Observational Study

Medicine®

OPEN

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 by 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 PDGFRA, 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.[3,5] In comparison, the proneural type is associated with improved survival, while the neural type has the genetic phenotype most like the normal brain.[3,5] 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.

Editor: Peng Luo.

The authors have no conflicts of interest to disclose.

[a] *Department of Neurosurgery, Jinan Central Hospital Affiliated to Shandong University, Jinan,* [b] *Nursing Department, Jinan Vocational College of Nursing,* [c] *Department of Neurosurgery, Shanghe County People's Hospital, Jinan, Shandong, 251600, China.*

[*] *Correspondence: Shugang Xie, Shanghe County People's Hospital, Jinan, Shandong, China (e-mail: shugangxie@tom.com).*

Medicine (2018) 97:1(e9538)

Received: 2 October 2017 / Received in final form: 20 November 2017 / Accepted: 11 December 2017

http://dx.doi.org/10.1097/MD.0000000000009538

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, age 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 χ^2 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: 1.118–1.652, $P = .002$) or setting it as a continuous variable (HR = 1.178, 95%CI: 1.101–1.260, $P < .001$) (Table 2).

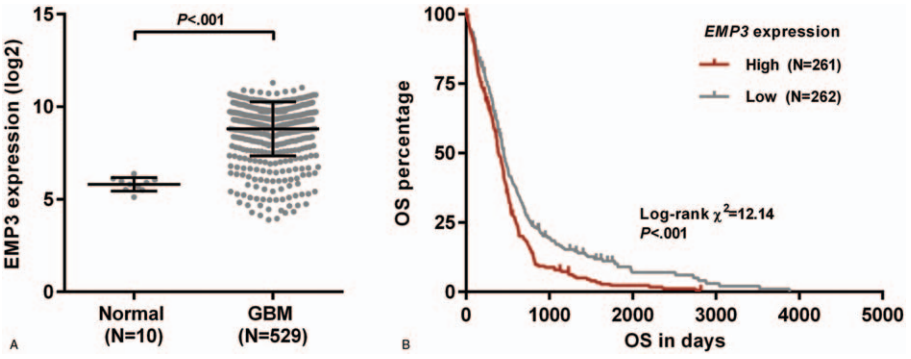

Figure 1. *EMP3* expression is upregulated and is negatively associated with OS in patients with GBM. (A) *EMP3* expression in normal brain tissues (N = 10) and GBM tissues (N = 529). (B) Kaplan–Meier curves of OS in GBM patients. Patients were subjected to two-group analysis according to median *EMP3* expression. Log-rank test was performed to assess the significance of the difference. EMP3 = epithelial membrane protein 3, GBM = glioblastoma multiforme, OS = overall survival.

Table 1

Demographic and clinicopathological parameters of patients with primary GBM in TCGA-GBM.

Parameters		*EMP3* mRNA expression High (N = 261)	Low (N = 262)	*P* value
Age (mean ± SD)		59.39 ± 12.90	55.87 ± 15.89	.006
Gender	Female	105	100	.63
	Male	156	162	
KPS score	≤ 80	157	168	.17
	> 80	39	29	
	Null	65	65	
Temozolomide chemotherapy	True	151	150	.85
	False	102	98	
	Null	8	14	
Living status	Living	33	56	.008
	Dead	228	206	

EMP3 = epithelial membrane protein 3, KPS = Karnofsky performance score.

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 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 differentiated 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

Table 2

Univariate and multivariate analyses of OS in patients with primary GBM in TCGA-GBM.

Parameters	Univariate analysis HR	95%CI	*P*	Multivariate analysis HR	95%CI	*P*
Age ≥ 57 vs < 57	1.953	1.602–2.381	<.001	1.859	1.517–2.278	<.001
Gender female vs male	0.851	0.700–1.034	.105	0.823	0.675–1.003	.053
KPS score < 80 vs ≥ 80	1.462	1.090–1.961	.011	1.393	1.030–1.885	.032
Temozolomide chemotherapy true vs false	0.557	0.458–0.678	<.001	0.572	0.466–0.702	<.001
EMP3 expression high vs low	1.401	1.158–1.696	.001	1.359	1.118–1.652	.002
Age ≥ 57 vs < 57	1.953	1.602–2.381	<.001	1.775	1.447–2.178	<.001
Gender female vs male	0.851	0.700–1.034	.105	0.815	0.668–0.993	.043
KPS score ≤ 80 vs >80	1.462	1.090–1.961	.011	1.455	1.073–1.972	.016
Temozolomide chemotherapy true vs false	0.557	0.458–0.678	<.001	0.547	0.445–0.673	<.001
EMP3 expression (Continuous)	1.180	1.105–1.260	<.001	1.178	1.101–1.260	<.001

EMP3 = epithelial membrane protein 3, GBM = glioblastoma multiforme, HR = hazard ratio, KPS = Karnofsky performance score.

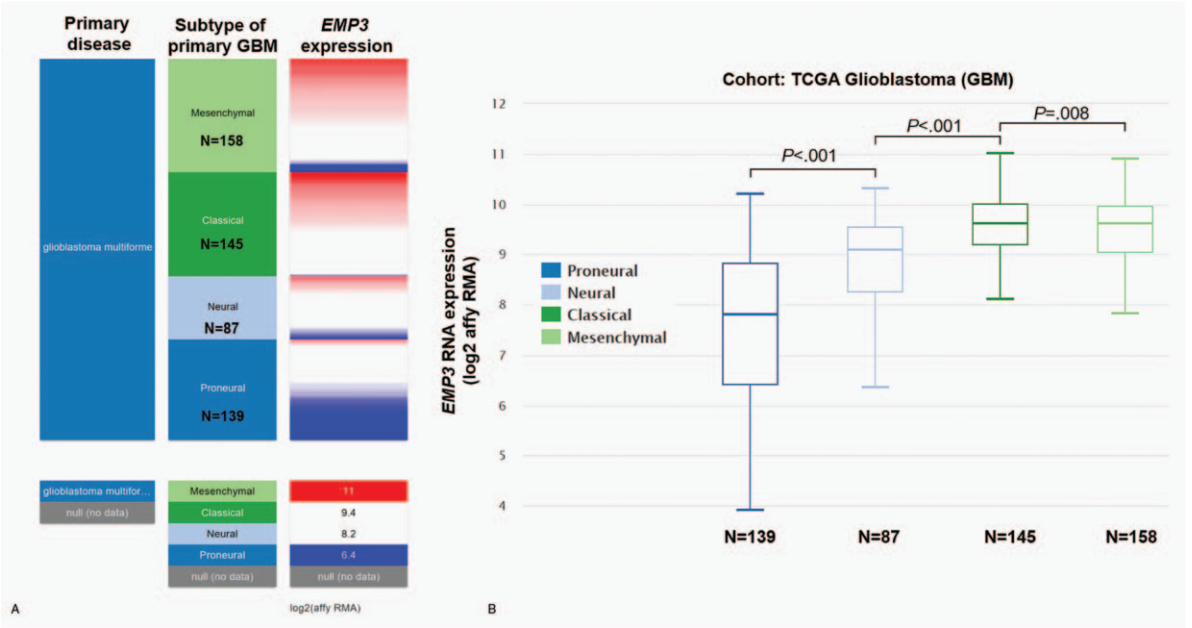

Figure 2. *EMP3* expression varies significantly among different subtypes of GBM. (A, B) The heat map (B) and box plots (B) of *EMP3* expression in different subtypes of GBM. EMP3 = epithelial membrane protein 3, GBM = glioblastoma multiforme.

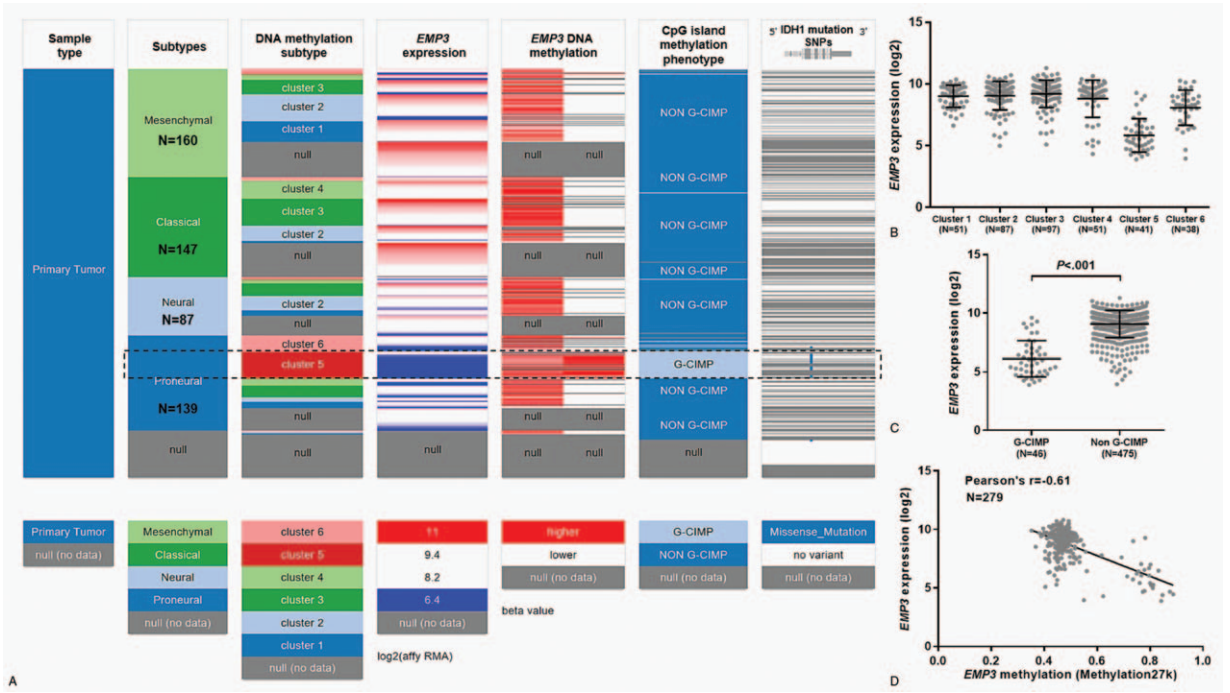

Figure 3. *EMP3* expression in GBM might be modulated by DNA methylation. (A) The heat map of DNA methylation subtype (syn1701558) (cluster 1 to 6, the lowest to the highest), *EMP3* expression, *EMP3* DNA methylation (methylation 27k), CpG island methylation phenotype (G-CIMP and non-G-CIMP) and IDH1 mutation SHPs in different subtypes of GBM. Black frame indicates the correlation among cluster 5 DNA methylation, low *EMP3* expression, high *EMP3* DNA methylation, G-CIMP and IDH1 mutation. (B) The expression of *EMP3* in different cluster of DNA methylation. (C) The expression of *EMP3* in G-CIMP and non-G-CIMP groups. (D) Regression analysis of the correlation between *EMP3* expression and its DNA methylation. CIMP = the CpG island methylation phenotype, EMP3 = epithelial membrane protein 3, GBM = glioblastoma multiforme.

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 subtype (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

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.[21] HCC patients with high CIMP had about a 3.6-fold increase in recurrence risk after liver transplantation compared to patients with low CIMP.[24] However, in patients with poorly infiltrated colorectal cancer, CIMP-low was associated with particularly poor prognosis.[26] In patients with glioma, CIMP is enriched in the proneural subgroup and is usually associated with improved survival outcome.[25] These results suggest that methylation status of the whole cancer genome does not necessarily indicate prognosis. Instead, it is the specific genes regulated by methylation determine survival outcomes.[27]

TCGA-GBM was initiated in 2005 and had over 10 years' survival data and genomic deep-sequencing data in around 500 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 demonstrate the direct regulative 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.

References

[1] Kloosterhof NK, de Rooi JJ, Kros M, et al. Molecular subtypes of glioma identified by genome-wide methylation profiling. Genes Chromosomes Cancer 2013;52:665–74.

[2] Johnson DR, O'Neill BP. Glioblastoma survival in the United States before and during the temozolomide era. J Neurooncol 2012;107: 359–64.

[3] Verhaak RG, Hoadley KA, Purdom E, et al. Integrated genomic analysis identifies clinically relevant subtypes of glioblastoma characterized by abnormalities in PDGFRA, IDH1, EGFR, and NF1. Cancer Cell 2010; 17:98–110.

[4] Erdem-Eraslan L, Gravendeel LA, de Rooi J, et al. Intrinsic molecular subtypes of glioma are prognostic and predict benefit from adjuvant procarbazine, lomustine, and vincristine chemotherapy in combination with other prognostic factors in anaplastic oligodendroglial brain tumors: a report from EORTC study 26951. J Clin Oncol 2013;31:328–36.

[5] Taylor LP. Diagnosis, treatment, and prognosis of glioma: five new things. Neurology 2010;75(18 suppl 1):S28–32.

[6] Taylor V, Suter U. Epithelial membrane protein-2 and epithelial membrane protein-3: two novel members of the peripheral myelin protein 22 gene family. Gene 1996;175:115–20.

[7] Alaminos M, Davalos V, Ropero S, et al. EMP3, a myelin-related gene located in the critical 19q13.3 region, is epigenetically silenced and exhibits features of a candidate tumor suppressor in glioma and neuroblastoma. Cancer Res 2005;65:2565–71.

[8] Fumoto S, Hiyama K, Tanimoto K, et al. EMP3 as a tumor suppressor gene for esophageal squamous cell carcinoma. Cancer Lett 2009;274: 25–32.

[9] Xue Q, Zhou Y, Wan C, et al. Epithelial membrane protein 3 is frequently shown as promoter methylation and functions as a tumor suppressor gene in non-small cell lung cancer. Exp Mol Pathol 2013;95: 313–8.

[10] Jun F, Hong J, Liu Q, et al. Epithelial membrane protein 3 regulates TGF-beta signaling activation in CD44-high glioblastoma. Oncotarget 2016;8:14343–58.

[11] Scrideli CA, Carlotti CGJr, Okamoto OK, et al. Gene expression profile analysis of primary glioblastomas and non-neoplastic brain tissue: identification of potential target genes by oligonucleotide microarray and real-time quantitative PCR. J Neurooncol 2008;88:281–91.

[12] Hartmann C, Hentschel B, Wick W, et al. Patients with IDH1 wild type anaplastic astrocytomas exhibit worse prognosis than IDH1-mutated glioblastomas, and IDH1 mutation status accounts for the unfavorable prognostic effect of higher age: implications for classification of gliomas. Acta Neuropathol 2010;120:707–18.

[13] Turcan S, Rohle D, Goenka A, et al. IDH1 mutation is sufficient to establish the glioma hypermethylator phenotype. Nature 2012;483: 479–83.

[14] Feng Y, Wang Z, Bao Z, et al. SOCS3 promoter hypermethylation is a favorable prognosticator and a novel indicator for G-CIMP-positive GBM patients. PLoS One 2014;9:e91829.

[15] Ma X, Shang F, Zhu W, et al. CXCR4 expression varies significantly among different subtypes of glioblastoma multiforme (GBM) and its low expression or hypermethylation might predict favorable overall survival. Expert Rev Neurother 2017;17:941–6.

[16] Zhang W, Yan W, You G, et al. Genome-wide DNA methylation profiling identifies ALDH1A3 promoter methylation as a prognostic predictor in G-CIMP- primary glioblastoma. Cancer Lett 2013;328: 120–5.

[17] Cancer Genome Atlas Research N. Comprehensive genomic characterization defines human glioblastoma genes and core pathways. Nature 2008;455:1061–8.

[18] Wang YW, Li WM, Wu WJ, et al. Potential significance of EMP3 in patients with upper urinary tract urothelial carcinoma: crosstalk with ErbB2-PI3K-Akt pathway. J Urol 2014;192:242–51.

[19] Hsieh YH, Hsieh SC, Lee CH, et al. Targeting EMP3 suppresses proliferation and invasion of hepatocellular carcinoma cells through inactivation of PI3K/Akt pathway. Oncotarget 2015;6:34859–74.

[20] Ernst A, Hofmann S, Ahmadi R, et al. Genomic and expression profiling of glioblastoma stem cell-like spheroid cultures identifies novel tumor-relevant genes associated with survival. Clin Cancer Res 2009;15: 6541–50.

[21] Chen HY, Zhu BH, Zhang CH, et al. High CpG island methylator phenotype is associated with lymph node metastasis and prognosis in gastric cancer. Cancer Sci 2012;103:73–9.

[22] Dahlin AM, Palmqvist R, Henriksson ML, et al. The role of the CpG island methylator phenotype in colorectal cancer prognosis depends on microsatellite instability screening status. Clin Cancer Res 2010;16: 1845–55.

[23] Strathdee G, Appleton K, Illand M, et al. Primary ovarian carcinomas display multiple methylator phenotypes involving known tumor suppressor genes. Am J Pathol 2001;158:1121–7.

[24] Wu LM, Zhang F, Zhou L, et al. Predictive value of CpG island methylator phenotype for tumor recurrence in hepatitis B virus-associated hepatocellular carcinoma following liver transplantation. BMC Cancer 2010;10:399.

[25] Noushmehr H, Weisenberger DJ, Diefes K, et al. Identification of a CpG island methylator phenotype that defines a distinct subgroup of glioma. Cancer Cell 2010;17:510–22.

[26] Dahlin AM, Henriksson ML, Van Guelpen B, et al. Colorectal cancer prognosis depends on T-cell infiltration and molecular characteristics of the tumor. Mod Pathol 2011;24:671–82.

[27] Weisenberger DJ, Siegmund KD, Campan M, et al. CpG island methylator phenotype underlies sporadic microsatellite instability and is tightly associated with BRAF mutation in colorectal cancer. Nat Genet 2006;38:787–93.