

Role and mechanism of RPA1 in the development and progression of glioma

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Abstract. Glioma is a highly malignant primary tumor of the central nervous system, characterized by highly invasive behavior and a high recurrence rate. The current treatment options for glioma often have unsatisfactory outcomes. Replication protein A1 (RPA1), a component of the RPA heterotrimer, has been identified as a potential oncogene implicated in the development and clinical prognosis of various types of solid tumors, including liver cancer, nasopharyngeal carcinoma and gastrointestinal cancer. However, studies on the associations between RPA1 expression and glioma cell proliferation or patient prognosis are limited, and the underlying mechanisms remain unclear. The aim of the present study was to explore the expression of RPA1 in glioma and its clinical relevance in the clinicopathology and prognosis of glioma. In addition, the biological functions and signal transduction pathways mediated by RPA1 were predicted using bioinformatics analysis to provide basic theoretical support for further mechanistic research and *in vivo* experiments. The Cancer Genome Atlas (TCGA) data analysis, and reverse transcription-quantitative PCR, western blot analysis and immunohistochemical (IHC) staining were performed to analyze RPA1 expression in glioma cells or tissues of different grades. The associations between RPA1 expression and various pathological parameters related to tumor cell proliferation and patient prognosis were

also investigated. Furthermore, the biological processes and signaling pathways influenced by RPA1-related target genes were predicted. The results revealed that RPA1 expression varies among different grades of gliomas, with higher World Health Organization (WHO) grades exhibiting higher RPA1 expression levels. Furthermore, both bioinformatics analysis and IHC staining results revealed that high RPA1 expression was associated with a shorter overall survival (OS). In addition, RPA1 expression exhibited a significant association with WHO glioma grade and key markers of malignancy, namely Ki-67 expression and p53 mutation status. Gene Ontology and Kyoto Encyclopedia of Genes and Genomes enrichment analyses suggested that RPA1 may promote the development of glioma through pathways including 'DNA replication', 'Fanconi anemia pathway', 'mismatch repair', 'homologous recombination', 'nucleotide excision repair' and 'cell cycle'. In conclusion, RPA1 expression in glioma tissues and cells is positively associated with the degree of glioma malignancy, and high RPA1 expression is associated with a shorter OS in patients with glioma.

Introduction

Gliomas, which originate from neuroepithelial tissue, account for 31% of all central nervous system (CNS) tumors and 81% of all malignant CNS tumors; they are the most common type of primary brain tumors worldwide (1,2). Great progress has been made in the treatment of gliomas, with the most commonly used treatments including tumor resection, adjuvant radiotherapy, chemotherapy and immunotherapy. However, gliomas, particularly glioblastoma (GBM), which is a grade IV tumor according to the glioma grading standards of the World Health Organization (WHO) (1), are highly malignant, strongly invasive and prone to recurrence. As a result, patients with GBM have a median survival time of only 1.5 years, and a 5-year survival rate of only 5.5% (3,4). Gliomas severely affect the quality of life and health of patients; therefore, it is important to identify novel therapeutic mechanisms and possible therapeutic targets for gliomas.

Replication factor A (RPA) is a heterotrimer that comprises RPA1 (70 kDa), RPA2 (32 kDa) and RPA3 (14 kDa). RPA1 is the main subunit of the RPA complex and the main contributor to its biological function; it also contributes to the stabilization of the complex formed by subunits RPA2

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Abbreviations: CNS, central nervous system; GBM, glioblastoma; GO, Gene Ontology; HGG, high-grade glioma; IHC, immunohistochemical; KEGG, Kyoto Encyclopedia of Genes and Genomes; LGG, low-grade glioma; OS, overall survival; TCGA, The Cancer Genome Atlas; RPA1, replication protein A1; MCODE, molecular complex detection

Key words: glioma, RPA1, clinical pathology, prognosis

and RPA3 (5-8). RPA1 has the following important roles: i) Maintains the correct replication of DNA (7); ii) participates in DNA repair, homologous recombination and DNA damage monitoring (8,9); and iii) participates in the occurrence and development of numerous kinds of tumors (10-13). To date, a number of studies on the association of RPA1 with tumors have shown that RPA1 serves as an oncogene, promoting the occurrence and development of liver cancer, bladder urothelial carcinoma, nasopharyngeal carcinoma, esophageal cancer, gastrointestinal cancer and other tumors (10-13). However, to the best of our knowledge, there have been no studies on the associations of RPA1 expression with glioma cell proliferation or the prognosis of patients with glioma.

The main aim of the present study was to explore the expression of RPA1 in glioma and its clinical relevance in the clinicopathology and prognosis of glioma. In addition, the biological functions and signal transduction pathways mediated by RPA1 were predicted using bioinformatics analysis to provide basic theoretical support for further mechanistic research and *in vivo* experiments.

Materials and methods

Bioinformatics analysis. All data used in the bioinformatics analysis were obtained from and analyzed using the following public databases: the UALCAN (<http://ualcan.path.uab.edu/index.html>), The Cancer Genome Atlas (TCGA; <https://cancergenome.nih.gov/>), STRING (<https://string-db.org/>) and the DAVID online analysis tool (<https://davidbioinformatics.nih.gov>). Gene Ontology (GO) and Kyoto Encyclopedia of Genome (KEGG) via the DAVID online analysis tool. Gene expression levels were compared and Kaplan-Meier survival analyses performed using the UALCAN online tool, which provides access to cancer transcriptome data from TCGA.

RPA1-related data were retrieved from TCGA, and differentially expressed genes were screened using the limma package in R (3.56.0; <https://bioconductor.org/packages/limma/>). The differentially expressed genes were subjected to protein-protein interaction (PPI) network analysis using the STRING database, which provides known and predicted protein interactions. The results were visualized using Cytoscape, and the molecular complex detection (MCODE) plug-in was used to identify clustered sub-networks within the PPI network. In the GO and KEGG gene enrichment analysis conducted via DAVID, the significance threshold was set at $P < 0.05$.

Cell culture. The HA1800 normal astrocyte cell line, two low-grade glioma (LGG) cell lines, namely U251 and SF295, and two GBM cell lines, namely A172 and TG905, were acquired. The normal astrocyte cell lines HA1800 and human glioma cell lines U251, SF295, A172 and TG905 were purchased from the Cell Bank of the Chinese Academy of Science. All cell lines were cultured in Dulbecco's modified Eagle's medium (DMEM) (HyClone; Cytiva) supplemented with 10% fetal bovine serum (HyClone; Cytiva), 100 g/ml streptomycin and 100 U/ml penicillin (Gibco; Thermo Fisher Scientific, Inc.) in a humidified atmosphere of 5% CO₂ at 37°C. The cells were collected and homogenized, and the cellular proteins and RNA were extracted as described below.

Sample acquisition and general data collection. Glioma tissue samples (WHO grades I-IV) were collected by neurosurgeons from 70 patients who underwent brain glioma resection and were diagnosed with glioma by postoperative histopathological examination at Linyi People's Hospital (Linyi, China) between June 2014 and December 2016 (Table I). In addition, 10 normal brain tissue samples were obtained from patients with craniocerebral trauma during frontal lobe decompression at Linyi People's Hospital (Linyi, China) between June 2014 and December 2016 (male:female, 6:4; 3 were <44 years old and 7 were ≥44 years old). Basic patient characteristics and clinical diagnosis information were obtained from all participants. The inclusion criteria comprised the availability of imaging data and complete details of the specific treatment process. Patients with a clear imaging diagnosis and postoperative pathological diagnosis were included in the experimental group. The study was approved by the Human Ethics Committee of Linyi People's Hospital (approval no. 30036), and all patients provided written informed consent.

Western blot analysis. Western blot analysis was performed to determine the RPA1 expression levels of the cell lines. Total protein was extracted from the cell homogenate in RIPA lysis buffer (Wanleibio) containing protease inhibitors, and the protein concentration was determined using the bicinchoninic acid method. Following electrophoresis (25 µg per lane; 10% SDS-PAGE), the proteins were transferred to a PVDF membrane. After blocking with 5% skimmed milk for 2 h at a room temperature (20-25°C), the membrane was incubated with an RPA1 antibody (1:400; cat. no. DF6172; Wuhan Sanying Biotechnology) overnight at 4°C. GAPDH mAb (1:2,000; cat. no. AF7021; Wuhan Sanying Biotechnology) was used as a control. The membrane was then washed by PBST (0.05% Tween-20) and incubated with horseradish peroxidase-labeled secondary antibody (1:2,500; cat. no. S0001; Cell Signaling Technology, Inc.) for 2 h at a room temperature. After washing the membrane three times, an ECL Supersensitive Detection kit (Shanghai Biyuntian Biotechnology Co., Ltd.) was used to visualize the protein bands. QuantityOne densitometric analysis software (version 4.6; Bio-Rad Laboratories, Inc.) was used to calculate the expression level of RPA1 using GAPDH as the loading control. Each western blot experiment was repeated at least three times to ensure the reproducibility of the results.

Immunohistochemical (IHC) staining. The glioma tissue was embedded in paraffin with 4% paraformaldehyde for 24 h at room temperature (20-25°C), sectioned (4 µm) and dried at 70°C for 45 min. The paraffin sections were routinely dewaxed and hydrated, followed by antigen retrieval in citrate buffer (PH 6.0) for 15 min at 95-100°C. The sections were then subjected to quenching with a peroxidase blocker (3% H₂O₂), washed with PBS, and blocked with 5% normal goat serum (Vector Laboratories, Inc.) for 1 h at 4°C. The sections were then incubated with primary RPA1 antibody (1:400; cat. no. DF6172; Wuhan Sanying Biotechnology), anti-Ki67 (1:400; cat. no. 27309-1-AP; Proteintech Group, Inc.) and anti-p53mut (1:300; cat. no. AF0879; Affinity Biosciences) overnight at 4°C. After washing with PBS, the sections were incubated with a goat anti-mouse IgG (H+L) HRP secondary antibody (1:2,000;

Table I. Analysis of the associations between RPA1 expression and clinicopathological parameters in 70 patients with glioma.

Clinicopathological parameters	Cases, n (%)	RPA1 expression, n (%)		χ^2	P-value
		Low	High		
Sex				0.043	0.836
Male	36 (51.4)	15 (41.7)	21 (58.3)		
Female	34 (48.6)	15 (44.1)	19 (55.9)		
Age, years				0.019	0.890
<44	38 (54.3)	16 (42.1)	22 (57.9)		
≥44	32 (45.7)	14 (43.8)	18 (56.2)		
Tumor diameter, cm				2.337	0.126
<4	17 (24.3)	10 (58.9)	7 (41.1)		
≥4	53 (75.7)	20 (37.7)	33 (62.3)		
Tumor location				2.705	0.259
Frontal lobe	33 (47.1)	16 (48.5)	17 (51.5)		
Temporal lobe	21 (30.0)	10 (47.6)	11 (52.4)		
Other	16 (22.9)	4 (25.0)	12 (75.0)		
KPS				1.544	0.214
<80	36 (51.4)	18 (50.0)	18 (50.0)		
≥80	34 (48.6)	12 (35.3)	22 (64.7)		
WHO grade				8.4	0.037
I-II	35 (50.0)	21 (60.0)	14 (40.0)		
III-IV	35 (50.0)	9 (25.7)	26 (74.3)		
Ki-67 expression				11.748	0.001
Low	26 (37.1)	18 (69.2)	8 (30.8)		
High	44 (62.9)	12 (27.3)	32 (62.7)		
p53 mutation status				7.099	0.008
Wild type	25 (35.7)	16 (64.0)	9 (36.0)		
Mutant	45 (64.3)	14 (31.1)	31 (68.9)		

RPA1, replication protein A1; KPS, Karnofsky performance status; WHO, World Health Organization.

cat. no. S0002; Affinity Biosciences) for 1 h at room temperature (20-25°C). Finally, the sections were counterstained with hematoxylin (5 min at normal temperature), digested with hydrochloric acid, washed, stained with ammonia bluing solution, dehydrated in an alcohol gradient, cleared and mounted. The IHC staining was interpreted independently by two pathologists using a light microscope. Positive expression of RPA1 was identified based on the appearance of brown granules in the cytoplasm and/or nucleus. The counting method was to count all tumor cells in the field of view one by one, and calculate the percentage of positive cells in the total cells, such as 'the RPA1-positive cell rate was ~30%'. An expression score was determined based on the percentage of positive cells and the staining intensity, which were scored as follows: 0 points, no positive cells in any visual fields; 2 points, ++ staining intensity and <10% positive cells; 3 points, + staining intensity and >50% positive cells; 4 points, ++ staining intensity and <50% positive cells; 5 points, ++ staining intensity and >50% positive cells; and 6 points, +++ staining intensity and 100% positive cells. A score of >4 was defined as high RPA1 expression and a score of ≤4 was defined as low RPA1

expression (14). In this immunohistochemistry of glioma cells, the high/low Ki-67 groups were classified based on the proportion of Ki-67 positive cells to total cells. Glioma samples with a positive cell proportion ≤5% were defined as the low Ki-67 groups, while those with a positive cell proportion >5% were classified as the high Ki-67 groups.

Reverse transcription-quantitative PCR (RT-qPCR) analysis. Total RNA was extracted from cells using an RNeasy Mini Kit (Takara Bio, Inc.), and the quality of the RNA was assessed using a NanoDrop ND-1000 instrument. cDNA was synthesized from 1 µg RNA using a High-Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific, Inc.) was used according to the manufacturer's protocol. qPCR was then performed using the StepOnePlus system (Applied Biosystems; Thermo Fisher Scientific, Inc.) with SYBR Green Master Mix (Thermo Fisher Scientific, Inc.) and gene-specific primers (Fuzhou Phygene Biotechnology Co., Ltd.). The following PCR conditions were used: 95°C for 10 min followed by 40 cycles of 95°C for 15 sec and 60°C for 1 min, and melting curve analysis. Relative gene expression

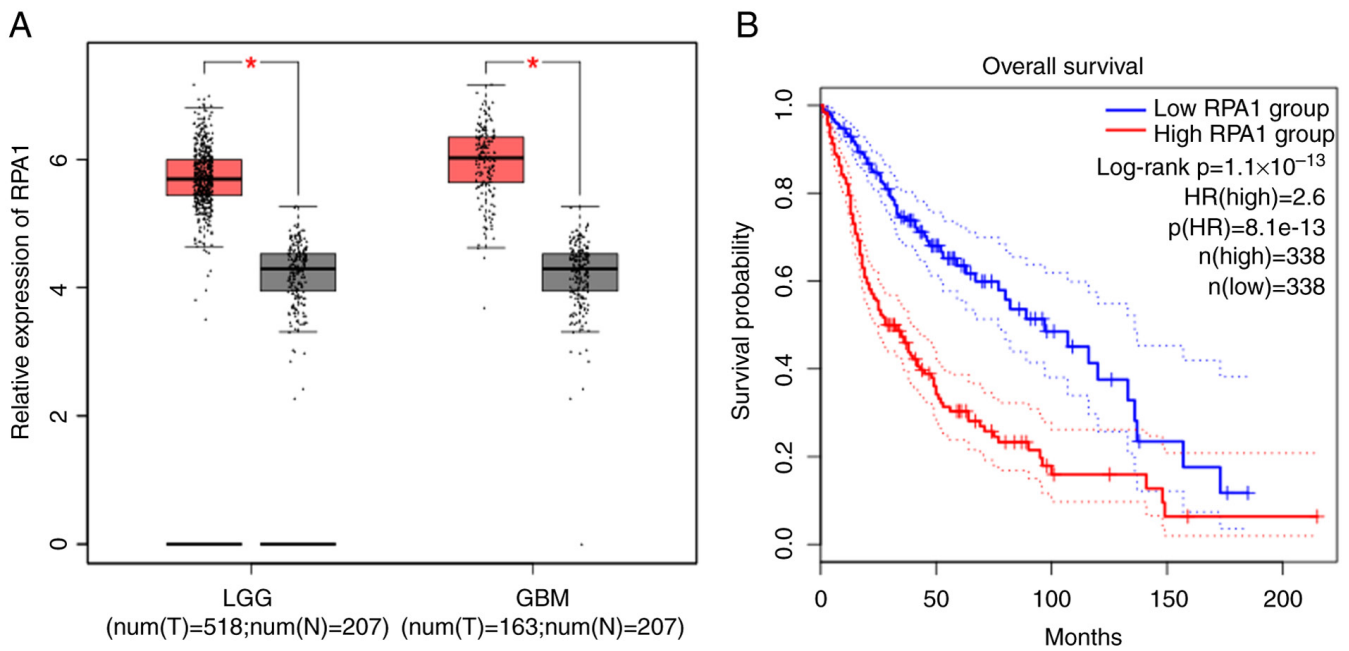


Figure 1. Bioinformatics analysis of RPA1 expression and survival in glioma. (A) Differential expression of RPA1 in LGG and GBM glioma tissues compared with normal brain tissue. (B) Kaplan-Meier curves for the comparison of overall survival in patients with gliomas according to RPA1 expression level. Both analyses were performed using data from The Cancer Genome Atlas via the UALCAN portal. RPA1, replication protein A1; LGG, low-grade glioma; GBM, glioblastoma; T, tumor; N, normal; HR, hazard ratio; $p(\text{HR})$, P-value of the HR. * $P < 0.05$.

was determined via the $2^{-\Delta\Delta C_q}$ method (15) using GAPDH as the reference gene. Triplicate reactions were performed, and a standard curve was used to verify 100% primer efficiency. The forward and reverse primer sequences were as follows: RPA1 forward, 5'-AAGTGGAGACCTACAACGAC-3' and reverse 5'-ACAACCACCTGAGCGTAT-3'; and GAPDH forward, 5'-GACCTCAACTACATGGTTTA-3' and reverse, 5'-AATGAGCCCCAGCCTTCTCC-3'. Primer synthesis was performed by Sangon Biotech Co., Ltd. The experiments were repeated three times under the same conditions.

Statistical methods. SPSS 22.0 (IBM Corporation) statistical analysis software was used. Unpaired Student's t-test was used to compare RPA1 expression between two groups. One-way ANOVA followed by Tukey's post hoc tests was used for the comparison of multiple groups. χ^2 test was used to analyze the associations of RPA1 expression with clinicopathological parameters. The relationship between RPA1 expression and overall survival (OS) in patients with glioma was evaluated using Kaplan-Meier survival analysis, and survival differences were compared using the log-rank test. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Differential expression analysis and survival analysis based on RPA1 gene expression data from TCGA. To clarify the relationships between RPA1 and glioma features, TCGA database and the UALCAN online tool were used to analyze the differences in RPA1 expression among 518 LGGs, 163 high-grade gliomas (HGGs) and 207 normal brain tissues. This revealed that the expression levels of RPA1 in both types of glioma were significantly higher than those in normal brain tissues. In

addition, Kaplan-Meier OS analysis was performed for patients with glioma and high or low RPA1 expression ($n=338/\text{group}$). This analysis revealed that high RPA1 expression was associated with a significant shortening of the duration of OS in patients with glioma (Fig. 1).

Expression of RPA1 in glioma cells. The expression of RPA1 in HA1800 normal astrocyte cell line, U251 and SF295 glioma cell lines and A172 and TG905 GBM cell lines was analyzed. According to the WHO tumor grading criteria, gliomas are classified into grades I-IV, based on their cytological characteristics and molecular biological features, where a higher grade indicates greater malignancy. The U251 and SF295 glioma cell lines are considered LGGs while the A172 and TG905 glioma cell lines are considered HGGs. RT-qPCR analysis revealed that the mRNA levels of RPA1 in the glioma cell lines were significantly greater than those in the normal astrocyte cell line, and significantly higher in the A172 and TG905 GBM cell lines compared with those in the U251 and SF295 LGG cell lines (Fig. 2A). These findings indicate that the mRNA expression levels of RPA1 in glioma cells were greater than those in normal astrocyte cells and increased with the degree of glioma malignancy. The upregulation of RPA1 in HGGs suggests that RPA1 may play a role in the progression of glioma.

Similarly, western blot analysis revealed that the protein expression levels of RPA1 in the glioma and GBM cells were significantly greater than that in normal astrocyte cells. Furthermore, the expression levels of RPA1 in the A172 and TG905 GBM cell lines were greater than that in the U251 glioma cell line, and the expression level of RPA1 in the A172 glioblastoma cell line was greater than that in the SF295 glioma cell lines. These observations support the mRNA results in

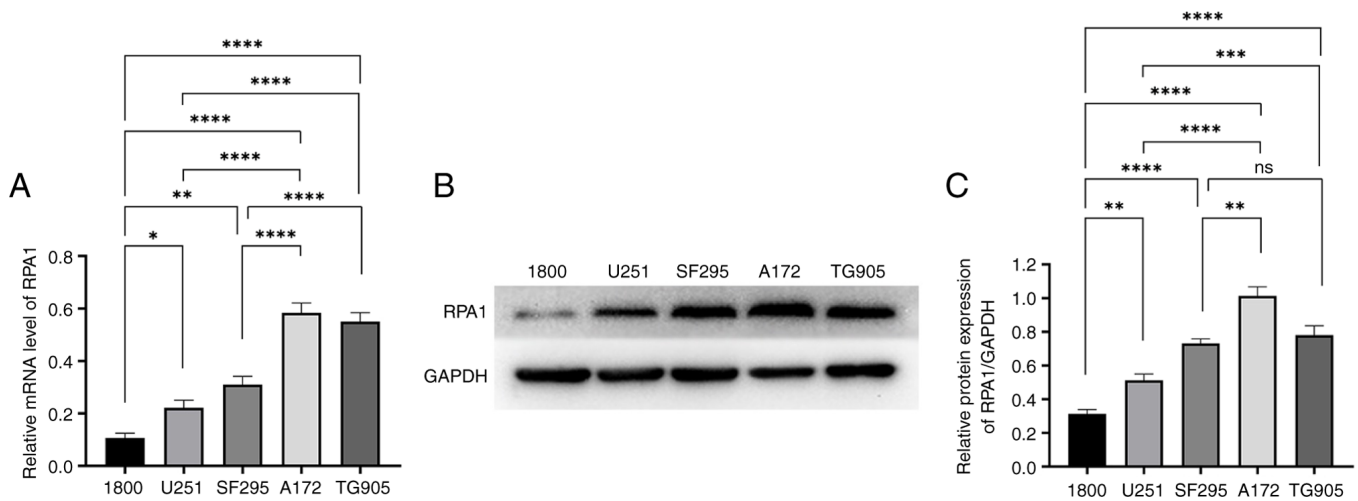


Figure 2. RPA1 expression is upregulated in glioma cell lines. (A) Relative mRNA expression of RPA1, (B) representative western blot images and (C) relative protein expression of RPA1 in a HA1800 astrocyte cell line and U251, SF295, A172, and TJ905 glioma cell lines. Quantified data are presented as the mean $\pm$ SD. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ and **** $P < 0.0001$ as indicated. ns, not significant ($P > 0.05$); RPA1, replication protein A1.

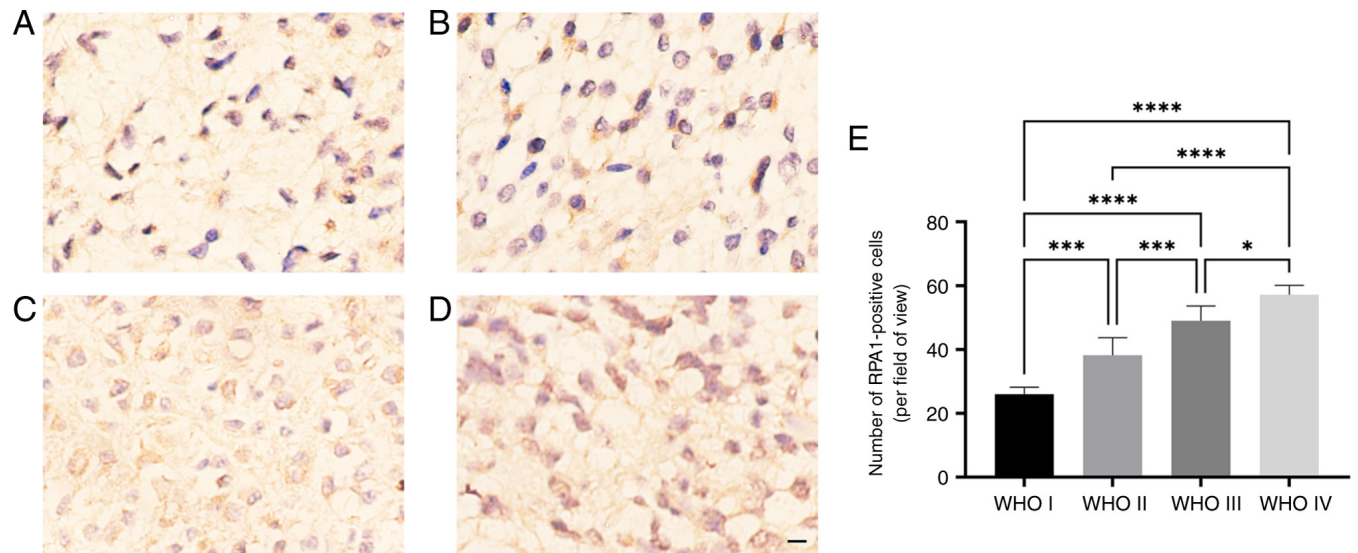


Figure 3. Representative immunohistochemical staining images and quantification of RPA1 protein expression in gliomas of different WHO grades. Representative staining images of glioma of WHO (A) grade I, (B) grade II, (C) grade III and (D) grade IV (magnification $\times 400$). (E) Quantification of RPA1 expression in the glioma samples of four different grades. Quantified data are presented as the mean $\pm$ SD. * $P < 0.05$, *** $P < 0.001$ and **** $P < 0.0001$ as indicated. RPA1, replication protein A1; WHO, World Health Organization.

suggesting that the RPA1 expression level is positively associated with the WHO grade in glioma cells (Fig. 2B and C).

Analysis of the association between RPA1 expression and clinicopathological parameters in patients with gliomas. RPA1 IHC staining was performed on 70 glioma tissue samples from patients with glioma of WHO grades I-IV. The results revealed that the expression of RPA1 increased as the degree of glioma malignancy increased. In addition, the expression of RPA1 in HGG was significantly greater than that in LGG (Fig. 3). The 70 glioma samples were divided into high and low RPA1 groups, and the associations between the expression of RPA1 and various clinicopathological parameters were analyzed (Table I, Fig. 4). The results revealed that RPA1 expression was significantly associated with tumor WHO grade, Ki-67

and p53mut ($P < 0.05$). However, no associations were found between RPA1 expression and patient sex distribution, age, tumor size, Karnofsky Performance Status (KPS; a commonly used tool for evaluating the overall health status and functional level of patients) score, or tumor location in 70 patients with glioma (Table I; Fig. 4).

Association between RPA1 expression and the prognosis of patients with gliomas. The survival of the 70 patients with gliomas was analyzed. KaplanMeier analysis revealed that low RPA1 expression was associated with a significantly prolonged OS (Fig. 5). These findings are consistent with the results of the initial database analysis, further confirming that high RPA1 expression is associated with the degree of malignancy of glioma.

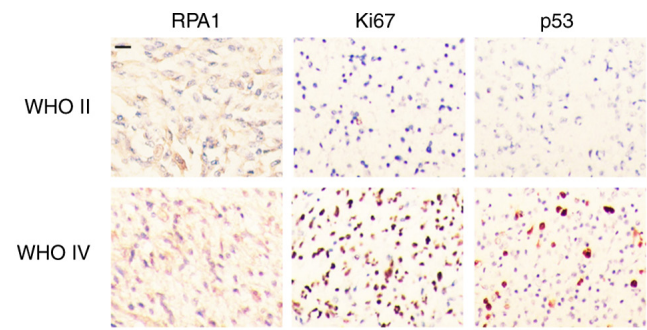


Figure 4. Immunohistochemical staining images of RPA1, Ki-67 and p53 in gliomas of different WHO grades. Representative staining images are shown (magnification, x200). RPA1, replication protein A1; WHO, World Health Organization.

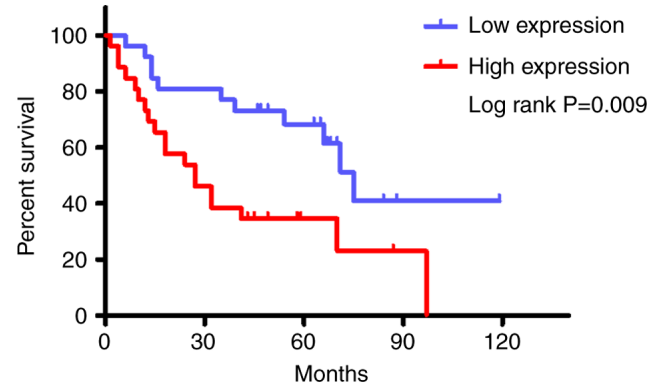


Figure 5. Kaplan-Meier survival curves for primary glioma patients with low and high expression levels of RPA1. The cohort comprised 30 patients with low RPA1 expression and 40 with high RPA1 expression. RPA1, replication protein A1.

Bioinformatics analysis of RPA1-related gene enrichment. To further explore the role of RPA1 in glioma proliferation, bioinformatics analysis was performed to predict the signaling pathways mediated by RPA1. A PPI network involving RPA1 was constructed using the STRING database (Fig. 6). Following identification of the top 10 genes associated with RPA1 (Table II), gene set enrichment analysis was performed using the DAVID online analysis tool. The results indicate that RPA1 is mainly involved in GO pathways associated with DNA replication monitoring, including ‘DNA replication initiation’, ‘regulation of mitotic cell cycle’ and ‘nucleosome assembly’ through its target genes (Table III). KEGG pathway enrichment analysis was also performed to explore pathways associated with the differentially expressed genes. The main enriched pathways were ‘DNA replication’, ‘mismatch repair’, ‘Fanconi anemia pathway’, ‘homologous recombination’, ‘nucleotide excision repair’ and ‘cell cycle’ (Table IV). The results of the bioinformatics analysis provide a theoretical basis for further study of the functional role of RPA1 in gliomas.

Discussion

Glioma is the most common type of primary intracranial malignant tumor. Current treatment strategies include maximum tumor resection combined with radiotherapy,

Table II. Gene set enrichment analysis of the top 10 genes in the RPA1 interaction network.

Node 1	Node 2	Combined score
RPA1	POLD3	0.76
RPA1	ELAVL1	0.75
HAT1	RPA1	0.75
NEDD1	RPA1	0.74
RPA1	USP1	0.73
RPA1	SF3A3	0.73
RBBP4	RPA1	0.73
TMEM237	RPA1	0.72
RPA1	MCM3	0.72
RPA1	BAZ1B	0.72

RPA1, replication protein A1; POLD3, DNA polymerase δ 3; ELAVL1, ELAV like RNA binding protein 1; HAT1, histone acetyltransferase 1; NEDD1, neuroepithelial cell differentiation protein 1; USP1, ubiquitin specific peptidase 1; SF3A3, splicing factor 3A subunit 3; RBBP4, retinoblastoma binding protein 4; TMEM237, transmembrane protein 237; MCM3, minochromosome maintenance complex component 3; BAZ1B, bromodomain adjacent to zinc finger domain 1B.

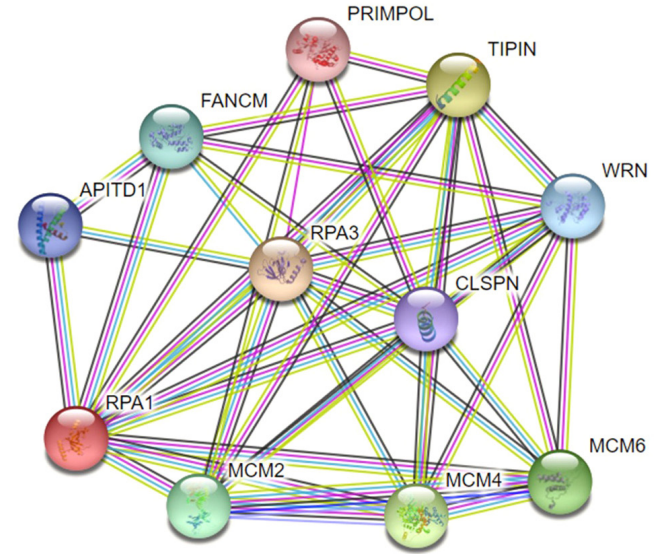


Figure 6. Protein-protein interaction network showing the RPA1 interaction network, generated using the STRING database. RPA1/3, replication protein A1/3; PRIMPOL, primase and DNA-directed polymerase; TIPIN, TIMELESS-interacting protein; FANCM, Fanconi anemia complementation group M protein; APITD1, apoptosis-inducing, TAF9-like domain-containing protein 1; WRN, Werner syndrome ATP-dependent helicase; RPA3, replication protein A1; CLSPN, claspin; MCM2/4/6, minichromosome maintenance complex component 2/4/6.

chemotherapy and immunotherapy; however, treatment outcomes remain unsatisfactory due to the invasive nature of gliomas, the protective effect of the blood-brain barrier, tumor drug resistance and recurrence (2,16). Patients with GBM have a particularly poor prognosis, with an average median survival time of 1.5 years and a 5-year survival rate of only 5.5%.

Table III. GO biological function cluster analysis of the top 10 replication protein A1-related genes.

ID	Description	Count	P-value
GO.0006268	DNA unwinding involved in DNA replication	3	3.9×10^{-7}
GO.0045005	DNA-dependent DNA replication maintenance of fidelity	5	1.1×10^{-9}
GO.0000712	Resolution of meiotic recombination intermediates	2	2.4×10^{-4}
GO.0031297	Replication fork processing	4	8.7×10^{-8}
GO.0000076	DNA replication checkpoint	2	2.9×10^{-4}
GO.0006270	DNA replication initiation	3	7.4×10^{-6}
GO.0032201	Telomere maintenance via semi-conservative replication	2	4.0×10^{-4}
GO.0007346	Regulation of mitotic cell cycle	3	1.5×10^{-2}
GO.0000731	DNA synthesis involved in DNA repair	4	3.3×10^{-7}
GO.0006334	Nucleosome assembly	2	1.0×10^{-2}

ID, identify; GO, Gene Ontology.

Table IV. Kyoto Encyclopedia of Genes and Genomes pathway enrichment analysis of genes associated with replication protein A1.

Pathway	Description	Count in network	P-value
Hsa03030	DNA replication	5	8.6×10^{-11}
Hsa03430	Mismatch repair	2	1.3×10^{-4}
Hsa03460	Fanconi anemia pathway	4	5.4×10^{-8}
Hsa03440	Homologous recombination	2	2.9×10^{-4}
Hsa03420	Nucleotide excision repair	2	3.2×10^{-4}
Hsa04110	Cell cycle	3	8.3×10^{-5}

Thus, gliomas are a major cause of cancer-related deaths in adults worldwide. Future directions for tumor therapy include molecular targeted therapy aimed at key tumor-related genes that affect tumor cell proliferation, invasion, angiogenesis and apoptosis through a variety of regulatory pathways.

RPA1 is the main subunit of the RPA complex in terms of biological function. It plays a central role in the maintenance of genome stability and is involved in multiple types of DNA metabolism (5-7). RPA interacts with numerous proteins, primarily through the N-terminal interaction of RPA1, which facilitates protein exchange and is important for DNA damage signaling (17,18). In addition, RPA1 binds to a number of DNA repair proteins and checkpoint regulators during DNA replication. Following DNA unwinding, RPA assists in the recruitment and function of DNA polymerase during the initiation of DNA replication. RPA1 also interacts with DNA2 helicases and endonucleases, which help to maintain cell viability (19,20). These findings underscore the important role of RPA1 in the maintenance of accurate DNA replication.

Previous studies have suggested that RPA1 functions as an oncogene and is significantly negatively associated with OS in patients with various types of cancer. For example, Zhang *et al* (10) demonstrated that RPA1 overexpression is associated with progression, metastasis and poor prognosis in nasopharyngeal carcinoma. Knockdown of RPA1 was found to impair DNA damage repair, inhibit cell proliferation and

induce cell cycle arrest in the G₂/M phase. In addition, in a xenograft model using nude mice, RPA1 knockdown increased the radiosensitivity of nasopharyngeal carcinoma cells and prolonged the DNA damage repair time (10). Similarly, Ni *et al* (21) have demonstrated that the suppression of RPA1 expression significantly disrupts DNA replication, thereby suppressing the proliferation of non-small cell lung cancer cells.

Few studies have investigated the effect of RPA1 on glioma. In the present study, differential expression analysis using TCGA data revealed that the expression level of RPA1 in glioma tissues was significantly higher than that in normal brain tissue. In addition, high RPA1 expression was significantly associated with a shorter OS time in patients with glioma. These data suggest that RPA1 is a tumor-promoting factor that contributes to the occurrence and progression of glioma. RT-qPCR and western blot analyses confirmed that RPA1 expression in glioma cell lines was significantly increased at both the mRNA and protein levels compared with that in a normal astrocyte cell line, and that the expression level increased as the degree of glioma malignancy increased. IHC staining of glioma tissue samples from 70 patients revealed that the number of RPA1-positive cells in the tissues increased as the pathological grade of the glioma increased. Statistical analysis also revealed a significant association of RPA1 expression level with the WHO tumor grade, Ki-67 proliferation

index and p53 mutation status. Furthermore, Kaplan-Meier survival analysis with a long-term follow-up was performed to monitor the survival of 70 patients with glioma. The findings were consistent with those of the bioinformatics analysis, and support the suggestion that RPA1 may serve as an important prognostic marker for predicting the prognosis and survival of patients with gliomas.

RPA1 is known to directly or indirectly regulate multiple downstream target genes in various types of tumors, contributing to tumor proliferation, apoptosis and drug resistance through signaling pathways associated with DNA metabolism (22,23). Previous studies have shown that RPA1 acts as an oncogene in gastrointestinal tumors, promoting tumorigenesis and proliferation. For example, a study on colorectal cancer demonstrated that RPA1 knockout significantly inhibited the formation of tumor cell colonies and arrested the cell cycle in the G₁ phase. In addition, silencing RPA1 expression increased the sensitivity of malignant colorectal tumor cells to 5-fluorouracil compared with that of control cells (13). Mechanistically, RPA1 silencing decreases the phosphorylation level of extracellular signal-regulated kinase, upregulates pro-apoptotic caspase 3 expression, and induces apoptosis in colorectal tumor cells (24,25). In cancer cells, hypoxia and DNA-dependent protein kinase induce the phosphorylation of p53, thereby disrupting the p53-RPA1 complex, which enhances RPA1-mediated NER/nonhomologous end joining and suppresses apoptosis (26). In addition, the study also showed that DNA damage in tumor cells is associated with a significant upregulation of RPA1 expression. RPA1 blocks p53 activity, allowing damaged DNA to evade cell cycle arrest (26). In colorectal cancer, the overexpression of RPA1 has been shown to decrease the activity of p53 and promote tumor progression (13,27), suggesting that targeting RPA1 may be a novel strategy for cancer treatment.

To further explore the function and molecular mechanisms of RPA1 in glioma, GO and KEGG gene enrichment analyses of RPA1-associated genes were performed in the present study using bioinformatics methods. The GO analysis indicated that RPA1 primarily affects processes associated with DNA replication surveillance, including 'DNA replication initiation', 'regulation of mitotic cell cycle' and 'nucleosome assembly' through its target genes. In addition, KEGG pathway analysis revealed enrichment of the 'DNA replication', 'Fanconi anemia pathway', 'mismatch repair', 'homologous recombination', 'nucleotide excision repair' and 'cell cycle' pathways, suggesting that RPA1 may mediate the proliferation, invasion and apoptosis of glioma cells through these pathways.

In conclusion, the present study demonstrated that the expression of RPA1 was significantly increased in glioma, especially GBM, and was strongly associated with malignancy. Therefore, RPA1 may serve as a promising target for reducing malignancy to increase the benefit of conventional multimodal therapies for human gliomas. These data can be further utilized through *in vitro* techniques and *in vivo* models to elucidate the role of RPA1 in differentiation, proliferation and other malignancy-related biological behaviors.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

ZZ, YS and JZ participated in all aspects of the experimental design and research procedure and wrote the manuscript. PY was involved in conducting and analyzing all experimental measurements. QZ participated in sample preparation and the molecular biology experiments. ZZ and JZ confirm the authenticity of all the raw data. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

All written informed consent was obtained from all patients. The experimental protocol was established in accordance with the ethical guidelines of the Declaration of Helsinki and approved by the Human Ethics Committee of Linyi People's Hospital (approval no. 30036).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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