Research on the Effects of Propofol and Sevoflurane on the Expression of Prognostic-Related Genes in Glioblastoma

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Research Article

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Abstract

Background: Glioblastoma is one of the most common malignant brain tumors with high recurrence and mortality. The first choice for treatment is surgical resection under general anesthesia. Previous studies have demonstrated that general anesthetics are correlated with the prognosis of patients with malignant tumors, but the mechanisms remain unclear.

Methods: In this study, surgical specimens were analyzed with gene microarray to explore the influences of propofol and sevoflurane on the expression of prognostic-related genes in glioblastoma. Through the construction of gene regulatory network and analyzing the network properties, we screened out the core genes related to the prognosis of glioblastoma and perform survival analysis to elucidate the potential effects of propofol and sevoflurane on the prognosis of glioblastoma patients.

Results: In this study, 16 Hub genes (NDUFA4, NDUFA6, NDUFV2, CYCS, NDUFB2, MGST2, CYC1, ATP5G1, MRPS2, PPP2R1A, GSTM3, GSTK1, MAPK3, NDUFB7, ATP5D and MGST3) related to the prognosis of glioblastoma were screened out and their expression was down-regulated. GO and KEGG pathway analysis showed the Hub genes were closely related to mitochondrial function and oxidative phosphorylation pathway (has 00190). The down-regulated expression of NDUFB2 and MGST2 genes after propofol treatment may be associated with better prognosis of glioblastoma.

Conclusions: General anesthetics can alter the expression of prognostic-related genes in glioblastoma. Changes in NDUFB2 and MGST2 gene expression levels can affect the overall survival of patients with glioblastoma, and other Hub genes may affect the prognosis of glioblastoma patients by interfering the energy metabolism process of tumor cells.

Trial registration:

Registration number: ChiCTR-IOR-16010180

Date of registration: 18th, December, 2016

Background

Glioma is the most common primary brain tumor with an incidence of 4/100,000–5/100,000 per year [1] and accounting for more than 70% of all intracranial tumors [2]. In the classification of brain tumors published by WHO in 2016, glioblastoma, as a category of glioma, was listed in the most malignant grade (Grade 4) [3]. Previous studies have shown that the prognosis of glioblastoma is extremely poor, and its overall median survival time is only about 14.4 months [4]. The high mortality and high disability of glioblastoma impose a heavy burden on families and society.

At present, surgical resection remains the first choice of the standardized treatment scheme for glioblastoma and assisted with postoperative radiotherapy, chemotherapy, and other comprehensive treatments [5]. Normally, glioblastoma resection is performed under general anesthesia. Previous studies
have suggested that propofol and inhalational anesthetics may have different effects on the prognosis of patients with malignant tumors. Retrospective analysis of clinical data showed that patients who received inhalational anesthetics intraoperatively had a significant reduction in overall survival than patients who received total intravenous anesthesia [6, 7], but the same conclusion was not acquired in prospective studies [8]. In cytological studies, Xu et al. found that propofol treatment significantly increased the level of miRNA-218 in U373 glioma cells, which played an important role in inhibiting glioma cell proliferation, migration, and promoting the apoptosis [9], whereas sevoflurane could promote the proliferation of glioma stem cells by activating HIF factor [10]. Therefore, previous researches have shown that propofol and sevoflurane have different effects on the biological process of glioma cells at the molecular level, but the regulatory mechanisms have not been clarified.

Gene microarray technology is used to explore unknown gene expression, and the association between differentially expressed genes (DEGs) and diseases is obtained through subsequent analysis. In previous studies, gene microarray was used to compare the changes in signaling pathways in human atrial tissue [11] and the changes in miRNA expression patterns in rat brain [12] after propofol and sevoflurane treatment, and the results showed that propofol and sevoflurane affected differently in gene expression and regulation. However, no researches showed the effects of propofol and sevoflurane on the gene expression of glioblastoma cells in vivo or in vitro.

In this study, propofol and sevoflurane were used during the glioblastoma surgery respectively, and the tumor tissues were analyzed with mRNA microarray analysis to observe the effects of the two anesthetics on gene expression in patients with glioblastoma. The correlation between DEGs and patients’ prognosis was further analyzed, and a gene regulatory network was constructed to explore the influences of propofol and sevoflurane on the prognosis of patients with glioblastoma and the possible mechanisms to provide new targets for diagnosis and treatment.

Methods

This research was conducted from June 2017 to July 2018 at the Xuanwu Hospital, Beijing, China, after approval from Xuanwu Hospital Ethics Committee. Written informed consents were obtained from all patients before participation. The trial was registered with the China Clinical Trial Registry (registration number: ChiCTR-IOR-16010180, principal investigator's name: Lei Zhao, date of registration: 12/18/2016). 6 patients scheduled for glioblastoma resection were included and divided into the intravenous anesthesia group (TIVA, n = 3) and the inhalation anesthesia group (INHA, n = 3) after matched with the tumor sites.

All methods in this manuscript were carried out in accordance with relevant guidelines and regulations. Inclusion criteria were patients aged 18–65 years, with an American Society of Anesthesiologists (ASA) physical status I–II, primary glioblastoma, and undergo craniotomy for the first time. Patients with severe systematic disease, history of tumors, and history of radiotherapy or chemotherapy were excluded. Patients in the TIVA group received propofol-remifentanil for maintenance intraoperatively, while patients
in the INHA group received sevoflurane-remifentanil intraoperatively. The operations were performed by the same neurosurgeon and patients who received a combination of propofol and sevoflurane intraoperative and had cardiovascular or cerebrovascular accidents were eliminated from this trial. One piece of glioblastoma tissue was obtained by the surgeon under aseptic conditions and rapidly stored at -80°C.

RNA was extracted from tumor tissue using TRIlol Reagent (Invitrogen), according to the manufacturer's instructions, and following purification with an RNeasy kit (Qiagen, Valencia, CA, USA). cDNA was generated using One-Cycle Target Labeling and Control Reagents (Affymetrix, Santa Clara, CA, USA), and cRNA was created with a GeneChip IVT Labeling Kit (Affymetrix, Santa Clara, CA, USA). Biotin-labeled, fragmented(≤ 200nt) cRNA was hybridized for 16 hours at 45°C to Affymetrix GeneChip Clariom D arrays (Affymetrix). GeneChips were washed and stained in the Affymetrix Fluidics Station 450, then scanned by using Affymetrix® GeneChip Command Console (AGCC) which installed in GeneChip® Scanner 3000 7G. The data were analyzed with Robust Multichip Analysis (RMA) algorithm using Affymetrix default analysis settings and global scaling as normalization methods. The values presented are log2 RMA signal intensity.

Statistical Analysis

DEGs between the two groups were screened according to $P< 0.05$ and the absolute value of fold change (FC) ≥ 2.0, which were considered statistically significant. Functional enrichment analysis of DEGs was carried out to obtain significant Gene Ontology (GO) functions, and the Pathway analysis of DEGs was conducted in the KEGG database. Extract the genes contained in significant GO and Pathway, use the STRING protein interaction database (version 10.5) to construct the protein-protein interaction (PPI) network of DEGs, and analyze the topology of the network. The Hub genes contained in the DEGs were screened out from the network, as well as the proteins with crucial physiological functions encoded by the Hub genes. The Hub genes were searched in the TCGA database, and the survival curve was drawn to explore their correlation with the prognosis of patients.

Results

1. DEGs

With $P< 0.05$ and the absolute value of fold change (FC) ≥ 2.0 were considered statistically significant, a total of 1,596 DEGs were obtained between the two groups, including 214 up-regulated genes and 1,382 down-regulated genes (Fig. 1).

2. Functional enrichment analysis of DEGs

The results of functional enrichment analysis showed that the DEGs in glioblastoma tissues had definite functional enrichment. According to the $P$-value of the enriched GO functions, the top 20 GO functions
with the highest significance level were selected (Fig. 2A and 2B), and their biological functions were analyzed and demonstrated (Table 1A and 1B).

Table 1
A. Functional enrichment of up-regulated DEGs in GBM

<table>
<thead>
<tr>
<th>GO ID</th>
<th>GO name</th>
<th>Gene number</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BP GO:0006366</td>
<td>RNA polymerase II promoter transcription</td>
<td>12</td>
<td>0.000044</td>
</tr>
<tr>
<td>GO:0035914</td>
<td>skeletal muscle cell differentiation</td>
<td>5</td>
<td>0.000074</td>
</tr>
<tr>
<td>GO:0019805</td>
<td>quinolinate biosynthetic process</td>
<td>2</td>
<td>0.000269</td>
</tr>
<tr>
<td>GO:0030593</td>
<td>neutrophil chemotaxis</td>
<td>5</td>
<td>0.000334</td>
</tr>
<tr>
<td>GO:0045944</td>
<td>positive regulation of RNA polymerase II promoter</td>
<td>21</td>
<td>0.000373</td>
</tr>
<tr>
<td>GO:0051412</td>
<td>response to corticosterone</td>
<td>3</td>
<td>0.000625</td>
</tr>
<tr>
<td>GO:0031668</td>
<td>cellular response to extracellular stimulus</td>
<td>3</td>
<td>0.000625</td>
</tr>
<tr>
<td>GO:0007186</td>
<td>G-protein coupled receptor signaling pathway</td>
<td>19</td>
<td>0.000652</td>
</tr>
<tr>
<td>GO:0001666</td>
<td>response to hypoxia</td>
<td>7</td>
<td>0.000810</td>
</tr>
<tr>
<td>GO:0071376</td>
<td>cellular response to corticotropin-releasing hormone</td>
<td>2</td>
<td>0.000886</td>
</tr>
<tr>
<td>GO:0034097</td>
<td>response to cytokine</td>
<td>4</td>
<td>0.001119</td>
</tr>
<tr>
<td>GO:2000503</td>
<td>positive regulation of natural killer cell chemotaxis</td>
<td>2</td>
<td>0.001321</td>
</tr>
<tr>
<td>GO:0071353</td>
<td>cellular response to interleukin-4</td>
<td>3</td>
<td>0.001678</td>
</tr>
<tr>
<td>GO:0043401</td>
<td>steroid hormone mediated signaling pathway</td>
<td>4</td>
<td>0.001740</td>
</tr>
<tr>
<td>GO:0071639</td>
<td>positive regulation of monocyte chemotactic protein-1</td>
<td>2</td>
<td>0.001838</td>
</tr>
<tr>
<td>CC GO:0031528</td>
<td>microvillus membrane</td>
<td>3</td>
<td>0.001147</td>
</tr>
<tr>
<td>MF GO:0001077</td>
<td>transcriptional activator activity of RNA polymerase II</td>
<td>12</td>
<td>0.000003</td>
</tr>
<tr>
<td>GO:000982</td>
<td>transcription factor activity of RNA polymerase II core</td>
<td>4</td>
<td>0.000051</td>
</tr>
<tr>
<td>GO:000978</td>
<td>RNA polymerase II core promoter proximal region</td>
<td>12</td>
<td>0.000186</td>
</tr>
<tr>
<td>GO:0003707</td>
<td>steroid hormone receptor activity</td>
<td>4</td>
<td>0.001623</td>
</tr>
</tbody>
</table>

BP-biological process, CC-cellular component, MF-molecular function
The results showed that the top 20 GO functions enriched by up-regulated DEGs were mainly related to RNA polymerase II promoter transcription, transcriptional activator activity of RNA polymerase II core...
region, G-protein coupled receptor signaling pathway, response to hypoxia, etc. While the top 20 GO functions enriched by down-regulated DEGs were mainly related to mitochondrial structure, respiratory chain functions, proton transport, response to oxidative stress, heme biosynthetic process, etc.

3. Pathway analysis of DEGs

Pathway analysis of DEGs demonstrated that the up-regulated and down-regulated DEGs in glioblastoma were significantly enriched in different pathways. The top 10 enriched pathways of up-regulated and down-regulated DEGs (Fig. 3A and 3B) and their biological significance were shown (Table 2).
### Table 2
Pathway enrichment of DEGs in GBM

<table>
<thead>
<tr>
<th>Pathway ID</th>
<th>KEGG pathway name</th>
<th>Gene number</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>up</td>
<td>hsa05132 Salmonella infection</td>
<td>6</td>
<td>0.000493</td>
</tr>
<tr>
<td></td>
<td>hsa04630 Jak-STAT signaling pathway</td>
<td>7</td>
<td>0.002483</td>
</tr>
<tr>
<td></td>
<td>hsa05323 Rheumatoid arthritis</td>
<td>5</td>
<td>0.004029</td>
</tr>
<tr>
<td></td>
<td>hsa04380 Osteoclast differentiation</td>
<td>6</td>
<td>0.004471</td>
</tr>
<tr>
<td></td>
<td>hsa04933 AGE-RAGE signaling pathway in diabetic complications</td>
<td>5</td>
<td>0.006567</td>
</tr>
<tr>
<td></td>
<td>hsa05142 Chagas disease (American trypanosomiasis)</td>
<td>5</td>
<td>0.007419</td>
</tr>
<tr>
<td></td>
<td>hsa05020 Prion diseases</td>
<td>3</td>
<td>0.007850</td>
</tr>
<tr>
<td></td>
<td>hsa04620 Toll-like receptor signaling pathway</td>
<td>5</td>
<td>0.008027</td>
</tr>
<tr>
<td></td>
<td>hsa00500 Starch and sucrose metabolism</td>
<td>3</td>
<td>0.008492</td>
</tr>
<tr>
<td></td>
<td>hsa00603 Glycosphingolipid biosynthesis - globo and isoglobo series</td>
<td>2</td>
<td>0.013014</td>
</tr>
<tr>
<td>down</td>
<td>hsa00190 Oxidative phosphorylation</td>
<td>30</td>
<td>0.000000</td>
</tr>
<tr>
<td></td>
<td>hsa05016 Huntington's disease</td>
<td>37</td>
<td>0.000000</td>
</tr>
<tr>
<td></td>
<td>hsa05012 Parkinson's disease</td>
<td>29</td>
<td>0.000000</td>
</tr>
<tr>
<td></td>
<td>hsa05010 Alzheimer's disease</td>
<td>32</td>
<td>0.000000</td>
</tr>
<tr>
<td></td>
<td>hsa00330 Arginine and proline metabolism</td>
<td>15</td>
<td>0.000001</td>
</tr>
<tr>
<td></td>
<td>hsa04932 Non-alcoholic fatty liver disease (NAFLD)</td>
<td>25</td>
<td>0.000069</td>
</tr>
<tr>
<td></td>
<td>hsa00340 Histidine metabolism</td>
<td>7</td>
<td>0.001145</td>
</tr>
<tr>
<td></td>
<td>hsa00051 Fructose and mannose metabolism</td>
<td>8</td>
<td>0.001924</td>
</tr>
<tr>
<td></td>
<td>hsa04146 Peroxisome</td>
<td>14</td>
<td>0.002235</td>
</tr>
<tr>
<td></td>
<td>hsa00250 Alanine, aspartate and glutamate metabolism</td>
<td>8</td>
<td>0.002871</td>
</tr>
</tbody>
</table>

Pathway analysis showed that the up-regulated DEGs were mainly involved in JAK-STAT, Toll-like receptor, and other signaling pathways, while the down-regulated DEGs were mainly involved in oxidative phosphorylation, amino acid metabolism, and other signaling pathways. These pathways are important in the formation of glioblastoma.

4. **Construction of DEGs networks and screening of Hub genes**
Significant GO functions and Pathways were selected, the genes contained in them were extracted, and the protein-protein interaction (PPI) network was constructed online with the STRING database (version 10.5). The network was edited using Cytoscape, the up-regulated DEGs were shown in red and the down-regulated DEGs were shown in green (Figs. 4 and 5). Cytohubba Plug-in was used to screen the top 10 Hub genes according to the degree among the DEGs (Table 3).

Figure 4. Hub genes in GO function analysis. The up-regulated DEGs were shown in red and the down-regulated DEGs were shown in green. After removing the unconnected nodes, a total of 5 DEGs were up-regulated, and the top 10 Hub genes from PPI analysis were down-regulated.

Figure 5. Hub genes in Pathway analysis. The up-regulated DEGs were shown in red and the down-regulated DEGs were shown in green. After removing the unconnected nodes, a total of 23 DEGs were up-regulated, and the top 10 Hub genes from PPI analysis were down-regulated.
### Table 3

**Hub Genes in GO function and Pathway analysis**

<table>
<thead>
<tr>
<th>Gene Symbol</th>
<th>Up/Down</th>
<th>FC</th>
<th>P Value</th>
<th>Degree</th>
</tr>
</thead>
<tbody>
<tr>
<td>GO</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NDUFA4*</td>
<td>down</td>
<td>-2.23</td>
<td>0.033936</td>
<td>17</td>
</tr>
<tr>
<td>NDUFA6*</td>
<td>down</td>
<td>-2.50</td>
<td>0.045149</td>
<td>17</td>
</tr>
<tr>
<td>CYCS*</td>
<td>down</td>
<td>-2.09</td>
<td>0.048825</td>
<td>16</td>
</tr>
<tr>
<td>CYC1</td>
<td>down</td>
<td>-1.88</td>
<td>0.028932</td>
<td>16</td>
</tr>
<tr>
<td>NDUFB2</td>
<td>down</td>
<td>-3.58</td>
<td>0.001380</td>
<td>13</td>
</tr>
<tr>
<td>PPP2R1A</td>
<td>down</td>
<td>-1.62</td>
<td>0.028192</td>
<td>13</td>
</tr>
<tr>
<td>ATP5G1</td>
<td>down</td>
<td>-2.55</td>
<td>0.020204</td>
<td>13</td>
</tr>
<tr>
<td>NDUFV2*</td>
<td>down</td>
<td>-2.34</td>
<td>0.036314</td>
<td>13</td>
</tr>
<tr>
<td>NDUFB7</td>
<td>down</td>
<td>-1.64</td>
<td>0.005584</td>
<td>13</td>
</tr>
<tr>
<td>MRPS2</td>
<td>down</td>
<td>-1.50</td>
<td>0.045865</td>
<td>13</td>
</tr>
<tr>
<td>Pathway</td>
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<td></td>
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</tr>
<tr>
<td>NDUFA4*</td>
<td>down</td>
<td>-2.23</td>
<td>0.033936</td>
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<tr>
<td>NDUFA6*</td>
<td>down</td>
<td>-2.50</td>
<td>0.045149</td>
<td>16</td>
</tr>
<tr>
<td>CYCS*</td>
<td>down</td>
<td>-2.09</td>
<td>0.048825</td>
<td>14</td>
</tr>
<tr>
<td>ATP5D</td>
<td>down</td>
<td>-2.35</td>
<td>0.042369</td>
<td>13</td>
</tr>
<tr>
<td>MGST2</td>
<td>down</td>
<td>-2.07</td>
<td>0.033136</td>
<td>12</td>
</tr>
<tr>
<td>MGST3</td>
<td>down</td>
<td>-1.80</td>
<td>0.012601</td>
<td>12</td>
</tr>
<tr>
<td>GSTM3</td>
<td>down</td>
<td>-4.55</td>
<td>0.006505</td>
<td>12</td>
</tr>
<tr>
<td>GSTK1</td>
<td>down</td>
<td>-2.37</td>
<td>0.019189</td>
<td>12</td>
</tr>
<tr>
<td>MAPK3</td>
<td>down</td>
<td>-1.69</td>
<td>0.022645</td>
<td>12</td>
</tr>
<tr>
<td>NDUFV2*</td>
<td>down</td>
<td>-2.34</td>
<td>0.036314</td>
<td>12</td>
</tr>
</tbody>
</table>

*means this gene is a Hub gene both in GO function and in Pathway analysis

The 16 Hub genes were all down-regulated. NDUFA4, NDUFA6, and NDUFV2 are involved in the formation of mitochondrial respiratory chain complexes IV and I, CYCS is a pro-apoptotic gene. These are the Hub genes shared by GO function and Pathway analysis. In other Hub genes, CYC1 and ATP5G1 are closely related to electron transport and ATP production in mitochondria. GSTM3 and GSTK1 are related to cell
metabolism, and their expression may impact the prognosis of patients with malignant tumors. PPP2R1A is involved in encoding the protein phosphatase 2, which regulates the dephosphorylation of the protein. The mitogen-activated protein kinase 3 encoded by the MAPK3 gene is an important member of the Ras/MEK/ERK tumor proliferation pathway and plays a role in regulating tumor cell proliferation.

5. Survival analysis of Hub genes

Survival analysis of Hub genes showed a statistically significant association between the expression levels of NDUFB2 and MGST2 genes and the prognosis of patients with glioblastoma. The overall survival of patients with high expression of NDUFB2 was significantly lower than that of patients with low expression (Fig. 6, P = 0.0028), and the overall survival of patients with high expression of MGST2 was also lower than that of patients with low expression (Fig. 7, P = 0.041). However, there was no statistically significant correlation between the expression levels of other Hub genes and the prognosis.

Discussion

Propofol and sevoflurane can be safely used in neurosurgical anesthesia [13]. Previous studies have suggested that propofol provides a positive effect on the prognosis of patients with malignant tumors, but the potential mechanisms are unclear.

This study focused on the changes in Hub genes’ expression after exposure to propofol or sevoflurane and their association with the prognosis of patients with glioblastoma. Survival analysis showed that the upregulation of NDUFB2 and MGST2 genes may be poor prognostic factors in patients with glioblastoma, and there was no statistical difference between the expression level of other Hub genes and prognosis. The function of the NDUFB2 gene and its association with tumors have not been reported in previous studies. The MGST2 protein induces the synthesis of leukotriene C4, which in turn triggers DNA damage [14]. In studies related to gliomas, Yang et al. observed that patients with an up-regulated MGST2 gene have a shorter survival time, and MGST2 also could be used in predicting the survival of glioma patients when combined with other genes [15]. In this study, the expressions of NDUFB2 and MGST2 genes in the sevoflurane group were up-regulated compared with the propofol group, which indicates that sevoflurane is more related to the poor prognosis of patients with glioblastoma.

Among the Hub genes, NDUFA4, NDUFA6, NDUFV2, and CYCS genes are the Hub genes shared by significant GO and Pathway. The NDUFA4 protein encoded by the NDUFA4 gene is a subunit of the mitochondrial respiratory chain complex IV [16]. Li et al. confirmed that the NDUFA4 in gastric cancer tissues and cells was up-regulated compared with normal tissues and cells. The up-regulated NDUFA4 gene activated the IncMIF-AS1/miR-212-5p/NDUFA4 signaling pathway and the oxidative phosphorylation pathway, which significantly promoted the proliferation of gastric cancer cells and reduced apoptosis [17]. CYCS is a pro-apoptosis-related gene, and its encoding product, cytochrome c (CYC) protein, is a component of the mitochondrial electron transport chain. Previous studies have suggested that ferulic acid (FA) inhibits the proliferation of prostate cancer cells by increasing the expression of CYCS, CASP1/2/8 and other genes [18]. Besides, Bredel et al. believed that glioma was the
cumulative result of repeated abnormal changes in multiple chromosomes, and the genes in these altered regions had synergistic effects and tumor promotion relationships, while the co-changes of genes with the strongest interaction, including CYCS, POLD2, MYC, etc., might be detrimental to the survival of patients [19]. NDUFV2 gene encodes the NDUFV2 subunit of the core unit of the respiratory chain complex I, which is involved in the mitochondrial electron transfer process, but its function has not been clarified [20]. No association has been found between the NDUFA6 gene and tumor prognosis.

In non-shared Hub genes, CYC1 is closely related to tumor prognosis, and its products participate in the formation of mitochondrial respiratory chain cytochrome bc1 complex, which binds with CYC to maintain mitochondrial current [21]. Previous studies have suggested that CYC1 and other 10 genes can be used as prognostic biomarkers for uveal melanoma, and are associated with shorter metastasis-free survival time [22]. MiRNA-661 can also accelerate the apoptosis of osteosarcoma cells by down-regulating the expression of CYC1 gene [23]. Han et al. observed that in breast cancer patients, up-regulation of CYC1 gene could inhibit the activation of AMPK, thereby promoting tumor metastasis, and the large amount of ATP generated by the up-regulated gene could also promote the growth of tumor cells [24]. Thus, the down-regulation of CYC1 gene may be associated with a better prognosis.

ATP5G1 gene encodes the ATP synthase F0 subunit. The study of Muluhngwi et al. confirmed that the inhibition of miRNA-29 on the proliferation of temozolomide resistant breast cancer cells was mediated by the inhibition of ATP5G1 and ATPIF1 genes to a certain extent [25]. In RCC cells, the researchers observed that the increased levels of the ATP5G1/G2/G3 protein were associated with lower survival. Decreased ATP synthase subunit in tumor cells was the basis of decreased mitochondrial electron transport chain activity, and the change of ATP5G1/G2/G3 gene expression might affect the patients’ prognosis [26]. In this study, the expression of ATP5G1 gene in tumor tissues exposed to propofol is down-regulated compared with tumor tissues exposed to sevoflurane, which may indicate that propofol is associated with better prognosis.

In other Hub genes, the MRPS2 gene encodes the mitochondrial ribosomal protein S2. Tang et al. showed that benzyl isothiocyanate induced morphological changes in glioma GBM8401 cells and promoted apoptosis by inhibiting the expression of 7 mitochondrial ribosome genes including MRPS2, which might be a potential biomarker for glioma [27]. In our study, the expression of MRPS2 gene is down-regulated, which suggests that the use of propofol may correlate with a better prognosis for patients with glioblastoma. The PPP2R1A gene encodes a structural subunit of the protein phosphatase 2 (PP2A). Current studies have confirmed that without mutations, the PPP2R1A gene plays an anticancer role in alveolar rhabdomyosarcoma cells [28]. Meanwhile, PPP2R1A protein is involved in the formation of PP2A protein, which is considered as a tumor suppressor and is involved in a variety of biological processes such as cell signaling pathway construction and cell apoptosis [29]. Our study observed the down-regulation of PPP2R1A gene expression in patients exposed to propofol, which might indicate that the tumor-suppressive effect of PP2A protein was weakened after propofol treatment. The GSTM3 gene encodes GSTM3 protein in the glutathione s-transferase (GSTs) family, which is mainly expressed in brain tissue [30]. Previous studies suggested that the silencing of GSTM3 gene induced the growth and
invasion of RCC cells, and the down-regulation of GSTM3 gene promoted tumorigenesis and was associated with poor prognosis [31]. GSTM3 also significantly reduce the tolerance of hepatic cancer cells (HCC) to radiotherapy by stimulating apoptosis-related genes (Bcl-2, p53, etc.), which may be a potential target for HCC cells to radiotherapy [32]. Therefore, GSTM3 is considered as a potential tumor suppressor. While in our study, the expression of GSTM3 gene in the propofol group was down-regulated compared with the sevoflurane group, whether it indicated that the patients in the propofol group had a poor prognosis should be further verified. The GSTK1 gene encodes the glutathione S-transferase κ1 protein. In colon cancer tissues, researchers found that the expression of GSTK1, GSTT1 and CYP1A1 genes in tumor tissues was higher than normal colon tissues, which might be related to the occurrence and development of colon cancer [33]. It suggested that GSTK1 gene was related to tumor progression, and the down-regulation after propofol exposure might be detrimental to the survival of tumor cells. The MAPK3 gene encodes mitogen-activated protein kinase 3, which is an important member of the Ras / MEK / ERK tumor proliferation pathway and regulates tumor cell proliferation. Previous studies have shown that the upregulation of miRNA-206 can inhibit the invasion and angiogenesis in triple-negative breast cancer cells, and the upregulation of miRNA-206 is accompanied by the downregulation of MAPK3, VEGF, and SOX9 levels [34]. In non-small cell lung cancer, blocking the MAPK3/1 (ERK1/2) signaling pathway can enhance the therapeutic response of EGFR receptor inhibitors and overcome the drug resistance of lung cancer cells, thus achieving a better prognosis [35]. And in pancreatic cancer and endometrial cancer, PPI analysis showed that MAPK3 was one of the Hub genes in tumor tissues [36, 37]. Some studies have also confirmed that MAPK3, MAPK14, etc. can be used as potential biomarkers for early diagnosis and treatment of colorectal cancer [38]. According to current studies, MAPK3 gene is mainly involved in the process of cell signal transduction of tumor cells, but the relationship between the expression level of this single gene and tumor prognosis needs further investigation, and there is no evidence to prove that this gene is an independent factor related to the prognosis of glioma and other tumors.

Besides, the Hub genes NDUFB7, ATP5D, and MGST3 have not been retrieved in the relationship between anesthetic agents and the prognosis of patients with malignant tumors, which may be potential targets for future research.

Up to now, there are few studies focused on the effect of anesthetic agents on gene expression in tumor cells. Our study innovatively used mRNA microarray to analyze the effect of propofol and sevoflurane on gene expression in glioblastoma tissues and tried to reveal the influence of different anesthetic agents on the prognosis of glioblastoma patients from the view of gene expression. Meanwhile, our study also has some limitations. First of all, due to the limitations of experimental conditions, this study did not include a large number of glioblastoma specimens for mRNA microarray analysis. Secondly, although the location and pathological classification of tumors were strictly restricted in this study, its homogeneity was still weaker than that of cytological study.

Conclusions
In summary, general anesthetic agents can alter the expression of prognostic-related genes in glioblastoma to a certain extent. Changes in NDUFB2 and MGST2 gene expression levels can affect the overall survival of patients with glioblastoma, and Hub genes NDUFA4, NDUFA6, NDUFV2, CYCS, CYC1, ATP5G1, MRPS2, NDUFB7, NDUFB2, ATP5D may affect the prognosis of glioblastoma patients by interfering the energy metabolism process of tumor cells. Further research can focus on the above genes to verify the internal mechanism of their influence on the prognosis of patients to guide the application of anesthetic agents.

**Declarations**

**Ethics approval and consent to participate:** Approved by Xuanwu Hospital Ethics Committee in June 2017 ([2017]016)

**Consent for publication:** Not applicable

**Data availability:** The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

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**Authors' contributions:** YA conducted the study, analyzed the data and wrote the manuscript. LZ designed the study, conducted the study, analyzed the data and wrote the manuscript. TLW helped design the study and revise the manuscript. DGL helped design the study, analyze the data and revise the manuscript. LXL helped conducted the study and analyzed the data. ZJL helped conduct the study and wrote the manuscript. CYL helped conduct the study and wrote the manuscript. PW analyzed the data and wrote the manuscript. All authors read and approved the final manuscript.

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**Figures**
Figure 1

The plot of DEGs. Up-regulated DEGs are expressed in red and down-regulated DEGs are expressed in green.
Figure 2

GO functions enriched by DEGs. A: functional enrichment of up-regulated DEGs B: functional enrichment of down-regulated DEGs (BP-biological process, CC-cellular component, MF-molecular function)

Figure 3

Pathways enrichment of DEGs. A. pathways enrichment of up-regulated DEGs. B. pathways enrichment of down-regulated DEGs.
Figure 4

GO Hub Genes
Figure 5

KEGG Pathway Hub Genes
Figure 6

Relationship between NDUFB2 gene expression and survival time of patients with glioblastoma
Figure 7

Relationship between MGST2 gene expression and survival time of patients with glioblastoma