Glucuronidase beta is an early predictive marker for the use of antidepressant in the treatment of glioma patients

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Abstract

**Purpose:** To screen early targets and investigate the potential molecular mechanisms involved in the use of antidepressant in the treatment of glioma.

**Methods:** The GSE89873 dataset, including expression levels of C6 cells under antidepressant treatment, non-antidepressant drug treatment, and untreated cells (control), was downloaded from database. Differentially expressed genes (DEGs) between antidepressant treatment and untreated cells, and between non-antidepressant drug treatment and untreated cells were identified and annotated. Genes that were significantly related to different drug treatment conditions were screened.

**Results:** In all, 416 differentially expressed genes (DEGs) were selected between cells of the antidepressant treatment and control groups, while 650 DEGs were selected between cells of the non-antidepressant treatment and control groups. The 402 overlapping DEGs were significantly associated with the apoptotic process, transforming growth factor beta receptor signaling pathway, and cell cycle arrest (p < 0.05). The DEGs ACOX1, ACSL1, GSTM3, and GSTP1 were significantly related to hormonal therapy (p < 0.05). Glucuronidase beta (GUSB) was significantly associated with age and targeted molecular therapy (p < 0.05). The GUSB was also significantly associated with overall survival time (p < 0.05). It is one of the unique DEGs in the antidepressant treatment group that participates in the drug metabolism-cytochrome P450 metabolic pathway.

**Conclusion:** Glucuronidase beta may be a specific biomarker for the early response of antidepressants to glioma treatment. This should, however, be further investigated to validate this finding.

**Keywords:** Glioma, Antidepressant treatment, Biomarker

INTRODUCTION

Glioma, one of the most common intracranial tumors of the neuroectoderm, accounts for 50 % of primary brain tumors [1]. Previous evidence has shown that glioma patients suffer from anxiety and depression, and depression is significantly associated with worsened survival [2,3]. Currently, conventional treatments for glioma include cytotoxic chemotherapy, radiotherapy, and surgery. However, the effectiveness of these approaches remains poor [4].

For glioma patients, antidepressant treatment not only extends the survival period but also
improves the quality of life [5]. Evidence has shown the beneficial role of antidepressants in adjuvant cancer therapy. Golan et al [6] showed that one of the biomarkers for the neuroprotective effects of antidepressants is upregulated GDNF expression, and its role was developed through a beta-arrestin1-dependent, CREB interactive pathway. Despite extensive studies, the cellular and molecular mechanisms involved in the effects of antidepressants in gliomas remain largely unknown.

Therefore, to determine the potential molecular mechanisms involved in antidepressant treatment of glioma patients, GSE89873 was acquired from the Gene Expression Omnibus (GEO) database. The expression levels of rat C6 cells after antidepressant treatment, non-antidepressant drug treatment, and untreated cells (normal control) were compared and the differentially expressed genes (DEGs) between groups were screened and annotated. The correlation between the target genes and the clinical factors of the sample was also determined.

METHODS

Data pre-processing and RNA annotation

The GSE89873 dataset was downloaded from the NCBI GEO database [7]. The dataset was deposited by Czeh and Di Benedetto [5]. In this dataset, rat C6 glioma cells were treated with the antidepressants, desipramine (n = 6) and fluoxetine (n = 6), as well as drugs without antidepressant properties, haloperidol (n = 6) and diazepam (n = 4), for 2 h. Untreated C6 glioma cells served as the control group.

DEGs screening and functional enrichment analysis

The DEGs between the antidepressant treatment group and control group and between the non-antidepressant drug treatment group and control group were screened with R3.6.1 Limma Version 3.34.0 [8] with a false discovery rate (FDR) < 0.05 and |log2 fold change (FC)| > 0.263 used as the threshold. Bidirectional hierarchical clustering [9] was constructed based on Euclidean distance [10] using R3.6.1 pheatmap Version 1.0.8 [11] and displayed by a heatmap. Unique and overlapping DEGs of the antidepressant treatment and non-antidepressant drug treatment groups compared with the control samples were obtained.

Gene ontology (GO) functional analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) signaling pathway enrichment annotation were implemented using DAVID version 6.8 [12,13] with the threshold of P < 0.05.

Screening modules and DEGs associated with clinical characterization

The crucial modules and genes associated with clinical characterization were identified by weighted gene co-expression network analysis (WGCNA) package (version 1.61) [14] in R3.6.1 with minimum number of genes = 100 and cut Height = 0.99.

The DEGs were mapped to WGCNA color module, and the parameters “fold enrichment” and p-values were calculated as previously described [15]. The module with p < 0.05 and fold enrichment > 1 was selected.

Establishment of protein-protein interaction (PPI) network

The interaction relationships between proteins were screened using STRING (version 11.0) [16], and a PPI network was constructed and displayed using Cytoscape Version 3.6.1 [17]. The GO function and KEGG signal pathway were analyzed using DAVID.

Assisted analysis of human malignant glioma data in TCGA

The expression levels associated with malignant gliomas were downloaded from TCGA database, which contained 152 tumor samples with prognostic survival information. Human homologous gene conversion of screened target rat genes was performed based on the biomaRt program of R3.6.1 (Version 2.46.0). The expression levels of the target genes were extracted from TCGA database. The correlation between the target gene and the clinical factors of the sample was calculated using the correlation function. Finally, the correlation between target genes and survival time was calculated by Cox regression analysis using Survival package Version 2.41-1 [18].

RESULTS

Distribution of DEGs

In all, 416 DEGs were selected in C6 cells treated with antidepressants compared to the control group, and 650 DEGs were selected for comparison between the non-antidepressant drug treatment group and the control group. The volcano and hierarchical clustering heat maps of the DEGs are shown in Figure 1. As shown in the...
hierarchical clustering heat map, the colors of the genes in different groups were distinct, indicating that the screened DEGs exhibited different gene expression characteristics.

As shown in Figure 2, a total of 261 DEGs were uniquely found between the antidepressant treatment and control groups, 495 DEGs were uniquely identified between the non-antidepressant drug treatment and control groups, and 155 overlapping DEGs were found in both groups.

**Figure 2:** Venn diagram of DEGs in the two groups

**Functional enrichment analysis**

The unique DEGs between the antidepressant treatment group and the control group, the unique DEGs between the non-antidepressant drug treatment group and the control group, and the overlapping DEGs were significantly enriched in 20, 18, and 17 biological processes (BPs), and were significantly enriched in ten, four, and three KEGG pathways, respectively (Figure 3).

**Figure 3:** Significantly correlated gene ontology (GO) annotations and Kyoto Encyclopedia of Genes and Genomes (KEGG) signal pathway bar graphs of DEGs between the antidepressant treatment group and control group (A), DEGs between non-antidepressant drug treatment group and control group (B), and the overlapping DEGs (C)
The unique DEGs between the antidepressant treatment group and control group were significantly related to BPs, such as “drug response”, and KEGG pathways, such as “amino sugar and nucleotide sugar metabolism”. The unique DEGs between the non-antidepressant drug treatment group and control group were significantly related to BPs, such as “positive regulation of MAP kinase activity”, and KEGG pathways, such as “Toll-like receptor signaling pathway”. The overlapping DEGs were significantly related to BPs, such as “drug response” and “apoptosis regulation” apoptotic process and KEGG pathways, such as “MAPK and p53 signaling pathways”.

Modules and target genes significantly related to disease characterization

As shown in Figure 4 A, the value of “power” was 50 when the square value of the correlation coefficient reached 0.9 for the first time. As shown in Figure 4 B, a total of 13 modules were obtained. The correlation between drug treatment status (antidepressant treatment or non-antidepressant drug treatment) and the modules was further calculated (Figure 4 C).

The DEGs between the antidepressant treatment group and control group were mapped to WGCNA modules, and 402 DEGs were involved in the modules (Table 1). Three modules were significantly enriched by DEGs, including the black (45 DEGs), green (34 DEGs), and salmon (14 DEGs) modules. The black module was significantly negatively correlated with non-antidepressant treatment and positively correlated with antidepressant treatment. The salmon module was significantly positively correlated with antidepressant treatment, and negatively correlated with non-antidepressant drug treatment. The green module was significantly negatively correlated to treatment with both drugs.

Table 1: Characteristics of modules

| ID    | Color       | Module size | Number with anti-specific DEGs | Enrichment Information Enrichment fold (95%CI) | P<sub>hyper</sub> |
|-------|-------------|-------------|--------------------------------|-----------------------------------------------|------------------|
| Module 1 | black       | 240         | 45                             | 2.464 (1.723-3.462)                           | 1.24E-06         |
| Module 2 | blue        | 476         | 12                             | 0.331 (0.169-0.591)                           | 1.83E-05         |
| Module 3 | brown       | 415         | 3                              | 0.0950 (0.0194-0.281)                         | 1.29E-09         |
| Module 4 | green       | 269         | 34                             | 1.661 (1.111-2.418)                           | 1.19E-02         |
| Module 5 | greenyellow | 146         | -                              | -                                             | -                |
| Module 6 | grey        | 1948        | 265                            | 1.788 (1.512-2.112)                           | 1.05E-11         |
| Module 7 | magenta     | 171         | 5                              | 0.384 (0.123-0.922)                           | 2.38E-02         |
| Module 8 | pink        | 186         | 1                              | 0.0707 (0.00178-0.401)                        | 4.42E-05         |
| Module 9 | purple      | 167         | 8                              | 0.630 (0.266-1.282)                           | 2.31E-01         |
| Module 10 | red         | 243         | 3                              | 0.162 (0.0331-0.483)                          | 4.55E-05         |
| Module 11 | salmon     | 110         | 14                             | 1.673 (1.277-2.962)                           | 7.75E-03         |
| Module 12 | tan         | 130         | 1                              | 0.101 (0.00254-0.576)                         | 1.42E-03         |
| Module 13 | turquoise   | 495         | 11                             | 0.292 (0.144-0.533)                           | 2.57E-06         |
| Module 14 | yellow      | 289         | -                              | -                                             | -                |
Next, GO function and KEGG pathways were screened. The DEGs were significantly enriched in 25 BPs, such as regulation of the apoptotic process, the transforming growth factor beta (TGF-β) signaling pathway, and cell cycle arrest. Six KEGG signaling pathways were significantly enriched by these DEGs, including the TGF-β signaling pathway and peroxisome, porphyrin, and chlorophyll metabolism pathways (Figure 5B).

Assisted analysis of human malignant glioma data in TCGA

Fifteen genes participated in the enriched KEGG pathway analysis. As shown in Figure 6A, ACOX1, ACSL1, GSTM3, and GSTP1 were significantly related to hormonal therapy, whereas glucuronidase beta (GUSB) was significantly related to age and targeted molecular therapy.

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**Figure 5:** Interaction network and functional enrichment of target genes. A: The interaction network of target genes. The size of the node indicates the degree of the node. The color of the node corresponded to the WGCNA module color. B: Functional enrichment of target genes.
It was observed that GUSB was significantly associated with overall survival time (Figure 6 B). The GUSB is one of the unique DEGs in the antidepressant treatment group, which participates in the drug metabolism-cytochrome P450 metabolic pathway. Therefore, it was concluded that GUSB acts as a specific biomarker for the early response of antidepressants to glioma treatment and is involved in the drug metabolism pathway during the response process.

In the present study, DEGs in the with-anti vs. control groups were mainly enriched in cellular activity terms, such as apoptotic process, TGF-β receptor signaling pathway, and cell cycle arrest. In line with this study, evidence from previous studies showed that after antidepressant treatment, the proliferation of glial cells was activated and gliogenesis was upregulated in the hippocampus and prefrontal cortex [21]. Many studies have suggested that adult neurogenesis in the hippocampus can be stimulated by antidepressant treatment [22]. Therefore, it is concluded that the deleterious effects of glioma might be rescued by treatment with antidepressants.

The KEGG pathways associated with DEGs of the with-anti vs. control groups included TGF-β signaling pathway, peroxisomes, and porphyrin and chlorophyll metabolism. In animal models of depression, the antidepressant effects of (R)-ketamine of TGF-β has been documented and has been recommended as a new antidepressant [23]. In animal models, previous evidence showed peroxisome proliferator-activated receptor gamma has been implicated in stress and stress-induced depression [24,25]. Similarly, these pathways activated by antidepressant drugs have also been observed in patients with glioma, suggesting that these drugs might be helpful for glioma improvement by modulating the pathways involved in the genes associated with anti-depression.

Furthermore, it was demonstrated that GUSB was significantly related to age, targeted molecular therapy, and overall survival time by participating in the cytochrome P450 drug metabolic pathway. Although direct evidence of the association between GUSB and overall survival time has not been reported, the association between GUSB activity and central nervous system lesions in humans has been well documented [26]. Furthermore, Cubizolle et al [26] reported that the occurrence of severe deficiency in GUSB results in neurological defects. More studies should be conducted to verify the current role of GUSB in glioma patients.
CONCLUSION

Glucuronidase beta is a potential biomarker for the early response of antidepressants to glioma treatment by participating in the drug metabolism pathway during the response process. Although the clinical data supporting the role of GUSB were not specified, these changes should be demonstrated in further studies.

DECLARATIONS

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Ethical approval

None provided.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

We declare that this work was done by the authors named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Dayuan Xu carried out the conception and design of the research and drafted the manuscript. Dayuan Xu and Gang Cui participated in the acquisition of data and carried out the analysis and interpretation of data. Both authors read and approved the final manuscript.

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