

Molecular Characterization of AEBP1 at Transcriptional Level in Glioma

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Molecular characterization of AEBP1 at transcriptional level in glioma

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Abstract

Background: Glioma is the most common malignant tumor of brain in adult patients. The standardized treatment protocol was based on surgical therapy, supplemented with radiotherapy and chemotherapy. However, the prognosis was still unsatisfied. The chemoresistance is one of the most important reason for the poor prognosis of glioma patients. It has confirmed that glioma stem cell (GSC) are one of the reasons for chemoresistance.

Methods: In this study three datasets (GSE23806, COSMIC, TCGA) was used to perform the analysis to search for the key genes related to GSC, temozolomide (TMZ) resistance and prognosis. The key gene for further research was selected by reviewing the previous studies. The selected gene was investigated the relation between expression levels and clinical characteristics in both TCGA and CGGA dataset. The bioinformatics analysis was performed by Gene ontology (GO) analysis. The survival analysis was performed by Kaplan–Meier survival analysis.

Results: AE binding protein 1 (AEBP1) was selected for further analysis. AEBP1 was overexpressed in GSCs and TMZ resistance cells. In both TCGA and CGGA dataset, the results showed that the expression level of AEBP1 was increased in glioblastoma (GBM) samples, IDH wild-type samples and MGMT promoter unmethylated samples. Meanwhile, AEBP1 expression was positive related with several GSC markers. GO analysis showed that AEBP1 were related with immune response, cell adhesion, apoptotic process, inflammatory response, positive regulation of cell proliferation, angiogenesis, response to drug and response to hypoxia. The survival analysis showed that the overexpressed level of AEBP1 was correlated with short survival time in both glioma and GBM patients.

Conclusion: In summary, AEBP1 was related with GSC induced TMZ resistance. Our study showed that AEBP1 might be an oncogene and a new effective therapeutic target for the treatment of glioma.

Keywords: glioma, chemoresistance, glioma stem cells, AEBP1

Introduction

Glioma is the most prevalent type of primary brain tumor in adults which is devastated to patients. According to the 2016 World Health Organization (WHO) CNS tumors classification, glioblastoma multiforme (GBM) is the most malignant type of glioma with a median survival 14.4 months^{1,2}. Despite the therapies including surgery, chemotherapy and radiation are improved, the treatment effects are still unsatisfied especially in GBM patients. New effective treatments are urgent needed to further improve the treatment effect.

The standardized drug used in chemotherapy for glioma is temozolomide (TMZ) which has been defined to improve the clinical outcomes when used alone or in combination with radiotherapy. However, the effect often lasts a short time when patients suffered chemoresistance. The mechanism of chemical resistance remains unclear. Several researches have focused on the glioma stem cells (GSC) which may be associated with chemoresistance.

Glioma stem cells (GSCs) are designated as a subpopulation of tumor cells which show a similar characterization of neural stem cells³. It has been defined that GSCs have the characterization of self-renewal, long-term proliferation and multi-lineage differentiation potential in several researches⁴⁻⁶. GSCs may survive during chemotherapy and differentiate to tumor cells which proliferate rapidly. It could lead to chemoresistance and tumor recurrences⁷. Recent years, several genes have been reported as signatures of GSCs. CD133 (PROM1) is one of the most reported genes as a GSC biomarker⁸. It has been reported that glioma cells with CD133 positive can grow spheres in serum free medium, whereas CD133 negative glioma cells can not^{5,9}. It has been demonstrated that the tumorigenicity could be weakened when CD133 was knocking down⁹. SRY-box transcription factor 2 (SOX2) is another signature of GSCs which overexpression in GSCs. The glioma cells could be stopped proliferation and loss tumorigenicity when SOX2 was silenced¹⁰.

However, the researches for GSC related genes to predict prognosis and chemoresistance are insufficient. Under these circumstances, we focused on these GSC related genes to search for a new biomarker in glioma which was also related to chemoresistance. In the present study, we discovered that the mRNA expression of AE binding protein 1 (AEBP1) was overexpressed in TMZ chemoresistance cell lines and glioma stem cell lines. AEBP1 was a coding protein which encoded a member of carboxypeptidase A protein family. In the previous researches, it was defined that AEBP1 was overexpressed in GBM and related with many types of tumor such as colon adenocarcinoma and gastric cancer¹¹⁻¹³. So we focused on AEBP1 to research for its molecular and clinical characterization in glioma.

Methods

Samples

In this research, two datasets were used to select the target gene which related with the GSCs and chemoresistance. GSE23806 dataset contains RNA microarray data of glioma stem cell lines and conventional cell lines and was downloaded from the public website (<https://www.ncbi.nlm.nih.gov/geo/>). The COSMIC dataset contains the half maximal inhibitory concentration (IC50) to TMZ and the RNA microarray data of several cell lines and was obtained from the public website (https://cancer.sanger.ac.uk/cell_lines/). Another two datasets were used to do the analysis of the AEBP1 characterization. The Cancer Genome Atlas (TCGA) RNA sequencing dataset was obtained from the website (<http://cancergenome.nih.gov>) which containing 699 glioma samples. The Chinese Genome Atlas (CGGA) RNA sequencing dataset was obtained from the

website (<http://www.cgga.org.cn>) which containing 693 glioma samples. The expression value of the RNA sequencing data was log transformed before analysis. Thus, in total, there were 1392 samples include in this research.

Bioinformatics analysis

Gene ontology (GO) analysis was used to do the biological function analysis¹⁴. R programming language was used to analyze the correlation between AEBP1 expression and other genes expression with the Pearson correlation analysis. The positive related genes were selected to do the GO analysis by DAVID (<https://david.ncifcrf.gov/>). The figure was plot by R programming language.

Statistical analysis

In this study, R programming language software was used to perform the analysis and draw the figures. The analysis of the different expression levels of AEBP1 between different grades, IDH status and MGMT promoter status was used by Student's t-test. The survival analysis was performed by Kaplan–Meier (K-M) survival analysis. The circlize figure was analyzed and plot by the R programming language software.

Results

Gene selection

To search for the key gene related with GSCs and chemoresistance, the GSE23806 dataset and the COSMIC dataset were analyzed first. In GSE23806 dataset, the different expression genes between glioblastoma stem-like cell line (n=27) and conventional glioma cell line (n=36) were carried out by the Student's t-test. The genes which showed high expression in glioblastoma stem-like cell line (n=6752) were selected for further analysis. In COSMIC dataset the IC50 of TMZ was given, according to the median IC50, the samples were divided into two groups: TMZ sensitive group and TMZ resistant group. The Student's t-test was carried out to find out the different expression genes between TMZ sensitive group and TMZ resistant group. The high expression genes in TMZ resistant group (n=447) were selected for further analysis. In TCGA RNA sequencing dataset, the genes related with malignant survival in GBM patients which carried out by univariate Cox regression (n=1456) were selected for the next analysis. Only five genes were in all the three groups of the analysis result including AEBP1 which had been reported overexpressed in GBM (Fig.1A). As it was showed in the figures, the RNA expression level of AEBP1 was higher in GSCs and TMZ resistance cell lines than the non-GSCs and TMZ sensitive cell lines (Fig.1B, C).

AEBP1 expression characterization in glioma

During the analysis, two datasets (TCGA RNA sequencing dataset and CGGA RNA sequencing dataset) including 1392 samples were used to do the analysis. The expression level of AEBP1 was analyzed during different grades of glioma in two datasets and the results showed that it was higher in GBM patients than lower grade glioma patients (Fig.2A, B). This result implied that the overexpression of AEBP1 was related with higher malignancy. According to the previous reports, isocitrate dehydrogenase (IDH) mutation was related with the overall survival and could be used as a sub-classifier. So the relationship between IDH mutation and AEBP1 expression was detected in the datasets. As a result, it was showed that the higher AEBP1 expression was enriched in IDH

wildtype glioma, which indicated the possibility of AEBP1 to be used as a predictive factor (Fig.3A, B). The relation between MGMT promoter methylation and AEBP1 expression was also analyzed and the result showed that the expression of AEBP1 was higher in MGMT promoter unmethylated glioma (Fig.3C, D).

AEBP1 was a potential marker for GSC

During our research, it was found that AEBP1 was upregulated in GSCs. Thus, we chose several GSC related genes according to previous articles¹⁵ to get further research of the relation between AEBP1 and GSC markers. The relation between AEBP1 and GSC markers was analyzed by Pearson correlation analysis in the two datasets and the results showed that the expression of several GSC markers were positive related with AEBP1 expression, such as CD133, CD44, FUT4, IL6 and STAT3 (Fig.4A, B). IL6/STAT3 pathway had been demonstrated that played a contributing factor for multidrug resistance in cancer¹⁶. This result demonstrated that AEBP1 may play an important role in GSCs and lead to tumorigenicity and chemoresistance.

AEBP1 related biological process

Based on the above discoveries, it was supposed that AEBP1 may play an important role in the biological process of glioma. To investigate the potential function of AEBP1 in glioma, Gene Ontology (GO) analysis was performed in the datasets. The positive related genes were found out by Pearson correlation analysis which $R > 0.5$ and analyzed with DAVID. As the results showed, the positive related genes with AEBP1 were highly enriched in immune response, cell adhesion, apoptotic process, inflammatory response, positive regulation of cell proliferation, angiogenesis, response to drug and response to hypoxia in GO terms (Fig.5A-D). All the results showed above indicated that AEBP1 might play an important role in the malignant progression and chemoresistance in glioma.

AEBP1 was a prognostic factor for GBM patients

To evaluate the influence of survival, we tested the prognostic value of AEBP1 expression in the datasets. Kaplan–Meier (K-M) survival curve analysis was used for the analysis. The results showed that glioma patients with higher AEBP1 expression have a shorter overall survival (OS) time (Fig.6A, B). Because of the heterogeneity between different grades of glioma, we analyzed the prognostic value of AEBP1 in GBM patients additionally. Similar results were obtained in GBM patients that patients with higher AEBP1 expression have a shorter OS time (Fig.6C, D). These results demonstrated that AEBP1 might be an effective prognostic biomarker for glioma patients, especially GBM patients.

Discussion

Glioma is the most common primary malignant tumor in central nervous system. Even the treatment is advanced, the median survival time of GBM is still unsatisfied. The origin and development of glioma is a complex process with multiple factors and steps. Following the advent of second-generation sequencing technology, the abnormal metabolic reorganization and significant cellular and molecular heterogeneity was detected in glioma cells which explains the poor conventional treatment effects, easy tumor recurrence after treatment and difficulty in the

availability of effective and innovative treatments. TMZ is the first-line chemotherapeutic drug for malignant glioma. The effective rate of TMZ in treating glioma is about 45%. The main reason for the failure of chemotherapy is the resistance of TMZ. Several studies have found that TMZ resistance in glioma is a combination of multiple factors, including DNA damage repair, glioma stem cell and the expression of oncogenes and tumor suppressor genes in tumor cells.

In this study, we focused on TMZ resistance caused by GSCs. By analyzing the COSMIC and GSE23806 datasets, we found that the mRNA expression level of AEBP1 was upregulated in TMZ resistant cell lines and glioma stem cell lines. AEBP1 encoded a member of the carboxypeptidase A protein family located on Chr7. AEBP1 is widely present in fat, liver, brain and lung tissues, but rarely detected in blood¹⁷. The encoded protein might act as a transcriptional repressor and play an important role in adipogenesis and smooth muscle cell differentiation. In addition, some studies have found that the expression level of AEBP1 in the proliferative precursor adipocytes was higher, while in the terminally differentiated non-proliferative adipocytes disappeared¹⁸.

Further analysis was performed in TCGA and CGGA datasets and the results showed that AEBP1 expression in GBM patients was significantly higher than that in lower grade glioma patients. It was also found that AEBP1 expression was overexpressed in IDH wild type glioma and MGMT promoter unmethylation type glioma. In many previous researches, it has been demonstrated that the prognosis of IDH wild type was malignant. This result is consistent with the poor prognosis of patients with high expression of AEBP1. The status of MGMT promoter was demonstrated as a factor to predict TMZ sensitivity and the MGMT promoter unmethylation type glioma was not sensitive to TMZ. AEBP1 was overexpressed in MGMT promoter unmethylation type glioma which might be an important factor in predicting TMZ sensitivity.

Several studies have confirmed that glioma stem cell was an important cause of resistance to radiation therapy and chemotherapy in glioma¹⁹⁻²¹. It has been reported that glioma stem cells may survive after chemotherapy and then differentiate into tumor cells and cause tumor recurrence⁷. Through our research, it was demonstrated that the expression of AEBP1 was significantly upregulated in GSCs, and the expression of multiple GSC markers is positively correlated with the expression of AEBP1, such as CD133, CD44, FUT4, IL6 and STAT3. Several studies have confirmed that the IL6/STAT3 pathway is closely related to tumorigenesis and self-renewal caused by GSCs. This result indicated that AEBP1 might play an important role in maintaining the survival of GSCs and lead to tumorigenicity and chemotherapy resistance. These results suggested that the relevant targeted therapy can be used to block the signal transduction pathways related to GSCs growth, which might be a new direction of glioma treatment.

In this study, the bioinformatics analysis was also performed. The results showed that the genes positive related with AEBP1 was enriched in functions such as immune response, cell adhesion, apoptotic process, inflammatory response, positive regulation of cell proliferation, angiogenesis, response to drug and response to hypoxia. In these results, AEBP1 was closely related to inflammation response and immune response which might lead to immune escape of tumor cells. It indicated that the AEBP1 might suppress the immune response through certain pathways. Therefore, it was inferred that the antibody of AEBP1 may be a new select for effective treatment in glioma patients. In addition, AEBP1 is positively related with response to drug and apoptotic process, which may be one of the reasons for the high expression of AEBP1 causing resistance to TMZ chemotherapy. The positive related genes with AEBP1 were also enriched in positive regulation of cell proliferation, angiogenesis, response to hypoxia and cell adhesion which related with the

malignant progression of glioma.

The survival analysis was performed and the results showed that the patients with higher AEBP1 expression had a shorter overall survival time than the patients with lower AEBP1 expression. This result indicated that AEBP1 might be used as a prognostic biomarker for glioma patients. The relevant targeted therapy might be effectively in glioma patients with AEBP1 overexpression.

Conclusion

In conclusion, the results described above revealed that AEBP1 might be an oncogene in glioma. It might be related to GSC and chemoresistance. The upregulation of AEBP1 might indicate poor sensitivity to TMZ therapy. The study also points to AEBP1 as a new effective therapeutic target for the treatment of glioma.

Ethical approval and consent to participate

This study was approved by the Beijing Tiantan Hospital institutional review board (IRB), and informed consent was obtained from all individual participants who were included in this study.

Consent for publication

Not applicable.

Availability of data and materials

All data generated and analyzed during this study are included in this published article and its additional files.

Competing interests

The authors declare no conflict of interest.

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Authors' contributions

Kuanyu Wang and Chenxing Wu provided the design of the study, data analysis and wrote the manuscript. Ruoyu Huang, Zhiliang Wang and Xuezhi Tong participated in data downloading and preliminary analysis.

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Not applicable.

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Figure legend

Fig.1: (A) The gene selection process. (B) AEBP1 expression between GSCs and conventional GBM cell lines. (C) AEBP1 expression between TMZ-sensitive cell lines and TMZ-resistant cell lines. **P < 0.01

Fig.2: (A) AEBP1 expression with different grades in TCGA dataset. (B) AEBP1 expression with different grades in CGGA dataset. ***P < 0.001

Fig.3: (A) AEBP1 expression with different IDH status in TCGA dataset. (B) AEBP1 expression with different IDH status in CGGA dataset. (C) AEBP1 expression with different MGMT status in TCGA dataset. (D) AEBP1 expression with different MGMT status in CGGA dataset. ***P < 0.001, **P < 0.01, *P < 0.05

Fig.4: (A-B) The relation between AEBP1 expression and GSC markers expression in TCGA and CGGA datasets.

Fig.5: (A, C) Gene ontology analysis of AEBP1 in TCGA dataset. (B, D) Gene ontology analysis of AEBP1 in CGGA dataset. The samples were ranked according to AEBP1 expression from low to high.

Fig.6: (A-B) Survival analysis of glioma samples in TCGA and CGGA datasets. (C-D) Survival analysis of GBM samples in TCGA and CGGA datasets.

Figures

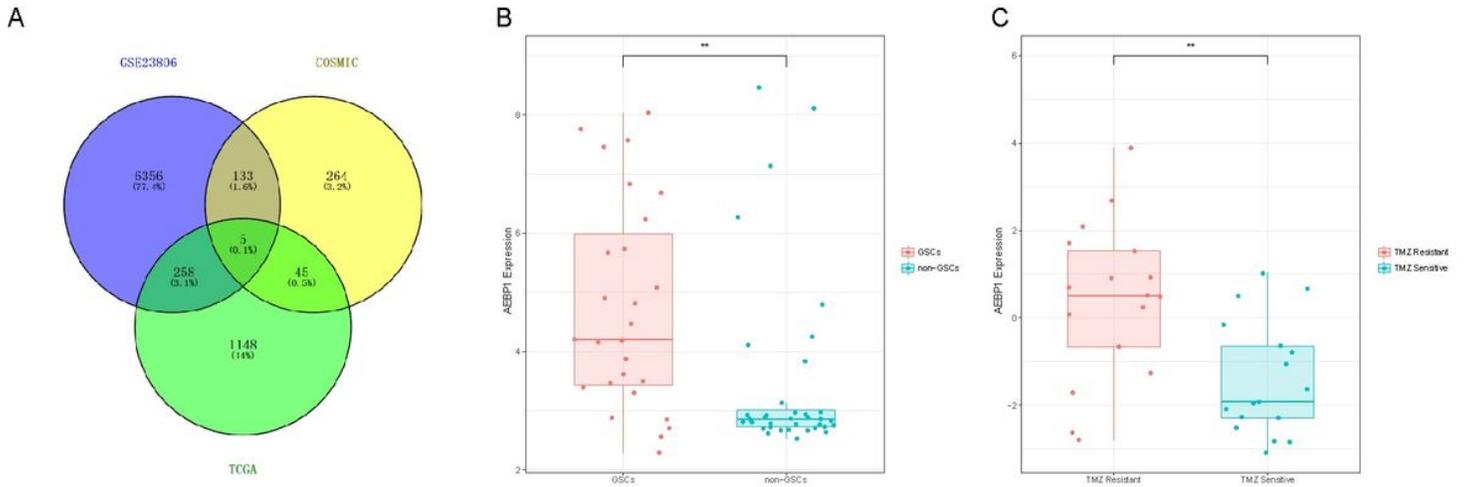


Figure 1

(A) The gene selection process. (B) AEBP1 expression between GSCs and conventional GBM cell lines. (C) AEBP1 expression between TMZ-sensitive cell lines and TMZ-resistant cell lines. ** $P < 0.01$

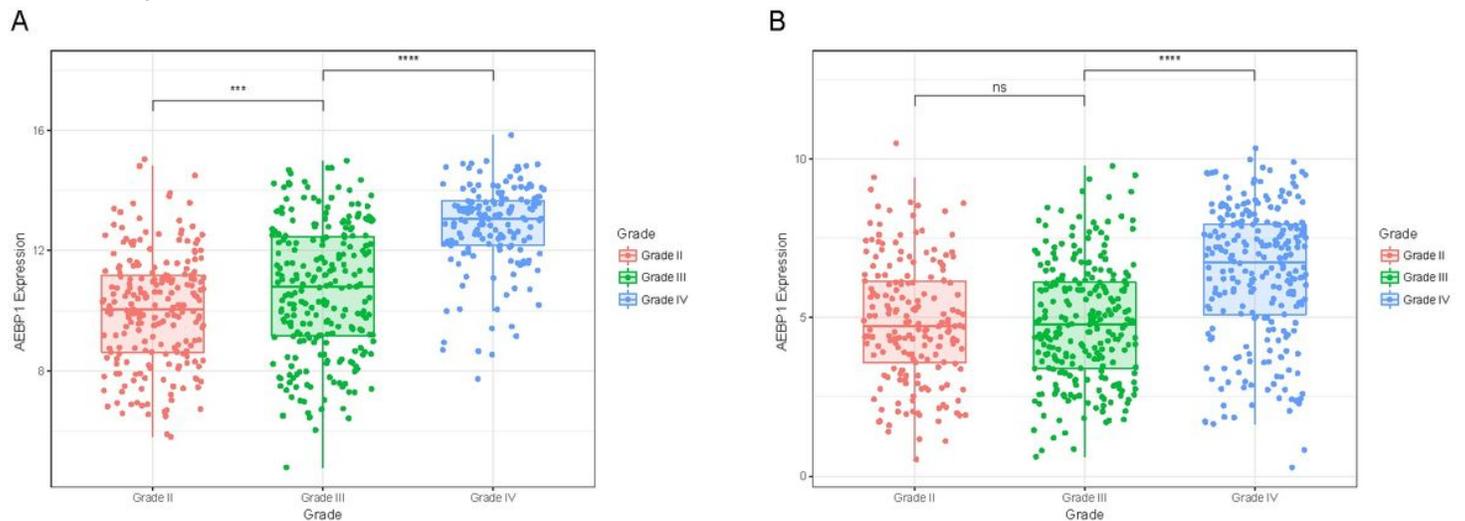


Figure 2

(A) AEBP1 expression with different grades in TCGA dataset. (B) AEBP1 expression with different grades in CGGA dataset. **** $P < 0.001$

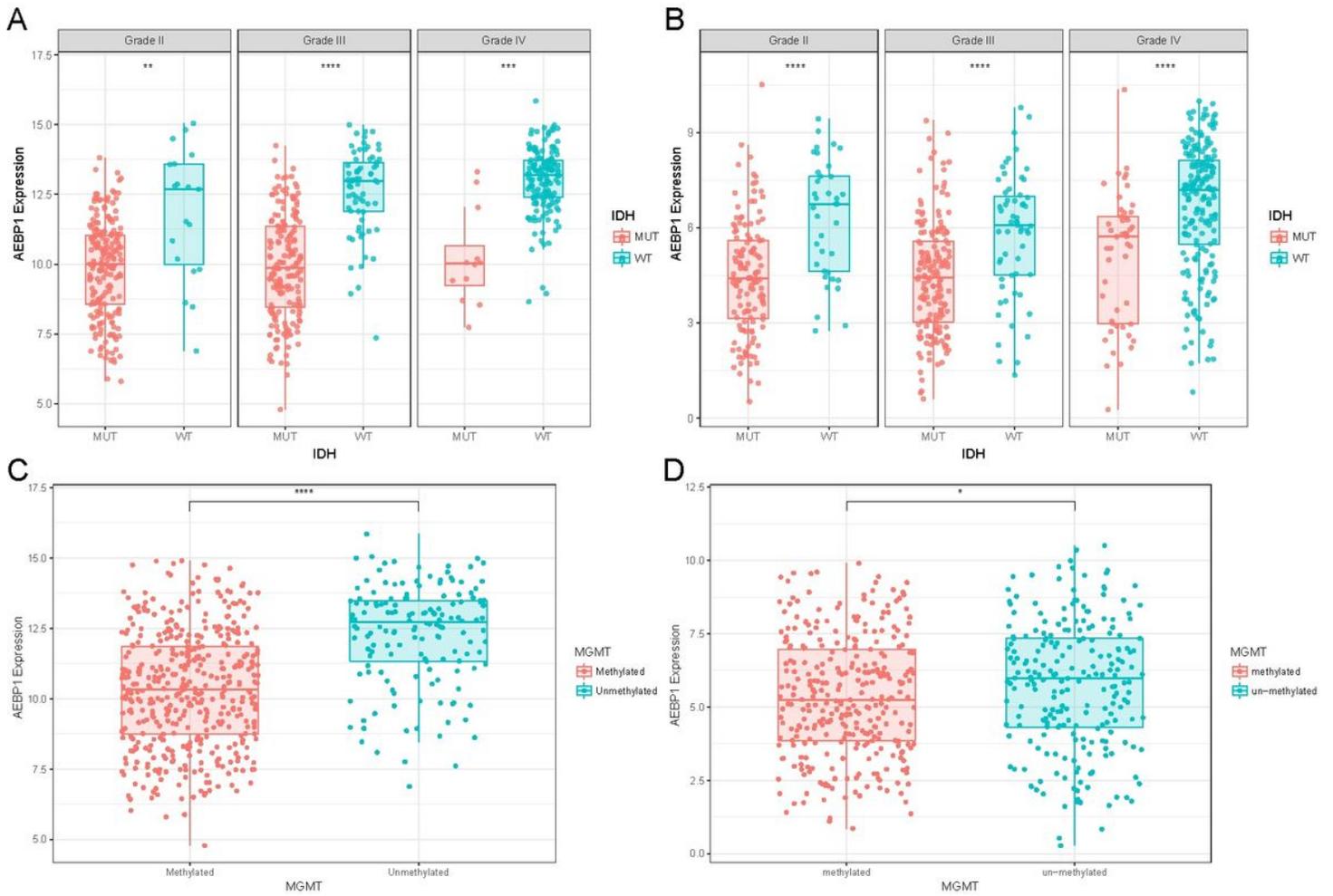
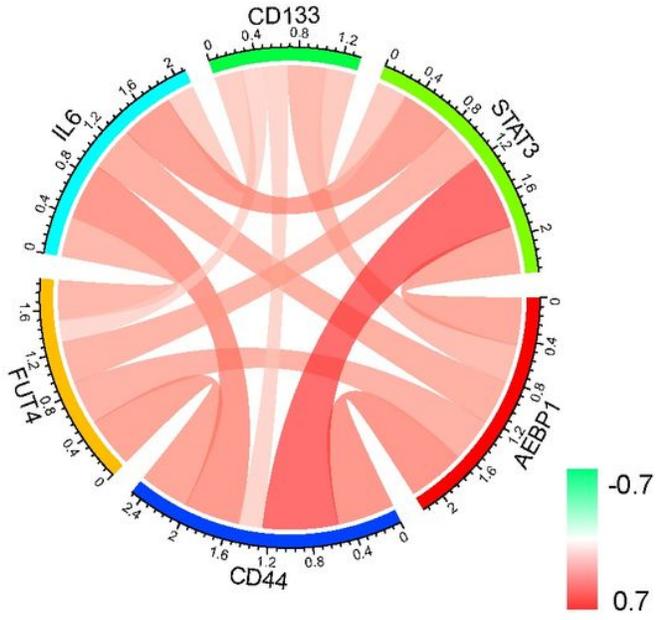


Figure 3

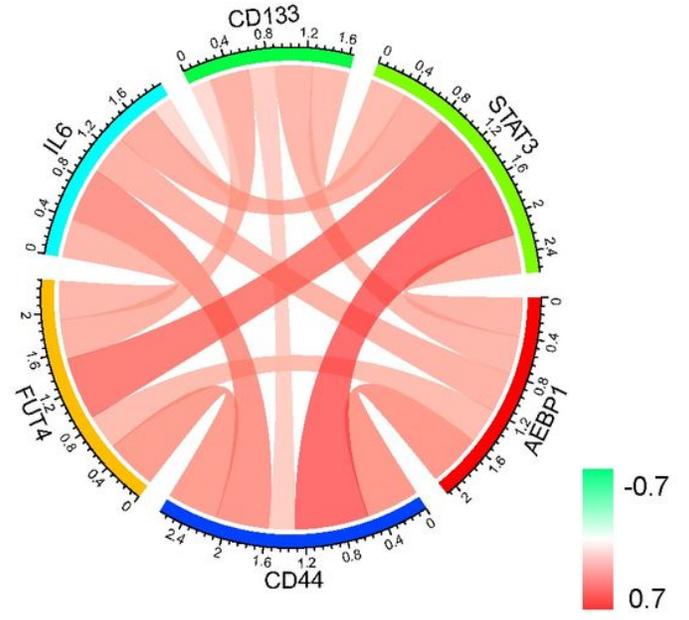
(A) AEBP1 expression with different IDH status in TCGA dataset. (B) AEBP1 expression with different IDH status in CGGA dataset. (C) AEBP1 expression with different MGMT status in TCGA dataset. (D) AEBP1 expression with different MGMT status in CGGA dataset. *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$

A

Correlation of TMEM71 and GSC markers in TCGA dataset

**B**

Correlation of TMEM71 and GSC markers in CGGA dataset

**Figure 4**

(A-B) The relation between AEBP1 expression and GSC markers expression in TCGA and CGGA datasets.

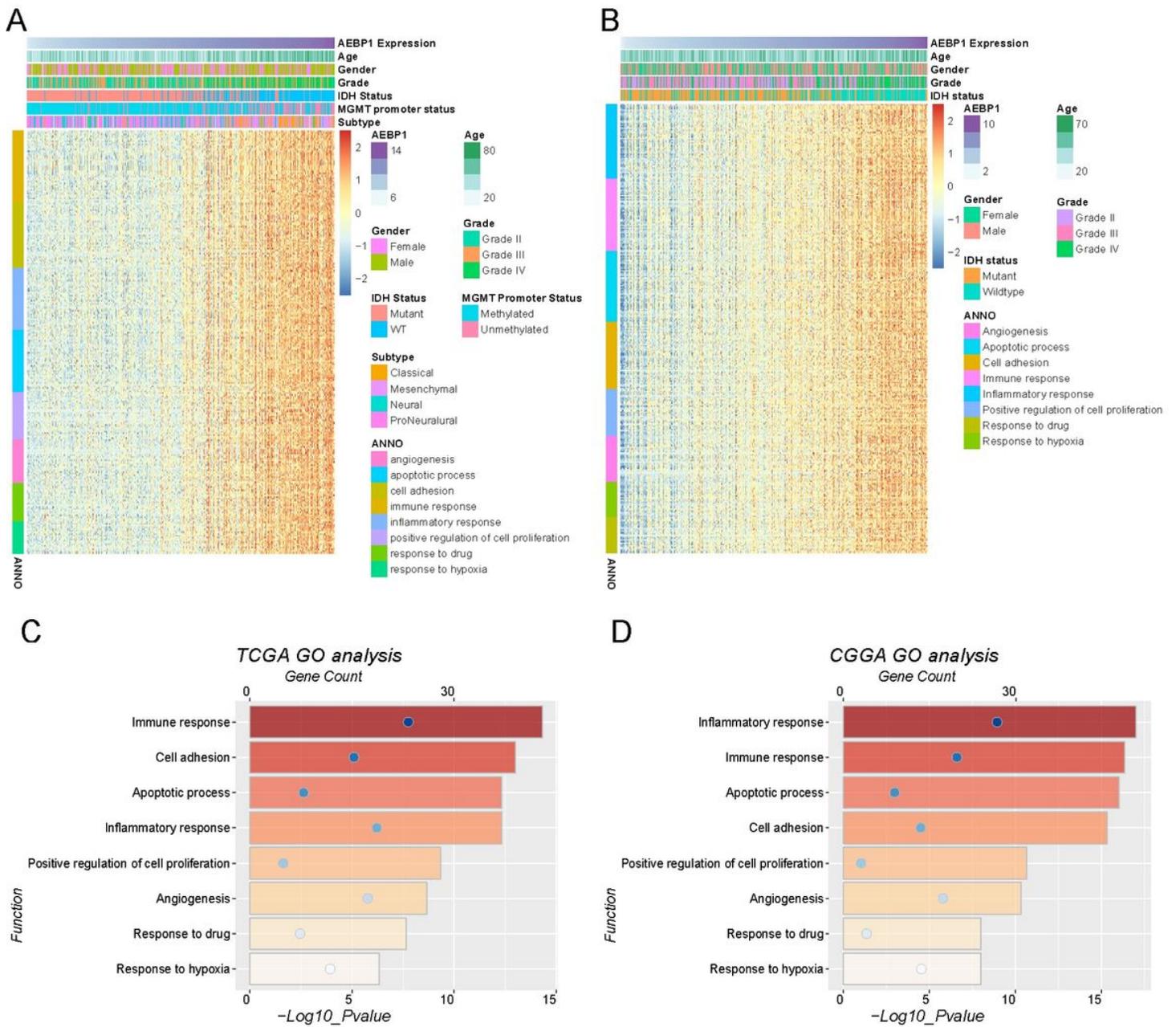


Figure 5

(A, C) Gene ontology analysis of AEBP1 in TCGA dataset. (B, D) Gene ontology analysis of AEBP1 in CGGA dataset. The samples were ranked according to AEBP1 expression from low to high.

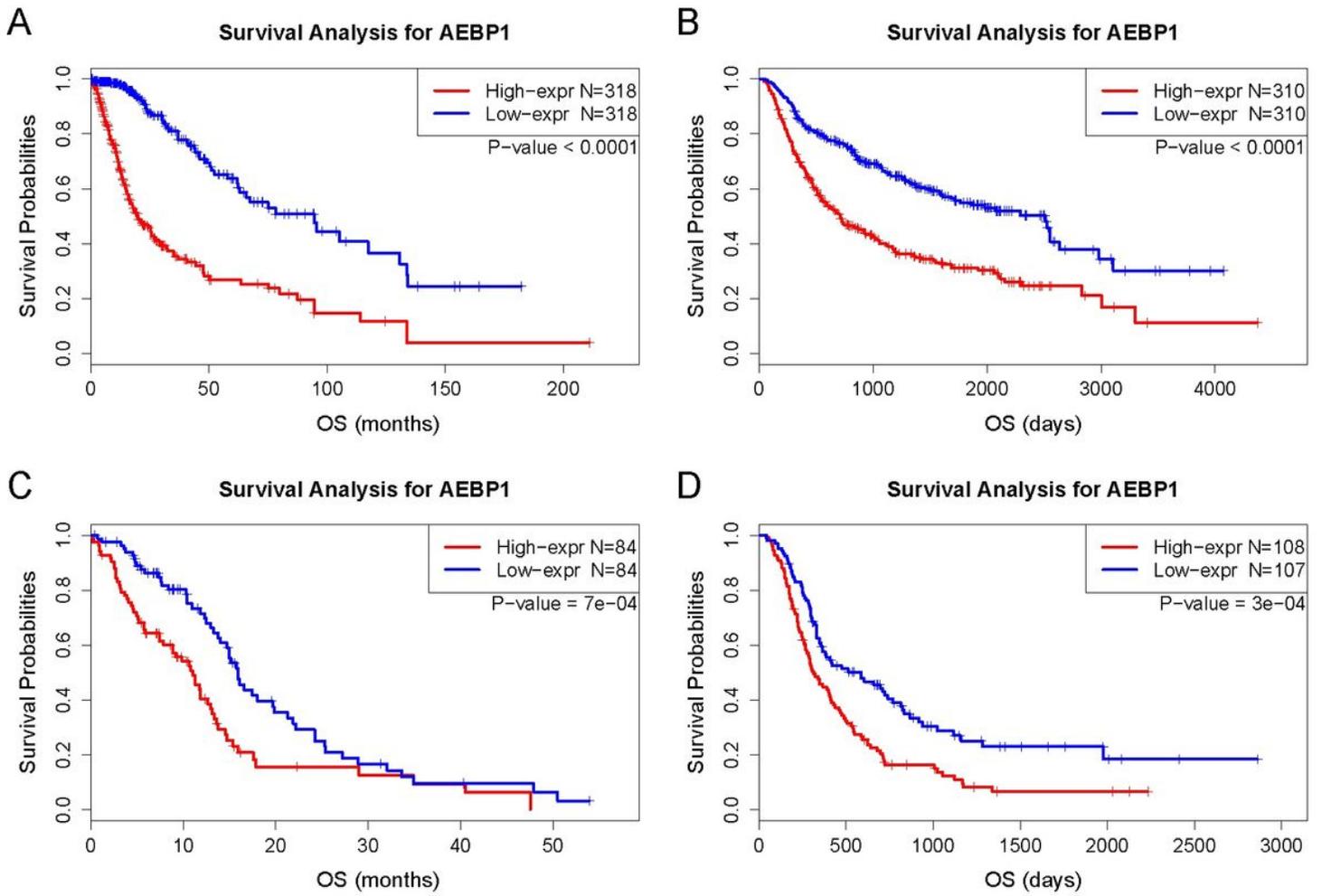


Figure 6

(A-B) Survival analysis of glioma samples in TCGA and CGGA datasets. (C-D) Survival analysis of GBM samples in TCGA and CGGA datasets.