High **EMP3** expression might independently predict poor overall survival in glioblastoma and its expression is related to DNA methylation

Hongsheng Yue, MS\(^a\), Qun Xu, BS\(^b\), Shugang Xie, BS\(^c\)*

**Abstract**

In this study, we analyzed the prognostic value of epithelial membrane protein 3 (**EMP3**) in terms of overall survival (OS) in glioblastoma multiforme (GBM) and the association between its expression and DNA methylation. Bioinformatic analysis was performed using data from The Cancer Genome Atlas (TCGA) database. **EMP3** expression was markedly higher in GBM tissues than in normal brain tissues. High **EMP3** expression was associated with significantly worse OS in patients with GBM. Univariate and multivariate analysis showed that **EMP3** expression was an independent prognostic factor of poor OS no matter converting its expression into categorical variables (Hazard Ratio [HR] = 1.359, 95% CI: 1.118–1.652, \(P = .002\)) or setting it as a continuous variable (HR = 1.178, 95% CI: 1.101–1.260, \(P < .001\)). Among different subtypes of GBM, proneural subtype had the lowest **EMP3** expression. The lowest **EMP3** expression was observed in cluster 5 DNA methylation, which all belong to G-CIMP phenotype. Regression analysis confirmed a moderate negative correlation between **EMP3** expression and its DNA methylation (Pearson’s \(r = -0.61\)).

Based on these findings, we infer that high **EMP3** expression might be an independent indicator of unfavorable OS in GBM. **EMP3** expression might be repressed by DNA methylation.

**Abbreviations:** CIMP = the CpG island methylation phenotype, **EMP3** = epithelial membrane protein 3, GBM = glioblastoma multiforme, IDH1 = isocitrate dehydrogenase 1, OS = overall survival, TCGA = The Cancer Genome Atlas.

**Keywords:** **EMP3**, glioblastoma, methylation, overall survival

1. **Introduction**

Glioblastoma multiforme (GBM) is the most aggressive and malignant intracranial tumor in human.\(^[1]\) The median survival was only around 12 months in the patients treated with surgery and a radiation-containing regimen with concomitant and/or adjuvant temozolomide chemotherapy.\(^[2]\) Verhaak et al\(^[3]\) using data from The Cancer Genome Atlas (TCGA) suggest that GBM has 4 distinct molecular subtypes, including mesenchymal, classical, neural, and proneural subtype characterized by differential expression of PDGFRα, IDH1, EGFR, and NF1.\(^[1]\)

The prognosis of each subtype varies significantly.\(^[4]\) For example, the mesenchymal type usually has overexpression of angiogenic markers and is the most malignant subtype.\(^[4,5]\) In comparison, the proneural type is associated with improved survival, while the neural type has the genetic phenotype most like the normal brain.\(^[1,5]\) Therefore, the study of the molecular mechanisms of different GBM subtypes is necessary for the development of targeted therapeutic strategy.

The epithelial membrane protein 3 (**EMP3**) is a myelin-related gene that belongs to the peripheral myelin protein 22-kDa (PMP22) gene family of small hydrophobic membrane glycoproteins.\(^[6]\) Previous studies reported that **EMP3** might be a tumor suppressor gene that is frequently inactivated by a hypermethylation-mediated transcriptional repression in several types of cancer, such as low-grade glioma,\(^[7]\) esophageal squamous cell carcinoma,\(^[8]\) and non-small cell lung cancer.\(^[9]\) However, one recent study reported that **EMP3** has oncogenic property in GBM, via activating the Transforming growth factor (TGF)-β/Smad2/3 signaling pathway.\(^[10]\) Its overexpression might also predict poor clinical outcome in primary GBMs.\(^[11]\)

Hypermethylation of oncogenes has been characterized as a favorable indicator for GBM patients.\(^[5]\) Isocitrate dehydrogenase 1 (IDH1) mutation has been verified as a favorable prognostic biomarker in patients with GBM,\(^[12]\) and is the molecular basis of the CpG island methylation phenotype (CIMP) in gliomas, which contributes to hypermethylation of a large number of genes.\(^[13]\) For example, hypermethylation of Suppressor of cytokine signaling 3 promoter is associated with favorable prognosis in GBM patients.\(^[14]\) CXCR4 hypermethylation might predict favorable overall survival (OS) in GBM patients.\(^[15]\) **ALDH1A3** promoter methylation may also confer a favorable prognosis in CIMP-primary GBMs.\(^[16]\) In this study, we analyzed the prognostic value of **EMP3** in terms of OS in GBM. In addition, we also examined its expression profiles in different subtypes of GBM and explored its association with DNA methylation and CIMP.
2. Materials and methods

2.1. Bioinformatic analysis of the association between EMP3 expression and OS in patients with GBM

The data of patients with GBM and the corresponding controls were obtained from TCGA-GBM, which was a database supervised by the National Cancer Institute’s Center for Cancer Genomics and the National Human Genome Research Institute.[17] This cohort included 12 biospecimens of normal tissues, 13 biospecimens of recurrent tumors, and 602 biospecimens of primary tumors. The pathological assessment of the biospecimens was performed by 2 independent pathologists to ensure the accuracy.[17] The clinicopathological parameters, including EMP3 expression, at initial diagnosis, gender, Karnofsky Performance Score (KPS), temozolomide chemotherapy, living status and OS in days of the patients in this cohort were downloaded using UCSC Xena Browser (http://xena.ucsc.edu/), which is a bioinformatics tool to visualize functional genomics data from multiple sources, including TCGA data.

Among the 602 cases of primary tumor, 529 had EMP3 expression measured by RNA array (AffyU133a). A total of 523 out the 529 cases that had intact OS data were included in survival analysis. The patients were divided into 2 groups by median EMP3 expression. Kaplan–Meier curves of OS were generated by using GraphPad Prism 6.0 (GraphPad Software, Inc.).

2.2. Bioinformatic analysis of EMP3 expression and its methylation status across different subtypes of GBM

Since GBM subtypes, RMP3 RNA expression, DNA methylation, CpG island methylation phenotype, and IDH1 mutation were measured in different patients, all primary patients were included in methylation related analysis to give an overall map. EMP3 expression, its methylation status and CIMP across different subtypes of GBM (proneural, neural, classical, and mesenchymal) were examined by data mining in TCGA-GBM using UCSC Xena Browser.

2.3. Statistical analysis

Statistical analysis was performed by using GraphPad Prism 6.0 and SPSS 19.0 (IBM SPSS Statistics). The association between EMP3 RNA expression and the clinicopathological features in patients with primary GBM was assessed by using χ² tests. Log-rank test was used to assess the significance of the difference between the Kaplan–Meier curves. Univariate and multivariate Cox regression models were used to assess prognostic significance. Welch’s t-test was conducted to compare EMP3 RNA expression between different subgroups. Regression analysis was performed to assess the correlation between EMP3 expression and its DNA methylation. \( P < .05 \) was considered statistically significant.

3. Results

3.1. EMP3 is significantly upregulated in GBM

By using data from TCGA-GBM, we characterized EMP3 expression in 10 cases of normal brain tissues and 529 cases of GBM (Fig. 1A). EMP3 expression was more than 10 times higher in GBM tissues than in normal brain tissues (\( P < .001 \)) (Fig. 1A).

3.2. EMP3 expression might be an independent predictor of poor OS in patients with GBM

One recent study reported that EMP3 has oncogenic properties in GBM.[10] Based on data in TCGA, we further assessed the association between EMP3 expression and OS curves among the patients. The patients were divided into high and low EMP3 expression groups according to the median EMP3 expression. The association between EMP3 expression and the clinicopathological features was summarized in Table 1. The high EMP3 expression group had a significantly older age (59.39 ± 12.90 vs 55.87 ± 15.89, \( P = .006 \)) and a substantially lower ratio of living (33/261 vs 56/262, \( P = .008 \)) (Table 1). Log-rank test of the Kaplan–Meier OS curves showed that high EMP3 expression was associated with significantly worse OS (\( P < .001 \), Fig. 1B). In univariate analysis, higher age (≥57), low Karnofsky Performance Score (KPS) (≤80), no temozolomide chemotherapy and high EMP3 expression was associated with shorter OS (Table 2). By setting EMP3 expression as a continuous variable, it was also associated with unfavorable OS (Table 2). Multivariate analysis showed that EMP3 expression was an independent prognostic factor of poor OS no matter converting its expression into categorical variables (HR = 1.359, 95% CI: 1.118–1.632, \( P = .002 \)) or setting it as a continuous variable (HR = 1.178, 95% CI: 1.101–1.260, \( P < .001 \)) (Table 2).
3.3. EMP3 expression varies significantly among different subtypes of GBM

By data mining in TCGA database, we characterized the expression profiles of EMP3 in different subtypes of primary GBM. Among the patients with characterized molecular subtypes and EMP3 expression, the proneural subtype had the lowest EMP3 expression, while the classical subtype and the mesenchymal subtype had the highest and 2nd highest EMP3 expression respectively (Fig. 2A and B).

3.4. EMP3 expression might be modulated by its DNA methylation

Then, we tried to explore the mechanism of EMP3 dysregulation in GBM. By grouping GBM patients according to DNA methylation subtype (syn1701558), we found that cluster 5 DNA methylation had the lowest EMP3 expression and the highest level of EMP3 DNA methylation (Fig. 3A and B). These results suggested that EMP3 expression might be modulated by its DNA methylation status in GBM. To further verify this finding, we assessed the association between EMP3 expression and glioma CIMP (G-CIMP). Patients in cluster 5 DNA methylation all belong to G-CIMP (Fig. 3A, black frame). G-CIMP was enriched in the proneural subgroup and had a significantly lower EMP3 expression than the non-G-CIMP group (P < .001) (Fig. 3C). In TCGA-GBM, 279 cases had EMP3 expression (AffyU133a) and DNA methylation (methylation 27k) measured at the same time. Regression analysis confirmed a moderate negative correlation between EMP3 expression and its DNA methylation (Pearson’s r = −0.61) (Fig. 3D).

4. Discussion

The relationship between EMP3 expression and tumor has been studied by a series of previous studies, with controversial results of tumor suppressive or oncogenic role in different cancers. For example, EMP3 might act as a tumor suppressor in esophageal squamous cell carcinoma and in nonsmall cell lung cancer.[8-9] In comparison, in upper urinary tract urothelial carcinoma, EMP3 can enhance cancer cell proliferation and migration through activating the ErbB2-PI3K-AKT pathway.[18] EMP3 upregulation and its correlation with differentiative degree were also observed in hepatocellular carcinoma (HCC).[19] Mechanistically, EMP3 can promote HCC progression via enhancing the PI3K/Akt pathway and uPA/MMP-9 cascade.[19] These findings suggest that the function of EMP3 in human cancers might be multi-facet, depending on specific type of cancer. Although EMP3 was initially identified as a tumor suppressor in low-grade glioma, its tumor suppressive role is still controversial. Previous studies found that EMP3 expression was significantly higher in GBM than in non-neoplastic white matter,[11] and was associated with significantly worse OS in WHO grade II-IV GBM.[20] Another recent study reported that in GBM cells, EMP3 directly interacts with TGFBR2 upon TGF-β stimulation, which subsequently activates TGF-β/Smad2/3 signaling activation and enhances cell proliferation in vitro and in vivo.[10] In this study, we compared EMP3 expression in GBM and in normal brain tissues in TCGA-GBM and confirmed significantly deregulated EMP3 in GBM. By generating Kaplan–Meier curves of OS, we found that high EMP3 expression was significantly associated with unfavorable OS. Univariate and multivariate analysis showed high EMP3 expression was an independent prognostic factor of poor OS. These findings imply that EMP3 upregulation might serve as a biomarker predicting patient prognosis.

In the 4 subtypes of GBM, we found that the proneural subtype had the lowest expression of EMP3. Since CIMP results in hypermethylation of a large number of genes in GBM, we further investigated whether the variation of EMP3 is related to CIMP in different subtypes of GBM. In our study, we observed that the...
IDH1 mutant cohort was in agreement with the G-CIMP phenotype. In addition, the G-CIMP phenotype had the highest level of EMP3 methylation and the lowest expression of EMP3. By comparing the expression of EMP3 in different DNA methylation subtypes (syn1701558), we observed that cluster 5 methylation had the lowest EMP3 expression. More importantly, regression analysis confirmed a moderate negative correlation between EMP3 expression and its DNA methylation. These correlations were evident in the heat maps and box plots shown in Figure 2 and Figure 3.
findings suggest that EMP3 expression might be repressed by DNA methylation in GBM.

CIMP indicates methylation status when a large number of gene loci are simultaneously hypermethylated.\[14\] CIMP was observed in several types of solid tumors, such as gastric cancer,\[21\] colorectal cancer,\[22\] ovarian cancer,\[23\] liver cancer,\[24\] and glioma.\[25\] In different types of cancer, CIMP might indicate different survival outcomes. For example, patients with high CIMP gastric cancer had significantly worse survival compared with patients with CIMP-low/CIMP-negative gastric cancer.\[24\] \[24\] In different types of cancer, CIMP might be developed by bioinformatic analysis in TCGA-GBM. Although methylation on studies to demonstrate the direct regulative effect of DNA is not recorded in the database.\[8\] logical information, such as treatment history of the patients were study also has some limitations. Firstly, the key patients, which ensure a relatively high reliability. However, this study also has some limitations. Firstly, the key findings were developed by bioinformatic analysis in TCGA-GBM. Although we identified a negative correlation between EMP3 expression and its DNA methylation status, we did not perform molecular studies to establish the direct regulatory effect of DNA methylation on EMP3 expression. Secondly, some clinicopathological information, such as treatment history of the patients were not recorded in the database.

5. Conclusion

High EMP3 expression might be an independent indicator of unfavorable OS in GBM. EMP3 expression might be repressed by DNA methylation, which is highly consistent with G-CIMP phenotype.

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