and validated this observation as an excellent indicator for the prognosis of glioma. Behind this clinical significance, various paracrine signalling factors have been reported to trigger the induction of an EMT programme by activating a corresponding diverse cohort of inter- and intracellular signalling

Table 2 Univariate and multivariate analyses of overall survival of TCGA glioma patients using the top 26 EMT-related immune genes

gene	HR	95% CI	p
Univariate			
CD44	1.61	(1.49–1.75)	**3.31e-32**
GBP2	1.09	(1.01–1.17)	**0.0204**
S100A10	1.65	(1.52–1.79)	**6.39e-33**
MS4A6A	1.39	(1.31–1.48)	**2.25e-27**
RAC2	1.63	(1.52–1.76)	**1.55e-40**
TIMP1	1.61	(1.52–1.72)	**2.29e-50**
DAB2	1.24	(1.12–1.38)	**3.22e-05**
CCL2	1.48	(1.37–1.61)	**5.25e-22**
FILIP1L	1.24	(1.16–1.33)	**1.03e-09**
ANXA1	1.73	(1.6–1.87)	**5.54e-45**
KLF6	1.37	(1.26–1.49)	**1.18e-13**
SERPINA3	1.44	(1.35–1.54)	**1.84e-28**
SRPX2	1.66	(1.54–1.78)	**6.39e-42**
C5AR1	1.56	(1.45–1.68)	**4.77e-34**
TNFRSF1A	1.51	(1.42–1.6)	**4.97e-41**
PRSS23	1.27	(1.2–1.35)	**1.33e-16**
FCGBP	1.42	(1.32–1.52)	**9.14e-23**
BACE2	1.68	(1.56–1.82)	**4.35e-40**
PTGER4	1.41	(1.31–1.5)	**8.92e-23**
ETV6	1.23	(1.16–1.3)	**1.57e-13**
CAV1	1.41	(1.31–1.51)	**4.47e-20**
OSMR	1.96	(1.8–2.12)	**7.37e-60**
SP100	1.69	(1.58–1.82)	**6.25e-48**
IFI30	1.52	(1.43–1.61)	**2.27e-43**
MGP	1.34	(1.25–1.44)	**3.43e-16**
CASP4	1.76	(1.65–1.88)	**7.86e-64**
Multivariate (adjusted by age, gender, histology and WHO grade)			
CD44	1.14	(1.02–1.28)	**0.023**
GBP2	0.948	(0.788–1.14)	0.572
S100A10	1.11	(0.986–1.25)	0.0836
MS4A6A	1.09	(0.988–1.21)	0.0839
RAC2	1.26	(1.12–1.43)	**0.000202**
TIMP1	1.23	(1.12–1.34)	**1.88e-05**
DAB2	1.2	(1.08–1.33)	**0.000812**
CCL2	1.14	(1.03–1.25)	**0.00884**
FILIP1L	1.03	(0.922–1.15)	0.587
ANXA1	1.15	(1.03–1.3)	**0.0166**
KLF6	1.13	(1–1.28)	**0.0441**
SERPINA3	1.11	(1–1.23)	**0.0435**
SRPX2	1.17	(1.06–1.29)	**0.00222**
C5AR1	1.17	(1.05–1.3)	**0.00554**
TNFRSF1A	1.13	(0.994–1.28)	0.0615
PRSS23	1.14	(1.02–1.26)	**0.0179**
FCGBP	0.998	(0.897–1.11)	0.964
BACE2	1.29	(1.17–1.43)	**8.12e-07**
PTGER4	1.12	(1–1.24)	**0.0421**
ETV6	1.09	(0.973–1.21)	0.141

Table 2 (continued)

gene	HR	95% CI	p
CAV1	1.06	(0.961–1.17)	0.242
OSMR	1.46	(1.3–1.65)	**1.99e-10**
SP100	1.15	(1.02–1.29)	**0.0196**
IFI30	1.25	(1.13–1.38)	**2.18e-05**
MGP	1.17	(1.06–1.3)	**0.0028**
CASP4	1.23	(1.09–1.39)	**0.000711**

Cox regression was used to assign significance. Significant ($p < 0.05$) results are marked in bold

cascades (Scheel et al. 2011). In response, an array of EMT-inducing transcription factors (TFs), such as SLUG, SNAIL, TWIST and ZEB1, becomes expressed and functionally activated (De Craene and Berx 2013). In addition, EMT can be activated by different extracellular signalling factors or their combinations, such as metabolic end products and immuno-reactive products in the TME.

With regard to the glioma microenvironment, its immune characteristic is most distinctive for the extremely powerful immunosurveillance escape ability (Ito et al. 2019; Lim et al. 2018; Weller and Le Rhun 2019). Compared with melanoma, in which approximately 50% of patients respond to a combination blockade of CTLA-4 and PD-1 immunotherapy and the response is durable in approximately 75% of patients, in glioma only 10% of patients respond to immunotherapy (Jackson et al. 2019). Therefore, in the context of the revolutionary clinical success of immunotherapy for a variety of solid tumours and haematological tumours, it is urgent to determine the molecular pathways underlying glioma immune escape and immunotherapy resistance. In the central nervous system, the first line of immune defence of glioma is the blood–brain barrier. Then, a large number of TAMs (constituting more than 30% of infiltrating cells in GBM) in the TME exert powerful effects to induce immunosuppression. Furthermore, preclinical studies have revealed that the intracranial location of a tumour leads to systemic immunotolerance against relevant tumour antigens (Chongsathidkiet et al. 2018; Hambardzumyan et al. 2016). Regarding the intrinsic resistance in glioma, tumour-infiltrating lymphocytes are scarce, and a previous report showed that the CD4^{+} T cell counts dropped below 300 cells/mm^{3} in most GBM patients who were undergoing temozolo-mide treatment and radiotherapy (Grossman et al. 2011). By preventing internalization of the G protein–coupled receptor sphingosine-1-phosphate receptor 1, GBM and other brain ma-lignancies can sequester a large number of functional T cells in the bone marrow (Chongsathidkiet et al. 2018). For the adaptive resistance of glioma, preclinical studies showed that T cells infiltrating glioma express a series of immune checkpoints and present a severe exhaustion signature. Even so, the response of glioma to checkpoint blockades is poor (Ito et al. 2019). There

is no question that all these resistance mechanisms are involved in the different stages of glioma immunosuppression and are vital for guiding the clinical practices of glioma treatment. There are also other mechanisms, for example, activation of EMT enables carcinoma cells to express several immunoevasive and immunosuppressive molecules that make themselves resistant to CD8^{+} T cell cytoplasmic attack (Akalay et al. 2013). Moreover, EMT can induce the expression of TSP1 and TGFβ, which promote the formation of Treg cells, while EMT-induced expression of CCL2 and LCN2 can pro-mote the formation of tolerance-inducing dendritic cells (DCs) (Kudo-Saito et al. 2013; Sangaletti et al. 2016). The expression of programmed cell death 1 ligand 1 (PD-L1) can be induced by EMT-specific TFs, while the expression of MHC class I can be decreased in mesenchymal neoplastic cells (Noman et al. 2017). Similarly, pro-tumorigenic M2 macrophages can be re-cruited into the TME through mesenchymal tumour cells (Hsu et al. 2014). Even though it is increasingly appreciated that EMT and tumour immunoresistance are inextricably linked in an array of malignant tumours, the interaction of EMT and the immuno-suppressive glioma microenvironment is largely unknown, let alone the molecular mechanisms behind it. Here, however, we revealed the close relationship between EMT scores and the glioma immune microenvironment at the gene level.

In summary, our studies shed new light on the underlying mechanism of EMT and the adaptation of the glioma immune microenvironment. We have not only uncovered the clinical relevance of EMT scores and their excellent prognostic value in glioma patients, but also raised the possibility that EMT signalling pathways may be at the core of glioma immune resistance and tumour relapse from immunotherapy. We found that all top 26 EMT-related immune genes played critical roles in oncobiology. Importantly, we validated their significance with regard to clinical outcomes of glioma patients. Clinically, our findings contribute to the underlying biological basis for the interaction of EMT and the glioma immune environment.

Compliance with Ethical Standards

Conflict of Interest The authors have no conflicts of interest to report.

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