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Original Research Article

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*

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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 Czéh 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 using R3.6.1 Limma Version 3.34.0 [8] with a false discovery rate (FDR) < 0.05 and $|\log_2$ 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.

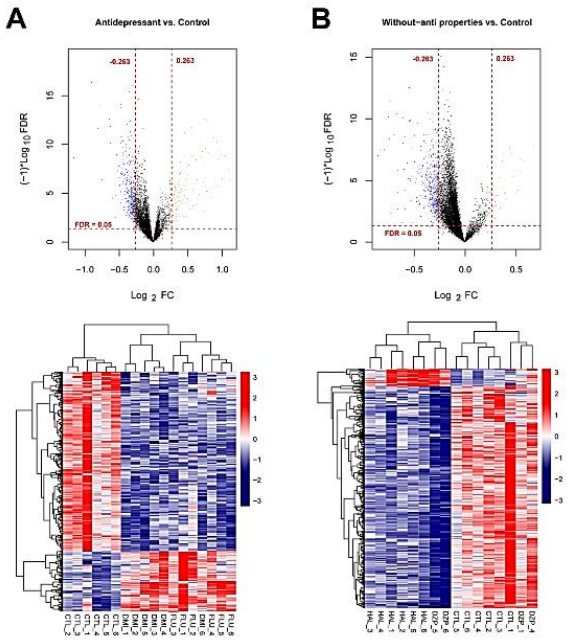

Figure 1: Volcano plot and heat map of differentially expressed genes (DEGs). Volcano plot (top) and heat map (bottom) of DEGs between the antidepressant treatment group and control group (A) and between the non-antidepressant drug treatment group and control group (B). The blue dots represent down-regulated DEGs and red dots indicate upregulated DEGs, respectively

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.

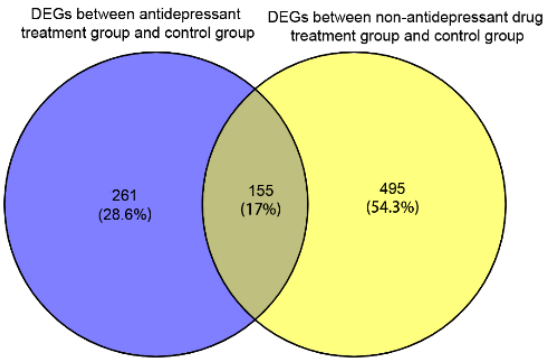

Figure 2: Venn diagram of DEGs in the two groups

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).

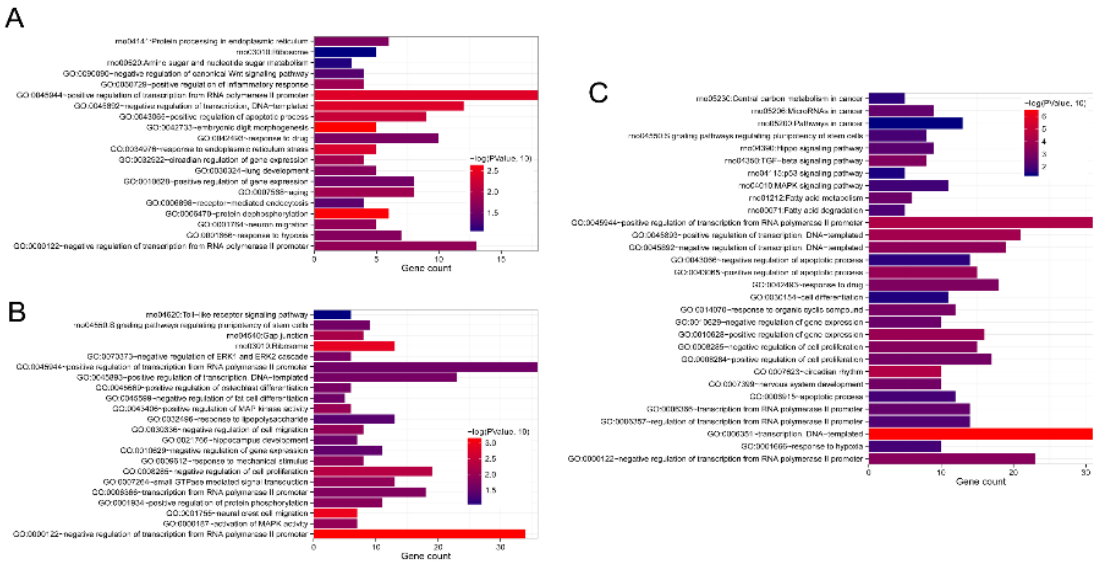

Figure 3: Significantly correlated gene ontology (GO) annotations and Kyoto Encyclopedia of Genes and Genomes (KEGG) signal pathway bar graphs of DEGs between the antidepressant treatment group and control group (A), DEGs between non-antidepressant drug treatment group and control group (B), and the 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 drug 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.

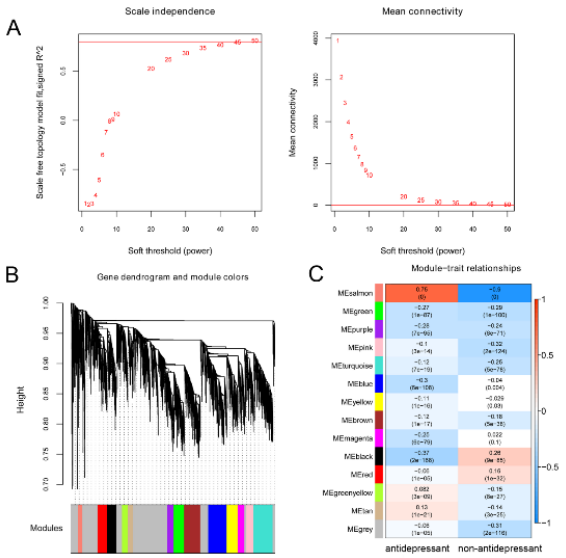

Figure 4: Modules and genes associated to disease characterization were screened using the WGCNA algorithm. A: Left; selection diagram of the adjacency matrix weight parameter power. Right; schematic diagram of the average connectivity of DEGs under different power parameters. B: Module division tree diagram. C: Heat map of the correlation between each module and the status of drug treatment

PPI network

In all, 402 interaction connection pairs of the 93 DEGs that were significantly enriched in the three modules remained (interaction score higher than 0.4). The PPI network contained 146 nodes and 402 edges (Figure 5 A).

Table 1: Characteristics of modules

ID	Color	Module size	Number with anti-specific DEGs	Enrichment information	
				Enrichment fold (95%CI)	P_{hyper}
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 5 B).

Assisted analysis of human malignant glioma data in TCGA

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

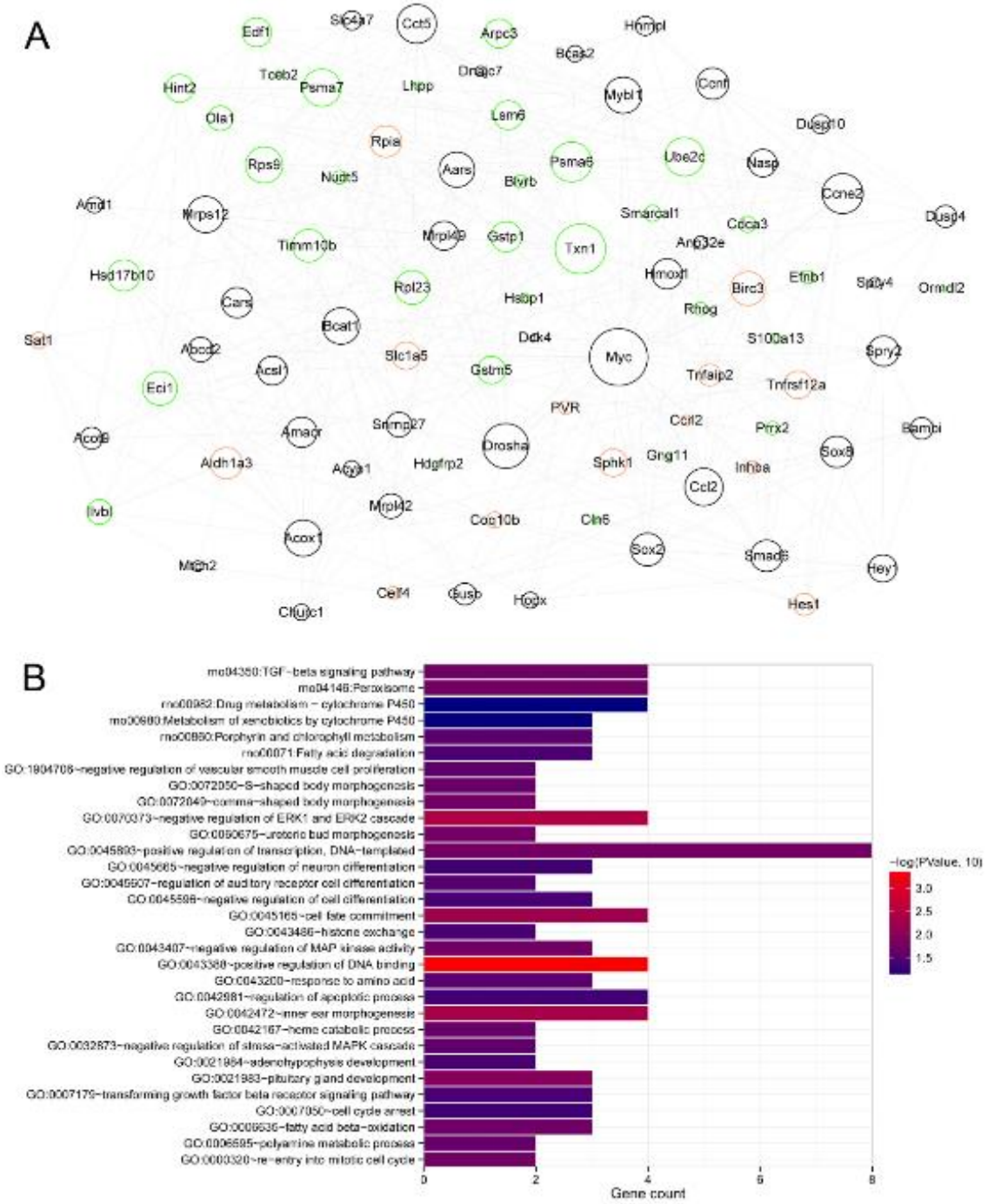

Figure 5: Interaction network and functional enrichment of target genes. A: The interaction network of target genes. The size of the node indicates the degree of the node. The color of the node corresponded to the WGCNA module color. B: Functional enrichment of target genes

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.

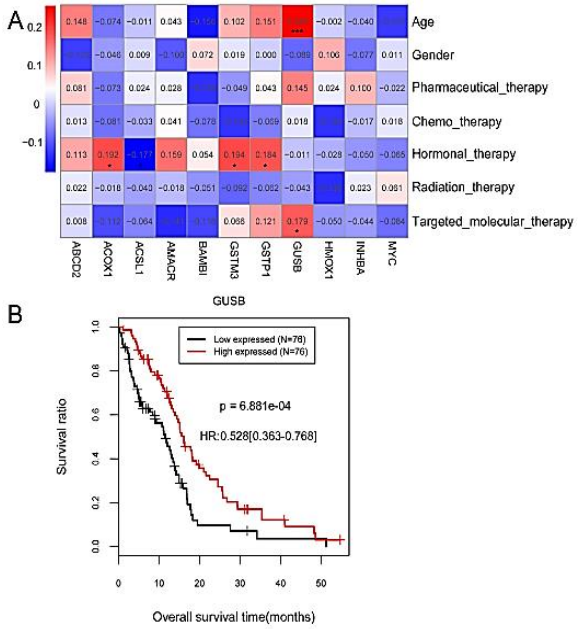

Figure 6: Heatmap of correlations between genes and clinical factors and the Kaplan-Meier (KM) curve of GUSB and overall survival time. A: Heatmap of correlations between genes and clinical factors; B: KM curve of GUSB and overall survival time

DISCUSSION

The anticancer properties of antidepressant drugs in adjuvant cancer therapy have been previously reported [19,20]. Although, previous data support the beneficial role of antidepressant drugs in glioma, the molecular mechanisms involved in these drugs have not been established. In this study, the transcriptomes of glioma cells after treatment with antidepressants and drugs without any antidepressant properties were investigated. In all, 416 DEGs were selected between the antidepressant treatment group and control group, and 650 DEGs were selected between the non-antidepressant drug treatment group and control group. Meanwhile, 93 DEGs between the antidepressant treatment group control group were significantly enriched in three modules. Furthermore, 25 BPs and six KEGG signaling pathways were significantly enriched by these genes. The DEGs of ACOX1, ACSL1, GSTM3, and GSTP1 were significantly associated with hormonal therapy, and GUSB was significantly associated with age, targeted molecular therapy, and overall survival time. Glucuronidase beta is one of the unique DEGs in the with-anti treatment group, which participates in the cytochrome P450 drug metabolic pathway. Therefore, GUSB may be a specific biomarker for the early response of antidepressants to glioma treatment.

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.

REFERENCES

1. *Chen R, Smith-Cohn M, Cohen AL, Colman H. Glioma subclassifications and their clinical significance. Neurotherapeutics 2017; 14: 284-297.*
2. *Hao A, Huang J, Xu X. Anxiety and depression in glioma patients: prevalence, risk factors, and their correlation with survival. Ir J Med Sci 2021; 190: 1155-1164.*
3. *Shi C, Lamba N, Zheng LJ, Cote D, Regestein QR, Liu CM, Tran Q, Routh S, Smith TR, Mekary RA, et al. Depression and survival of glioma patients: A systematic review and meta-analysis. Clin Neurol Neurosurg 2018; 172: 8-19.*
4. *Zhang L, Wu X, Xu T, Luo C, Qian J, Lu Y. Chemotherapy plus radiotherapy versus radiotherapy alone in patients with anaplastic glioma: a systematic review and meta-analysis. J Cancer Res Clin Oncol 2013; 139: 719-726.*
5. *Czéh B, Di Benedetto B. Antidepressants act directly on astrocytes: evidences and functional consequences. Eur Neuropsychopharmacol 2013; 23: 171-185.*
6. *Golan M, Schreiber G, Avissar S. Antidepressants elevate GDNF expression and release from C_6 glioma cells in a β-arrestin1-dependent, CREB interactive pathway. Int J Neuropsychopharmacol 2011; 14: 1289-1300.*
7. *Barrett T, Wilhite SE, Ledoux P, Evangelista C, Kim IF, Tomashevsky M, Marshall KA, Phillippy KH, Sherman PM, Holko M, et al. NCBI GEO: archive for functional genomics data sets--update. Nucleic Acids Res 2013; 41: D991-995.*
8. *Ritchie ME, Phipson B, Wu D, Hu Y, Law CW, Shi W, Smyth GK. limma powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic Acids Res 2015; 43: e47.*
9. *Nowak G, Tibshirani R. Complementary hierarchical clustering. Biostatistics 2008; 9: 467-483.*
10. *Bien J, Tibshirani R. Hierarchical clustering with prototypes via minimax linkage. J Am Stat Assoc 2011; 106: 1075-1084.*
11. *Wang L, Cao C, Ma Q, Zeng Q, Wang H, Cheng Z, Zhu G, Qi J, Ma H, Nian H, et al. RNA-seq analyses of multiple meristems of soybean: novel and alternative transcripts, evolutionary and functional implications. BMC Plant Biol 2014; 14: 169.*
12. *Huang da W, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat Protoc 2009; 4: 44-57.*
13. *Huang da W, Sherman BT, Lempicki RA. Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists. Nucleic Acids Res 2009; 37: 1-13.*
14. *Langfelder P, Horvath S. WGCNA: an R package for weighted correlation network analysis. BMC Bioinformatics 2008; 9: 559.*

15. Cao J, Zhang S. A Bayesian extension of the hypergeometric test for functional enrichment analysis. Biometrics 2014; 70: 84-94.
16. Szklarczyk D, Morris JH, Cook H, Kuhn M, Wyder S, Simonovic M, Santos A, Doncheva NT, Roth A, Bork P, et al. The STRING database in 2017: quality-controlled protein-protein association networks, made broadly accessible. Nucleic Acids Res 2017; 45: D362-D368.
17. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, Amin N, Schwikowski B, Ideker T. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res 2003; 13: 2498-2504.
18. Wang P, Wang Y, Hang B, Zou X, Mao JH. A novel gene expression-based prognostic scoring system to predict survival in gastric cancer. Oncotarget 2016; 7: 55343-55351.
19. Zhuo C, Xun Z, Hou W, Ji F, Lin X, Tian H, Zheng W, Chen M, Liu C, Wang W, et al. Surprising anticancer activities of psychiatric medications: old drugs offer new hope for patients with brain cancer. Front Pharmacol 2019; 10: 1262.
20. Avendaño-Félix M, Aguilar-Medina M, Bermudez M, Lizárraga-Verdugo E, López-Camarillo C, Ramos-Payán R. Refocusing the use of psychiatric drugs for treatment of gastrointestinal cancers. Front Oncol 2020; 10: 1452.
21. Manji HK, Quiroz JA, Sporn J, Payne JL, Denicoff K, A Gray N, Zarate CA Jr, Charney DS. Enhancing neuronal plasticity and cellular resilience to develop novel, improved therapeutics for difficult-to-treat depression. Biol Psychiatry 2003; 53: 707-742.
22. Lucassen PJ, Meerlo P, Naylor AS, van Dam AM, Dayer AG, Fuchs E, Oomen CA, Czéh B. Regulation of adult neurogenesis by stress, sleep disruption, exercise and inflammation: Implications for depression and antidepressant action. Eur Neuropsychopharmacol 2010; 20: 1-17.
23. Zhang K, Yang C, Chang L, Sakamoto A, Suzuki T, Fujita Y, Qu Y, Wang S, Pu Y, Tan Y, et al. Essential role of microglial transforming growth factor-β1 in antidepressant actions of (R)-ketamine and the novel antidepressant TGF-β1. Transl Psychiatry 2020; 10: 32.
24. Jiang B, Wang H, Xu H. Steroid receptor RNA activator affects the development of poststroke depression by regulating the peroxisome proliferator-activated receptor γ signaling pathway. Neuroreport 2020; 31: 48-56.
25. Ryan KM, Patterson I, McLoughlin DM. Peroxisome proliferator-activated receptor gamma co-activator-1 alpha in depression and the response to electroconvulsive therapy. Psychol Med 2019; 49: 1859-1868.
26. Cubizolle A, Serratrice N, Skander N, Colle MA, Ibanes S, Gennetier A, Bayo-Puxan N, Mazouni K, Mennechet F, Joussemet B, et al. Corrective GUSB transfer to the canine mucopolysaccharidosis VII brain. Mol Ther 2014; 22: 762-773.