Enhanced expression of TOP2A as a predictive biomarker in glioma patients

Tianmin Zhou1, Yan Wang2, Dongmeng Qian1, Qing Liang1, Bin Wang1

1Key Laboratory of Medicine and Biotechnology of Qingdao, Department of Microbiology, Medical College of Qingdao University, Qingdao, Shandong, P. R. China; 2Department of Pathology, The Affiliated Hospital of Qingdao University, Qingdao, Shandong, P. R. China

Received December 7, 2017; Accepted January 19, 2018; Epub March 1, 2018; Published March 15, 2018

Abstract: Topoisomerase II alpha (TOP2A), an enzyme that controls and alters the topologic state of DNA during transcription, is aberrant in many cancers. However, few studies have investigated expression of TOP2A and its clinical significance in glioma. We retrieved six independent investigations from the Oncomine database and identified that TOP2A is highly expressed in glioma tissues compared with corresponding normal controls. Similar results were also found in clinical specimens at the protein level. Immunohistochemical analysis indicated that TOP2A overexpression was highly correlated with grade stage, KI67 positive percentage, IDH1 mutation, and age, but other clinical parameters such as sex distribution and tumor size were barely associated with high TOP2A gene expression. Meanwhile, we used Prognoscan to assess the prognostic value of TOP2A expression in glioma patients, and found that high expression was associated with poor prognosis of patients with glioma. Furthermore, we used the Gene-Cloud of Biotechnology Information (GCBI) bioinformatics platform to predict the role of TOP2A in glioma. It was not only involved in DNA replication, chromosome condensation, and responses to DNA damage stimuli, but also promoted cancer cell mitotic cell cycle and apoptosis, and phosphatidylinositol-mediated signaling by regulating gene expression. By these approaches we demonstrate that TOP2A may be a reliable prognostic factor or therapeutic target in glioma.

Keywords: TOP2A, glioma, marker, prognosis

Introduction

Glioma remains the most common adult brain tumor which is slightly more common in men than in women [1], with 7.3 cases per 100,000 person-years. High-grade gliomas are present in 85% and low-grade glioma in 15% with 5-year overall survival of 82, 54, 22 and 3% for grade I, II, III and IV, respectively [2]. Within a five-year follow-up time, most patients had a recurrent tumor and died of the disease [3]. Despite much effort focused on improving the diagnosis and therapy of glioma, the clinical outcome of glioma is still unsatisfactory [4, 5]. Consequently, more studies are needed to explore the potential mechanism and detect effective diagnostic and prognostic biomarkers to prolong the lives of glioma patients.

TOP2A gene encodes a DNA topoisomerase that controls and alters the topologic state of DNA during transcription. This nuclear enzyme is involved in processes such as chromosome condensation, chromatid separation, and the relief of torsional stress that occurs during DNA transcription and replication [6]. Its aberrant expression has been associated with multiple tumor prognostics [7], including lung cancer, breast cancer, pancreatic adenocarcinoma, and colorectal cancer [8-11]. There are few investigations analyzing expression of TOP2A in glioma [12], and due to the limited number of studies and few clinical samples, no role has been detected.

In this study, we extended glioma research utilizing large databases and clinical samples, with the purpose of determining the expression level of TOP2A in glioma and its corresponding prognostic value. Using the Gene-Cloud of Biotechnology Information (GCBI) bioinformatics platform we investigated the role of TOP2A of glioma.
Materials and methods

Oncomine analysis

The Oncomine database (http://www.oncomine.org) incorporates 264 independent datasets including 35 cancer types and supports various methods of analysis, including molecular concepts, interactome, and meta-analysis [13]. Thus, we detected the level of TOP2A in different cancers including hematological malignancies and solid tumors and adopted the Oncomine database online tools meta-analysis to estimate the expression of TOP2A in glioma and normal brain control that come from 6 different datasets.

PrognoScan

PrognoScan (http://www.prognoscan.org) is a comprehensive platform for evaluating potential tumor biomarkers and therapeutic targets. Based on a large collection of cancer microarray datasets with clinical annotation, PrognoScan is a useful online tool for assessing the association between specific gene expression and prognosis in patients with cancer [14]. We used the PrognoScan platform to validate the prognostic value of TOP2A expression in patients with glioma.

Patients and specimens

Forty-six glioma tumor tissue samples were collected from the Affiliated Hospital of Qingdao University at Qingdao in China from July 2014 to December 2016. All the patients were with tumors suitable for resection according to the Chinese guideline of surgical treatment strategy for glioma in 2012, and among them, 16 cases were in grade II, 9 cases were grade III, and 21 cases were glioma. Altogether, 46 cases of formalin-fixed paraffin-embedded (FFPE) specimens were used for Immunohistochemistry.

In this study, the inclusion criteria were as the following: (1) Pathological diagnosis of glioma tumors; (2) A diagnosis that was consistent with the histological diagnostic criteria of the World Health Organization [15]; (3) Tumor is the primary tumor in the brain, and patients did not receive pre-operative anti-cancer treatment, and there were no extrahepatic metastases; (4) For the use of archived tissue specimens from these patients, permission from the Affiliated Hospital of Qingdao University was granted. (5) A representative sample was taken from each FFPE tissue and hematoxylin and eosin (HE) staining was performed. Results were reviewed by two experienced pathologists.

Immunohistochemical staining and evaluation

A traditional immunohistochemical (IHC) staining protocol was used in this study. Briefly, tissue slices was deparaffinized in xylene and rehydrated with different concentrations of ethanol alcohol. Then the section was treated with 3% hydrogen peroxide, followed by antigen retrieval with 10 mM citrate buffer (pH 6.0) with microwave. After being blocked with 10% goat serum for 30 min, incubation with the primary antibody was done overnight with anti-TOP2A antibody (mouse monoclonal antibody, 1:100 dilution, ZM-0245, Zhongshan jinqiao, China) at 4°C, followed by a peroxidase-labeled secondary antibody. Immunoreaction score distribution (IOD) measurement, as reported previously, was used to determine the positive-staining density [16]. A Leica CCD camera DFC420 connected to a Leica DM IRE2 microscope (Leica Microsystems Imaging Solutions, Ltd., Cambridge, UK) was used as an imaging system. High-power magnification (×40) was used to photograph representative fields. The IODs in each image were measured and counted using Image-Pro Plus v6.0 software (Media Cybernetics, Inc., Bethesda, MD, USA).

X-tile analysis

TOP2A expression was assessed with X-tile plots. TOP2A expression was expressed as IOD and the optimization of cutoff points was based on outcomes. The cutoff score derived from 46 cases of training set by a standard log-rank method was used to assess statistical significance. \( P \) values were obtained from a lookup table.

Gene-cloud of biotechnology information (GCBI)

GCBI (Shanghai, China, https://www.gcbi.com.cn) is a platform comprising a variety of research findings, genetic information, sample information, data algorithms and bioinformat-
Over-expression of TOP2A gene in patients with glioma

It creates a “gene knowledge base” which encompasses biology, medicine, informatics, computer science, mathematics, graphics, and other disciplines. GCBI includes more than 120 million copies of genomic samples, approximately 90,000 copies of tumor samples and more than 17 million copies of genetic information. Therefore, GCBI is a good bioinformatics analysis platform and has provided data analysis support for many studies on cancer research [17-20]. In this study, we used GCBI to identify differentially expressed genes (DEGs) between glioma tissue and normal brain control and finally predict the role of TOP2A in gliomas.

**Statistical analysis**

All the statistical analyses were carried out using SPSS 17.0 software (SPSS Inc, Chicago, IL, USA) and GraphPad Prism 5 (San Diego, CA) software. The relationship between TOP2A expression and clinicopathological factors was analyzed by Chi-squared test and Analysis of

---

**Figure 1.** Analysis of TOP2A expression by Oncomineonline database. A: The mRNA expression of TOP family members in different tumor types. This graphic shows the numbers of datasets with statistically significant mRNA over-expression (red) or down-expression (blue) of the target gene (cancer vs. normal tissue). The P value threshold is 0.01. The number in each cell represents the number of analyses that meet the threshold within those analysis and cancer types. The gene rank was analyzed by percentile of target gene in the top of all genes measured in each research. Cell color is determined by the best gene rank percentile for the analyses within the cell. B: TOP2A expression is up-regulated in glioma. A meta-analysis of TO2PA gene expression from six Oncomine databases where colored squares indicate the median rank for TOP2A (vs. Normal tissue) across 8 analyses. C: Comparison of TOP2A mRNA expression in TCGA dataset. D: Comparison of TOP2A mRNA expression in Sun L etal. dataset. Comparison the expression levels of TOP2A in paired glioma tumor and peri-tumoral tissues.
Over-expression of TOP2A gene in patients with glioma

Table 1. Meta-analysis of TO2PA gene expression from six Oncomine databases

<table>
<thead>
<tr>
<th>Datasets (sample size)</th>
<th>Comparison groups</th>
<th>Fold Change</th>
<th>P-value</th>
<th>Overexpression Gene Rank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sun Brain (180)</td>
<td>Glioblastoma vs. Normal</td>
<td>102.544</td>
<td>2.37E-27</td>
<td>22 (in top 1%)</td>
</tr>
<tr>
<td></td>
<td>Oligodendroglia vs. Normal</td>
<td>28.449</td>
<td>8.4E-18</td>
<td>27 (in top 1%)</td>
</tr>
<tr>
<td></td>
<td>Anaplastic Astocytoma</td>
<td>34.640</td>
<td>4.10E-12</td>
<td>27 (in top 1%)</td>
</tr>
<tr>
<td>Liang Brain (33)</td>
<td>Glioblastoma vs. Normal</td>
<td>2.202</td>
<td>6.27E-6</td>
<td>115 (in top 2%)</td>
</tr>
<tr>
<td>TCGA Brain (552)</td>
<td>Glioblastoma vs. Normal</td>
<td>28.903</td>
<td>2.59E-13</td>
<td>115 (in top 2%)</td>
</tr>
<tr>
<td>French Brain Statistics (29)</td>
<td>Anaplastic Oligodendroglia VS. Brain</td>
<td>9.685</td>
<td>1.15E-8</td>
<td>115 (in top 2%)</td>
</tr>
<tr>
<td>Shai Brain Statistics (34)</td>
<td>Glioblastoma vs. White Matter</td>
<td>2.291</td>
<td>1.17E-6</td>
<td>227 (in top 3%)</td>
</tr>
<tr>
<td>Murat Brain Statistics (84)</td>
<td>Glioblastoma vs. Normal</td>
<td>13.755</td>
<td>2.17E-11</td>
<td>262 (in top 2%)</td>
</tr>
</tbody>
</table>

Note: A meta-analysis of TO2PA gene expression from six Oncomine databases where colored squares indicate the median rank for TOP2A (vs. Normal tissue) across 8 analyses. French Brain (1), Liang Brain (2), Murat Brain (3), Shai Brain (4), Sun Brain (5-7) and TCGA (8). The P value is given for the median rank analysis.

Figure 2. TOP2A expression levels in peri-tumoral tissues (A) and glioma (B) (×40). (C) Box plot indicating the mean staining intensity of paired glioma and peri-tumoral tissues. (D) Paired peri-tumoral tissue and glioma tissue in the same section (D) (×10).

Variance. Survival curves for TOP2A expression were generated using the Kaplan-Meier method and compared using the log-rank test. P values < 0.05 were considered statistically significant. Each statistical analysis of online database was completed by usedits online tool.

Results

TOP2A is distinctively overexpressed in glioma than normal brain

Firstly we used Oncomine database to analyze the expression of TOP2A in human cancers, including hematological malignancies and solid tumors (Figure 1A). We found that TOP2A mRNA expression was high in different cancer types than corresponding normal samples across a wide variety of datasets. Then in order to assess the expression of TOP2A in glioma, we analyzed six independent microarray datasets from Oncomine database [21-26], and revealed statistically significant over expression of TOP2A in the majority of glioma tissues compared with normal brain controls (Table 1). The median rank of TOP2A in up-regulated genes of TOP2A was 126.0 based on a meta-analysis across the six datasets, including 8 analyzed using Oncomine algorithms [27] (P = 3.14*10-6, Figure 1B) (In total, 859 glioma samples and 53 healthy controls were used). TOP2A transcripts were 28.90 fold elevated in glioma samples as compared with
Over-expression of TOP2A gene in patients with glioma

Table 2. Association of TOP2A expression with pathological characteristics of glioma (46 cases)

<table>
<thead>
<tr>
<th>Pathological category</th>
<th>Case number</th>
<th>TOP2A Low</th>
<th>TOP2A High</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>29</td>
<td>11</td>
<td>18</td>
<td>0.1264</td>
</tr>
<tr>
<td>Female</td>
<td>17</td>
<td>11</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥ 50</td>
<td>23</td>
<td>5</td>
<td>18</td>
<td>0.0009</td>
</tr>
<tr>
<td>&lt; 50</td>
<td>23</td>
<td>17</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>Tumor size</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥ 3 cm</td>
<td>21</td>
<td>10</td>
<td>11</td>
<td>1.0000</td>
</tr>
<tr>
<td>&lt; 3 cm</td>
<td>25</td>
<td>12</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>IDH1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IDH1 (+)</td>
<td>15</td>
<td>12</td>
<td>3</td>
<td>0.0113</td>
</tr>
<tr>
<td>IDH1 (-)</td>
<td>16</td>
<td>5</td>
<td>11</td>
<td></td>
</tr>
</tbody>
</table>

normal tissues in a dataset with 552 samples that derived from TCGA database [21] (Figure 1C). In another dataset from Sun L study [22], TOP2A was 102.544 fold elevated in glioma samples as compared with normal tissues (P = 2.37*10-27) (Figure 1D).

Elevation of TOP2A in glioma compared with peri-tumoral tissues by immunohistochemistry

We also analyzed the expression levels of TOP2A protein in 46 glioma tumors and paired peri-tumoral tissues by immunohistochemistry (IHC). Expression of TOP2A protein levels was frequently higher in the glioma tumor tissues (Figure 2A) than in peri-tumoral tissues (Figure 2B). These differences were statistically significant (Figure 2C; P < 0.0001, paired t test). Meanwhile we found that expression of TOP2A is positive in all samples of glioma. In 43 cases, glioma tissues and peri-tumoral tissue were located in one area, and the picture clearly revealed that TOP2A expression was higher in peri-tumoral tissue than glioma tissue (Figure 2D).

Association of TOP2A expression with clinicopathological features of glioma patients

As show in Figure 3A and 3B, Immunoreaction score distribution (IOD) of TOP2A significantly increased along with grad stage of glioma (F = 11.91, P < 0.0001) and Ki67 positive percentage (Spearman’s r = 0.7788, P < 0.0001). We also learned the association of the expression of TOP2A with other clinicopathological features in glioma, as shown in Table 2. Expression of TOP2A association correlated with IDH1 mutation (P = 0.0113) and age (P = 0.0009), but other clinical parameters, such as sex distribution (P = 0.1264) and tumor size (P = 1.0000) were barely associated with high TOP2A gene expression.

High mRNA expression of TOP2A is an unfavorable prognostic factor for glioma

Because many of the above data show that TOP2A is highly expressed in gliomas. Ac-
Over-expression of TOP2A gene in patients with glioma

2925 (P < 0.01, Fold change > 2) significant DEGs were identified, of which 1041 over-presented and 1884 showed an attenuated behavior (Figure 5B and 5C). We found that TOP-2A was significantly overexpressed in glioma samples (Fold Change = 6.447, t = 17.9485, P < 0.01). We then performed a Gene Ontology analysis of 2925 differentially expressed genes (Figure 5D). Fortunately, we found that TOP2A in the enriched set was associated with mitotic cell cycle, positive regulation of transcription from RNA polymerase II promoter, positive regulation of apoptotic process, DNA replication, phosphatidylinositol-mediated signaling, response to DNA damage stimulus, chromosome condensation. So we speculate that the elevation of TOP2A not only takes part in DNA replication, chromosome condensation, and response to DNA damage stimulus, but also associates with mitotic cell cycle, apoptotic processes, and phosphatidylinositol-mediated signaling.

Figure 4. Kaplan-Meier survival curves generated from PrognoScan for TOP2A mRNA expression in patients with glioma. A: Overall survival curves for patients with glioma in the GEO dataset GSE4271. B: Patients with glioma in the GEO dataset GSE4412. HR = hazard ratio.

Discussion

Glioma, a tumor of central nervous system, which mainly develops from the macroglial cells, presents the highest prevalence and mortality risk. The difficult treatment of patients with glioma is primarily attributed to recurrence and resistance to chemoradiotherapy [29-31]. Despite the challenges of treatment, it is rewarding to illustrate the pathogenesis of glioma, as well as to develop novel prognostic strategies and discover effective therapeutic approaches. TOP2A is a nuclear enzyme that mainly involved in processes such as chromosome condensation, chromatid separation, and the relief of torsional stress that occurs during DNA transcription and replication [6]. It has been reported that TOP2A is a sensitive and specific marker in actively proliferating cells (in the late S, G2, and M phases of the cell cycle) which suggests a role in a wide range of human cancers [32, 33].

In the present study, through the Oncomine databases, we found that TOP2A was significantly over expressed in glioma compared with normal brain samples. Similar results were found in 46 gliomas and paired peri-tumoral tissues by immunohistochemistry by analysis of the association of TOP2A expression with clinicopathological features of glioma patients. TOP-2A significantly increased along with grade
Over-expression of TOP2A gene in patients with glioma

Figure 5. GCBI database Predict the role of TOP2A in glioma. A: Flow diagram of the study design; B: Volcano plot for potential DEGs (n = 180) (glioma n = 157, in yellow; normal lung tissues n = 23, in blue); X-axis was log2 (Fold Change), Y-axis was -log10 (p value). C: Heat map for potential DEGs (n = 180) (glioma n = 157, in yellow; normal lung tissues n = 23, in blue); D: Gene ontology (GO) enrichment analysis the DEGs in glioma.
stage of glioma, Ki67 positive percentage, IDH1 mutation and age, but other clinical parameters, such as sex distribution and tumor size were barely associated with high TOP2A gene expression. However, there was no evidence revealing a role of highly expressed TOP2A in the prognosis of glioma. In our study, according to the PrognoScan database, we could find its prognostic value: high expression of TOP2A caused shorter overall survival time and shorter disease-free survival time. Thus, the expression of TOP2A might become a potential biomarker for the prognosis of glioma.

As for the functional and pathway enrichment analysis, TOP2A was strongly emphasized in cell cycle pathways and DNA replication processes, which are connected with its physiological functions [34, 35]. In our study, GCBI analysis found that mitotic cell cycle, positive regulation of transcription from RNA polymerase II promoter, positive regulation of apoptotic process, DNA replication, phosphatidylinositol-mediated signaling, response to DNA damage stimulus, and chromosome condensation pathways were highly enriched in glioma samples with TOP2A up-regulated. Furthermore, earlier clinical data indicate that TOP2A mRNA and protein expression might participate in the process of chromosome condensation, chromatid separation, and the relief of torsional stress that occurs during DNA transcription and replication [6]. According to our analysis, we speculate that high expression of TOP2A at mRNA and protein levels might be one of the causes of cell cycle apoptotic process and phosphatidylinositol-mediated signaling.

In conclusion, we used bioinformatics and immunohistochemistry analysis to define the expression level of TOP2A in glioma. TOP2A was identified in association with the progression and prognosis of glioma, probably regulating cell cycle apoptotic processes and phosphatidylinositol-mediated signaling.

Disclosure of conflict of interest

None.

Address correspondence to: Tianmin Zhou, Key Laboratory of Medicine and Biotechnology of Qingdao, Department of Microbiology, Medical College of Qingdao University, 308 Ningxia Road, Qingdao, P. R. China. E-mail: 329637042@163.com; Bin Wang, School of Basic Medical Sciences, Qingdao University, Qingdao, P. R. China. Tel: +86-131-0772-05-70; E-mail: wangbinqingdao@163.com

References

Over-expression of TOP2A gene in patients with glioma


Over-expression of TOP2A gene in patients with glioma


