OncoTargets and Therapy

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ORIGINAL RESEARCH

# RETRACTED ARTICLE: miR-152-3p Sensitizes Glioblastoma Cells Towards Cisplatin Via Regulation Of SOS1

This article was published in the following Dove Press journal:
*OncoTargets and Therapy*

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**Background:** Accumulating evidences suggest that microRNAs (miRNAs) play key roles in mediating glioblastoma progression. Decreased expression of miR-152-3p was reported in several cancer types including glioblastoma.

**Methods:** The sensitivity of glioblastoma cells to cisplatin was assessed by the cell counting kit-8 assay and flow cytometry analysis. The expression of miR-152-3p was determined by RT-qPCR method. Bioinformatic analysis, dual luciferase reporter assay and Western blot were used to explore the target gene of miR-152-3p. The association between miR-152-3p and SOS1 was confirmed in glioblastoma tissues by Pearson correlation analysis.

**Results:** In the current study, we discovered that overexpression of miR-152-3p increased cisplatin sensitivity while inhibition of miR-152-3p decreased cisplatin sensitivity in glioblastoma cells (T98G and U87). In addition, miR-152-3p augmented cell apoptosis induced by cisplatin treatment. It was further predicted and validated that SOS1, a protein involved in regulating chemosensitivity, was a direct target gene of miR-152-3p. SOS1 was proven to suppress the cytotoxic effect of cisplatin in glioblastoma. Transfection of recombinant SOS1 could effectively reverse the increased cisplatin sensitivity induced by miR-152-3p overexpression in T98G. Furthermore, overexpression of SOS1 reduced the percentage of apoptotic cells increased by miR-152-3p mimic in the presence of cisplatin in T98G. More importantly, a significant negative correlation between miR-152-3p levels and SOS1 levels was observed in glioblastoma tissues collected from 40 patients.

**Conclusion:** Our study identified miR-152-3p as a chemotherapy sensitizer in glioblastoma.

**Keywords:** glioblastoma, miR-152-3p, SOS1, cisplatin

## Introduction

Glioblastoma is recognized as main primary tumor of central nervous system. Even with active treatment including surgery, radiotherapy, and chemotherapy, the survival time after diagnosis is approximately 1–2 years.[1] Brain tumors are a kind of highly invasive and fatal tumor disease,[2] the incidence is 6–7 new cases per 100,000 person-years.[3] Glioblastoma is poorly differentiated astrocytes, which are characterized by high mitotic activity, nuclear atypia, necrosis, cellular polymorphism, vascular proliferation, and thrombosis.[4]

Cisplatin is one of the most widely used cytotoxic drugs (particularly for bladder, ovarian and testicular carcinomas) with the best curative effect for the treatment of a variety of tumors.[5,6] Previous researches have showed that cisplatin is one of the first-line chemotherapeutic drugs adpoted for glioblastoma.[7,8] Cisplatin is a DNA damage agent, and its cytotoxic effect is based on the formation of platinum-DNA complex and cross-linking, which leads to cell cycle arrest and

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http://doi.org/10.2147/OTT.S210732

enables cells to repair damage, failed DNA reparation results in cell apoptosis through activation of signaling pathways.[9] Despite a certain initial response rate, cisplatin treatment often fails due to the development of resistance to chemotherapy.[10] The development of cisplatin resistance greatly limits its effectiveness in glioblastoma cancer treatment.[8] Therefore, it is of great importance to better understand the mechanism of cisplatin resistance and find an effective combination therapy to combat cisplatin resistance. Multiple studies have showed that miRNAs are involved in regulation of drug resistance in glioblastoma, which are potential biomarkers and therapeutic targets for patients with glioblastoma.[11–13]

MicroRNAs (miRNAs) are endogenously expressed short non-coding RNAs of 20–23 nucleotides,[14] which bind to target gene mRNAs' complementary sequences in the 3′-untranslated regions (UTRs), and involve in regulation of diverse biological processes, including proliferation, differentiation, and apoptosis.[15] MiRNAs' expression and activity are strictly regulated in time and space, and its aberrant expression is widely associated with the development of human diseases, including cancer.[16,17] MiRNAs have been reported to play key roles during tumorigenesis and function as oncogenes or tumor suppressors.[18] miR-152 has been proven to be abnormally expressed in several diseases, including cancer, and there is increasing evidences suggesting that miR-152 is a tumor suppressor associated with the proliferation, migration, and invasion of human cancer cells.[19,20] Recently, Sun et al has collected glioblastoma tissues and adjacent tissues from patients who underwent curative resection, and reported that the expression of miR-152-3p was decreased by more than half in glioblastoma tissues and glioblastoma cells compared with non-tumor samples and normal cells, and overexpression of miR-152-3p induced cell apoptosis and inhibited cell invasion.[14] In this study, we explored the function of miR-152-3p in cisplatin sensitivity of glioblastoma.

Son of sevenless 1 (SOS1) is a dual diguanine nucleotide exchange factor (GEF) for Ras and Rac1, which converts inactive Ras-GDP into active Ras-GTP in many EGF (Epidermal Growth Factor)-stimulated cells.[21] SOS1 is known to participate in EGF-dependent signaling pathways and promote cell survival and growth.[22] Moreover, dysregulation of SOS1 has been found in the progression of numerous cancers including hematological malignancies, breast cancer, skin cancer, and glioblastoma.[23,24] SOS1 has two Ras binding sites, one of which is an allosteric site distal to the active site, and activation of SOS1 by receptor tyrosine kinase (RTK) would mediate Ras activation.[25] It is widely accepted that Ras plays a critical role in cell growth related signaling pathways.[26] Lv Z and Yang L examined the mRNA and protein expression levels of SOS1 in glioblastoma cell lines and found that the mRNA and protein expression levels of SOS1 were higher than those of the HA cell line.[24] In SOS1 knockdown U87 glioblastoma cells, Ras, p-Raf, and p-ERK were reported to be significantly downregulated, and Lv et al reported that miR-124 was able to suppress the growth of U87 cells by targeting SOS1 through MAPK pathway.[24]

Several studies have reported that miRNA could regulate cell proliferation and apoptosis by targeting SOS1 in cancer cells.[24,27] In this research, we aimed to investigate the regulatory association between miR-152-3p and SOS1 as well as their roles in regulation of glioblastoma cisplatin sensitivity. We found that miR-152-3p could increase glioblastoma cisplatin sensitivity via SOS1.

## Materials And Methods

### Clinical Patient Tissue Samples

A total of 40 tumor tissue samples from patients with glioblastoma and the corresponding 40 non-tumor tissue samples from dead healthy volunteers were collected at Changzhou No.2 People's Hospital between 2016 and 2018. There were 22 males and 18 females with age ranging from 45 to 74 years, and the average age was 60.3 years. This clinical trial followed the Helsinki Declaration. Written informed consent was acquired from all patients and this study was approved by the Ethics Committee of Changzhou No.2 People's Hospital. All tissue samples were immediately frozen in liquid nitrogen after surgery and stored in a −80°C refrigerator prior to use.

### Cell Culture And Reagent

T98G and U87 human glioblastoma cell lines were purchased from American Type Culture Collection (ATCC, Rockefeller, MD) and cells were cultured in Dulbecco's modified essential medium (DMEM) (Life Technologies, Carlsbad, CA) supplemented with 10% fetal bovine serum (HyClone, Logan, UT) and 1% penicillin-streptomycin solution (Life Technologies). Cells were cultured in a 5% $CO_2$ humidified incubator at 37°C. Cisplatin and

temozolomide (TMZ) were bought from Sigma-Aldrich (St. Louis, MO).

## Overexpression And Inhibition Of miR-152-3p

MiR-NC inhibitor, miR-152-3p inhibitor, miR-NC mimic, and miR-152-3p mimic were synthesized and purchased from GenePharma (Suzhou, China). Their sequences were as follow: miR-NC inhibitor: 5′-UCGCUUGGUGCAGGUCGGGAA-3′; miR-152-3p inhibitor: 5′-CCAAGUUCUGUCAUGCACUGA-3′; miR-NC mimic: 5′-GGAACUUAGCCACUGUGAAUU-3′; miR-152-3p mimic: 5′-UCAGUGCAUGACAGAACUUGG-3′. Approximately $2\times10^6$ T98G or U87 cells were seeded in each well of 6-well plates. On the next day, 50 nM miR-NC inhibitor or miR-152-3p inhibitor or miR-NC mimic or miR-152-3p mimic was mixed with 10 μL Lipofectamine RNAiMax (Life Technologies) in 250 μL serum-free DMEM for 15 mins. The mixtures were then added into indicated well in 6-well plates. After 48 hrs, the cells were subjected to the following experiments.

## RNA Extraction And Reverse Transcription-Quantitative Polymerase Chain Reaction (RT-qPCR) For miRNA And mRNA Assay

Total RNA was extracted from tissues a[illegible] culture[illegible] T98G and U87 cells using TRIzol reagent (I[illegible]nitroge[illegible] arlsbad, CA) and cDNA synthesis was perf[illegible]ed using P[illegible]eScript RT reagent kit (TaKaRa, Otsu, [illegible]higa, [illegible]pan) according to the Manufacturer's instructi[illegible]n. Real-time [illegible]T-qPCR reactions were performed wit[illegible]SYBR Premix Ex Taq (TaKaRa) in a Bio-Rad CFX96 R[illegible]-Time [illegible]CR System (Bio-rad, Hercules, CA) in triplica[illegible] with T[illegible]Green™ [illegible]ast qPCR Mix (TaKaRa). The reacti[illegible]n cond[illegible]ons we[illegible]s[illegible]own as follows: pre-denature at 95°[illegible] for 30 [illegible]econds, denature at 95°C for 5 seconds followed b[illegible]nealing and elongation at 60°C for 10 seconds (repeated for [illegible]cycles). The relative levels of miR-152-3p and SOS1 were norm[illegible]alized by U6 small nucleolar RNA and GAPDH, respectively. $2^{-\Delta\Delta Ct}$ method was used to calculate gene expression level.[28] The primer sequences were listed as follow: stem loop primer: 5′-CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGCCAAGT-3′; miR-152-3p forward primer: 5′-TCGGCAGGTCAGTGCATGACAGAA-3′; miR-152-3p reverse primer: 5′-CTCAACTGGTGTCGTGGA-3′; U6 forward primer: 5′-GAGGGCCTATTTCCCATGATT-3′; U6 reverse primer: 5′-TAATTAGAATTAATTTGACT-3′; SOS1 forward primer: 5′-GAGTGAATCTGCATGTCGGTT-3′; SOS1 reverse primer: 5′-CTCTCATGTTTGGCTCCTACAC-3′; GAPDH forward primer: 5′-GGAGCGAGATCCCTCCAAAAT-3′; GAPDH reverse primer: 5′-GGCTGTTGTCATACTTCTCATGG-3′.

## Cell Viability And Apoptosis Assay

Cell viability assay was performed using Cell Counting Kit-8 kit (Dojindo Laboratories, Kumamoto, Japan) according to the manufacture's instruction. Probably $2\times10^6$ cells were seeded into each [illegible]ell of 6-well plates. After transfected with 50 nM [illegible]R-152-[illegible] mimic or miR-NC mimic, miR-152-3p inhi[illegible]r or miR-N[illegible] inhibitor, and 2μg pcDNA3-SOS1 or [illegible]cDNA3[illegible]lasmid f[illegible] 48 hrs, cells were harvested and [illegible]eeded i[illegible] 96-w[illegible]l [illegible]ate, then treated with different conc[illegible]trati[illegible]s of cisplatin (2.5, 5, 10, 20, 40 μM) for 48 [illegible]s. 10 μ[illegible]CCK-8 [illegible]olution was added into each well[illegible]tained for 2[illegible]r[illegible] and absorbance at 450 nm was measured [illegible] reflect cell viability. Annexin-V/Dead Cell [illegible]optosis Kit (Invitrogen) was used to conduct cell apoptosis assay according to the protocol recommended by the manufact[illegible]er's instruction. Briefly, the cells were harv[illegible]ted and [illegible]ashed in cold phosphate-buffered saline (PBS)[illegible]en, the cells were diluted to about $1\times10^6$ cells/[illegible] in 1XAnnexin-binding buffer to 100 μL per assay. 5 μL Alexa Fluor 488 annexin V and 1 μL 100 μg/mL PI working solution were added to each well of cell suspension. The cells were incubated at room temperature for 15 mins, and then 400 μL annexin-binding buffer was added. Following, we analyzed the stained cells by BD FACSCalibur flow cytometer (BD Biosciences, Franklin Lakes, NJ).

## Western Blot Analysis

SOS1 antibody (Cat No. PA5-78174, 1:1000) was obtained from Thermo Fisher Scientific (Waltham, MA), GAPDH mouse monoclonal antibody (Cat No. ab8245, 1:10000) was purchased from Abcam (Cambridge, United Kingdom). Anti-mouse (Cat No. CW0221S, 1:10000) and anti-rabbit (Cat No. CW0234S, 1:10000) secondary antibodies were bought from CWBiotech (Beijing, China). Western blotting was carried out as the following procedure. After collecting cells and washing twice with cold PBS, collected cancer cells were lysed in cold RIPA buffer (Beyotime, Shanghai, China) with protease inhibitor cocktail (Sigma-Aldrich, St. Louis, MO), and then incubated on ice for 30 mins. After that, lysates were centrifuged at 12,000×g with 4°C for 15 mins. Protein concentration

was measured by Bicinchoninic Acid Protein Assay kit (BCA, Thermo Fisher Scientific). Equal amounts (20 μg/well) of proteins were separated by 8% SDS-PAGE, transferred to polyvinylidene fluoride (PVDF, millipore, Billerica, MA) membranes, and immunoblotted with the respective antibodies as indicated above, blots were developed with SuperSignal West Femto Maximum Sensitivity Substrate (Thermo Fisher Scientific) and the images were obtained by ImageQuant LAS 4000 (GE Healthcare, Little Chalfont, United Kingdom).

## Dual-Luciferase Reporter Gene Assay

We used TargetScan (http://www.targetscan.org/) miRNA target prediction database to predict potential target genes of miR-152-3p. We amplified SOS1 3′-UTR region from cDNA of T98G cells and inserted into the pGL3 vector (Promega Corporation, Madison, WI). Two site mutations were introduced to pGL3-SOS1 3′UTR-WT to construct mutant SOS1-3′UTR (SOS1 3′UTR-Mut) with QuikChange Site-Directed Mutagenesis Kit (Agilent, Santa Clara, CA). The T98G cells were co-transfected with the 2μg SOS1 3′UTR-WT or SOS1 3′UTR-Mut plasmid and 50 nM miR-152-3p mimic (or negative control) using Lipofectamine™ 2000 reagent (Invitrogen) for 48 hrs. Luciferase activity was evaluated by using the Dual-Glo Luciferase assay system (Promega Corporation).

## Statistical Analysis

In this research, we performed all experiments thrice. GraphPad Prism 5.0 software (San Diego, CA) was used to analyze research data and data were presented as mean ±SD. Student's *t*-test was used to analyze differences between two groups. One-way ANOVA was used to compare differences among three or more groups, followed by Newman–Keuls analysis. $P<0.05$ was considered to indicate a statistically significant difference.

# Results

## Overexpression Of miR-152-3p Enhanced Cisplatin Sensitivity In Glioblastoma

miR-152-3p has been reported as a tumor suppressor, which is downregulated in many cancer tissues.[19,20,29] To explore the role of miR-152-3p in regulation of cisplatin sensitivity of glioblastoma and its underlying mechanisms, we firstly overexpressed miR-152-3p in T98G and U87 glioblastoma cancer cells, respectively, and results showed that miR-152-3p mimic successfully overexpressed miR-152-3p in the two cancer cell lines (Figure 1A). Overexpression of miR-152-3p in T98G and U87 cells did not affect glioblastoma cell viability (Figure 1B). However, overexpressed miR-152-3p significantly enhanced the cell viability inhibition in the presence of increasing concentration of cisplatin (0, 2.5, 5, 10, 20, 40 μM) in T98G and U87 glioblastoma cancer cells (Figure 1C and D). These findings suggested that overexpression of miR-152-3p would enhance cytotoxicity of cisplatin in glioblastoma.

## Inhibition Of miR-152-3p Decreased Cisplatin Sensitivity In Glioblastoma

Transfection of miR-152-3p inhibitor significantly decreased miR-152-3p levels in two cell lines (Figure 2A). Similarly, decreased expression of miR-152-3p did not influence cell viability of T98G and U87 cells (Figure 2B). In contrast to overexpression of miR-152-3p, decreased miR-152-3p observably attenuated the cytotoxicity of cisplatin (0, 2.5, 5, 10, 20, 40 μM) on cell viability in T98G and U87 cells (Figure 2C and D). These data collectively indicated that miR-152-3p could regulate cisplatin sensitivity in glioblastoma.

## miR-152-3p Enhanced Cisplatin-Induced Cell Apoptosis In Glioblastoma

The cytotoxic effect of cisplatin was mainly through inducing cell apoptosis in cancer cells.[10] Flow cytometry analysis was used to detect cell apoptosis upon miR-152-3p overexpression with or without cisplatin treatment in T98G cells. Results showed that both miR-152-3p overexpression and low concentration of cisplatin (5 μM) could induce cell apoptosis in T98G, and overexpression of miR-152-3p could dramatically enhance cisplatin-induced cell apoptosis (Figure 3A and B). Furthermore, similar results were observed in U87 cells (Figure 3C and D). In agreement with indicated results, knockdown of miR-152-3p lessened cell apoptosis rate increased by cisplatin (Figure S1A). In conclusion, miR-152-3p could intensify cell apoptosis induced by cisplatin in glioblastoma.

## miR-152-3p Heightened TMZ Sensitivity Of Glioblastoma Cells

Based on above findings, we surmised that miR-152-3p had the same effect on TMZ resistance of glioblastoma cells. CCK-8 assay demonstrated that enhanced expression of miR-152-3p overtly diminished cell viability suppressed by different concentrations of TMZ (0, 25, 50, 100, 200, 400 μM)

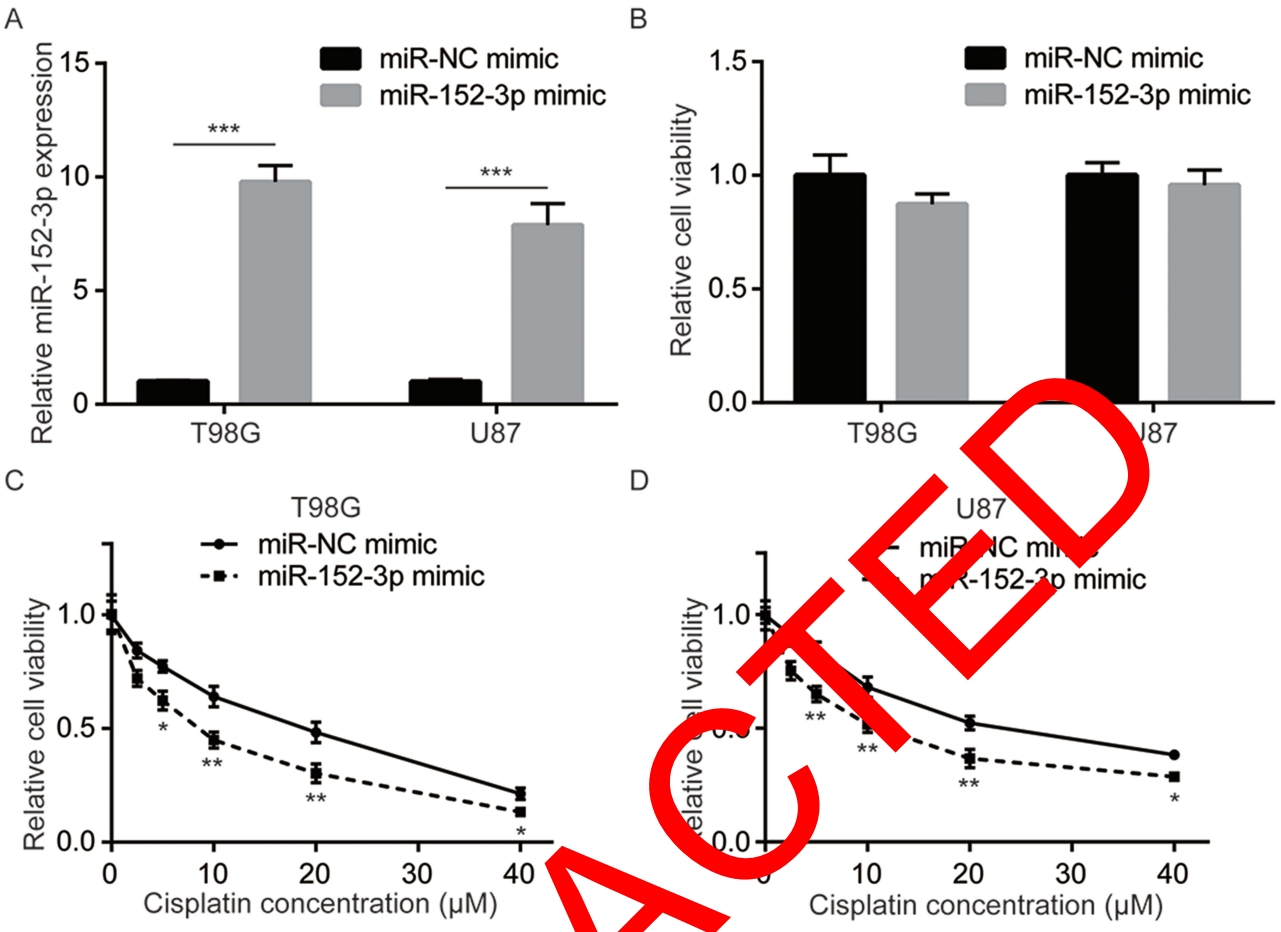




**Figure 1** Overexpressed miR-152-3p strengthened cisplatin-induced cell viability reduction in glioblastoma cells. (**A**) MiR-152-3p mimic significantly increased miR-152-3p expression in T98G and U87 glioblastoma cells. (**B**) MiR-152-3p mimic did not influence cell viability of T98G and U87 glioblastoma cells. (**C**) The cell viability assay results indicated that miR-152-3p mimic dramatically enhanced cisplatin-induced T98G cell reduction with the increasing concentration of cisplatin (0, 2.5, 5, 10, 20, 40 μM). (**D**) The cell viability assay results indicated that miR-152-3p mimic notably strengthened cisplatin-induced U87 cell reduction with the increasing concentration of cisplatin (0, 2.5, 5, 10, 20, 40 μM). *p<0.05, **p<0.01, ***p<0.0001.

in T98G and U87 cells (Figure 4A). It was indicated by flow cytometry analysis that overexpression of miR-152-3p fortified cell apoptosis caused by 200 μM TMZ in glioblastoma (Figure 4B). Inversely, silencing of miR-152-3p promoted cell viability inhibited by TMZ and reversed cell apoptosis enhanced by TMZ (Figure 4C and D). Taken together, miR-152-3p inhibited TMZ resistance in glioblastoma.

## miR-152-3p Negatively Regulated SOS1 By Binding To Its 3′-UTR

The above results indicated that miR-152-3p could enhance the anticancer effect of cisplatin, but its mechanism was still to be clarified. According to TargetScan database, SOS1 was predicted to be a potential target gene of miR-152-3p, there was a putative binding site of miR-152-3p at the SOS1 3′-UTR (Figure 5A). Overexpression of miR-152-3p dramatically restrained SOS1 mRNA and protein expression levels in T98G and U87 glioblastoma cells (Figure 5B–D). In contrast, decreased expression of miR-152-3p observably augmented SOS1 mRNA and protein expression in T98G and U87 glioblastoma cells (Figure 5E–G). We constructed WT or Mut of SOS1 3′-UTR luciferase reporter gene plasmids to verify the association between miR-152-3p and SOS1. The dual-luciferase reporter gene assay result showed that miR-152-3p mimic dramatically decreased the luciferase activity only in the SOS1 3′-UTR-WT co-transfection system in T98G (Figure 5H). The data suggested that miR-152-3p directly repressed SOS1 expression in glioblastoma cells. Moreover, cell viability had no significant alterations when SOS1 was knocked down (Figure S1B). However, we observed that the deleption of SOS1 strengthened the inhibitory effect of cisplatin on cell viability in glioblastoma (Figure S1C). Consistently, SOS1 inhibition facilitated cell apoptosis induced by cisplatin of glioblastoma cells (Figure S1D).

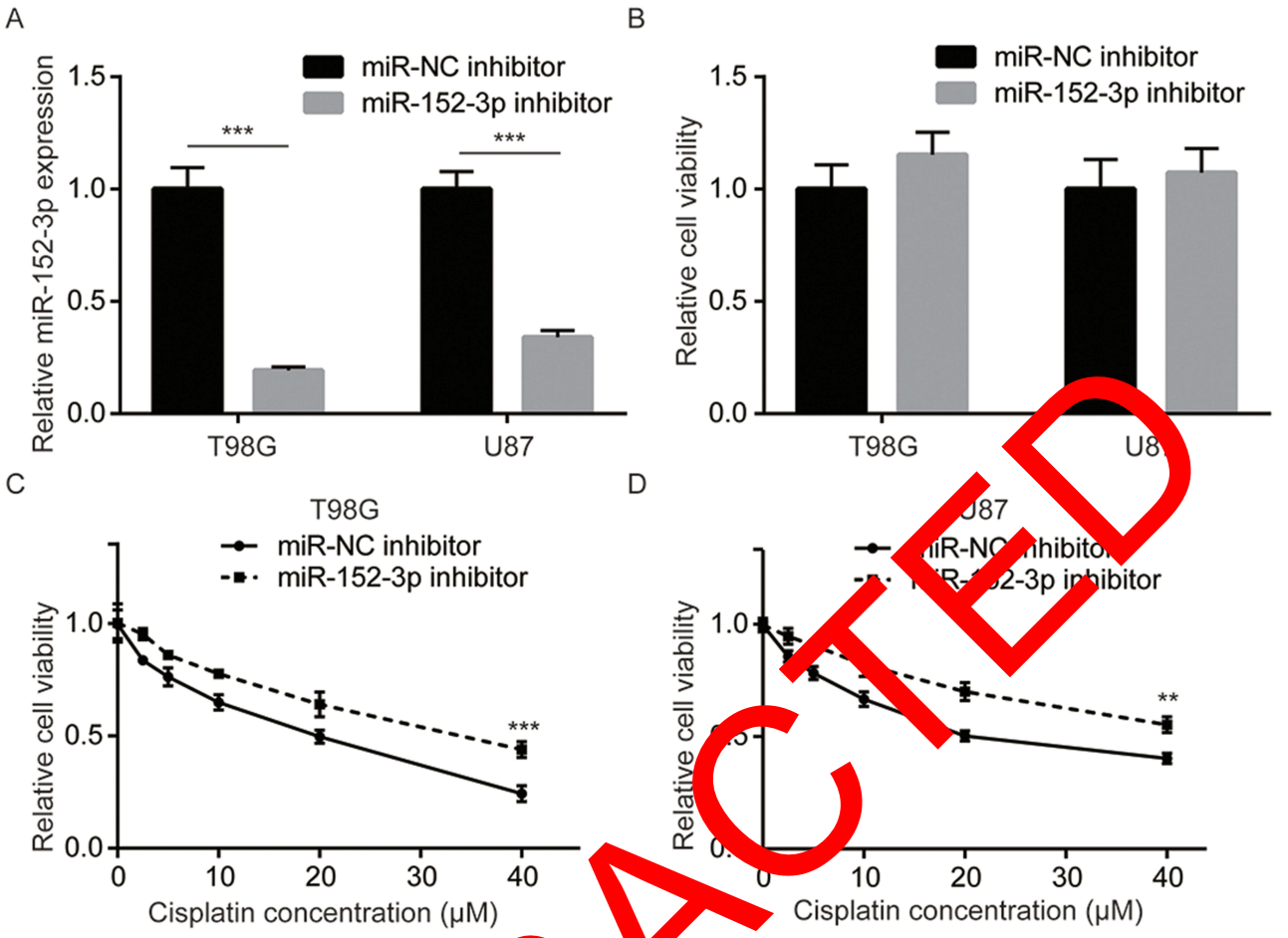


**Figure 2** Suppression of miR-152-3p weakened cisplatin-induced cell viability reduction in glioblastoma cells. (**A**) MiR-152-3p inhibitor prominently reduced miR-152-3p expression in T98G and U87 glioblastoma cells. (**B**) MiR-152-3p inhibitor did not affect cell viability of T98G and U87 glioblastoma cells. (**C**) T98G cells were treated with the increasing concentration of cisplatin (0, 2.5, 5, 10, 20, 40 μM) in advance added with miR-NC-inhibitor or miR-152-3p inhibitor. T98G cells treated with miR-152-3p inhibitor markedly decreased cisplatin-induced cell viability suppression. (**D**) U87 cells were pre-treated with the increasing concentration of cisplatin (0, 2.5, 5, 10, 20, 40 μM), and then treated with miR-NC-inhibitor or miR-152-3p inhibitor. U87 cells treated with miR-152-3p inhibitor significantly reduced cisplatin-induced cell viability suppression. **p<0.01, ***p<0.001.



## miR-152-3p Strengthened Cytotoxicity Of Cisplatin Via Repression Of SOS1

To further investigate whether miR-152-3p regulated cisplatin sensitivity via regulation of SOS1 expression, we constructed pcDNA-SOS1 recombinant plasmids and co-transfected with miR-152-3p mimic into T98G cells. Results proved that protein expression level of SOS1 dramatically reduced when overexpressed miR-152-3p; however, when co-transfected miR-152-3p with pcDNA3-SOS1 recombinant plasmid, the protein expression of SOS1 recovered (Figure 6A and B). In cell viability and apoptosis assays, data showed that overexpression of miR-152-3p would augment cisplatin-induced cell viability reduction and cell apoptosis which was reversed after transfection of recombinant SOS1 (Figure 6C–E). Considering that miR-152-3p has been justified to exert its role through targeting DMNT1 in glioblastoma, we further probed the function of miR-152-3p/DMNT1 pathway in glioblastoma. Our results revealed that overexpression of DMNT1 abolished the impact of miR-152-3p upregulation on glioblastoma cell viability and apoptosis (Figure S1E-F). In conclusion, miR-152-3p regulated cisplatin sensitivity through repression of SOS1/DMNT1 expression in glioblastoma.

## miR-152-3p Levels Negatively Correlated With SOS1 mRNA Expression In Glioblastoma Tumor Tissues

To explore whether regulatory association between miR-152-3p and SOS1 also exists in cancer patient samples, 40 glioblastoma tissues and paired normal samples were collected and analyzed via RT-qPCR. It was disclosed

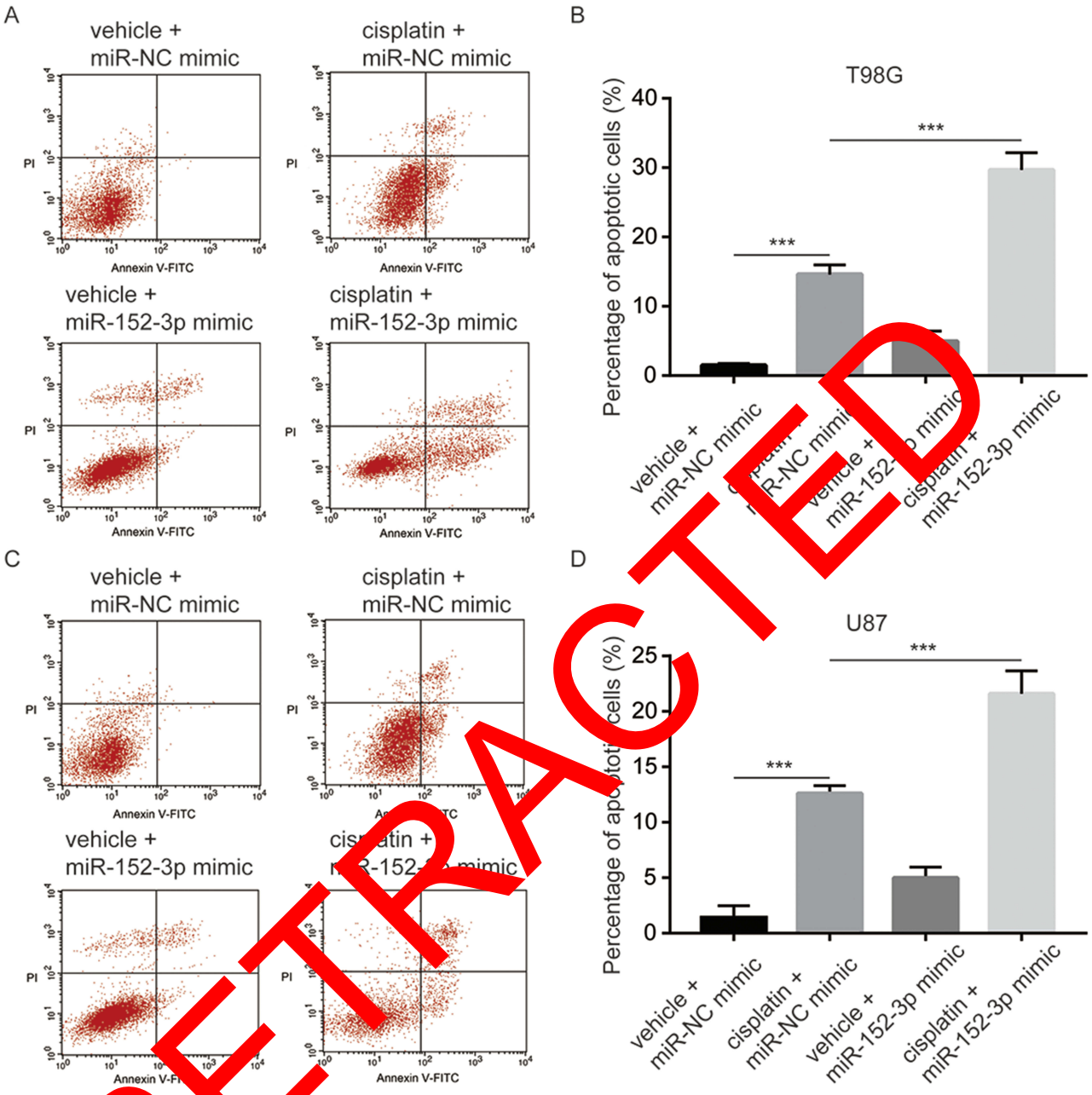




**Figure 3** MiR-152-3p could augment cisplatin-induced cell apoptosis in glioblastoma. (**A**) Compared to cisplatin group, T98G cells treated with cisplatin in combination with miR-152-3p mimic could significantly enhance cell apoptosis percentage. (**B**) Analyzed data from figure A, cisplatin could significantly increase T98G cell apoptosis percentage, and the effect notably enhanced by miR-152-3p overexpression. (**C**) Compared to cisplatin group, U87 cells treated with cisplatin in combination with miR-152-3p mimic could significantly enhance cell apoptosis percentage. (**D**) Analyzed data from figure C, cisplatin could observably increase U87 cell apoptosis percentage, and the anti-tumor effect prominently increased by miR-152-3p overexpression. ***p<0.001.

that miR-152-3p was weakly expressed in tumor specimens compared with normal tissues (Figure 7A). The expression of SOS1 in glioblastoma samples was higher than that in non-tumor tissues (Figure 7B). Results revealed that SOS1 mRNA expression was negatively correlated with miR-152-3p levels (Figure 7C).

## Discussion

Glioblastoma is a grade IV astrocytoma defined by the world health organization (WHO) which is defined as the most aggressive glioma, and is characterized by poorly differentiated neoplastic astrocytes.[30] Although significant achievements have been made in the past, clinical

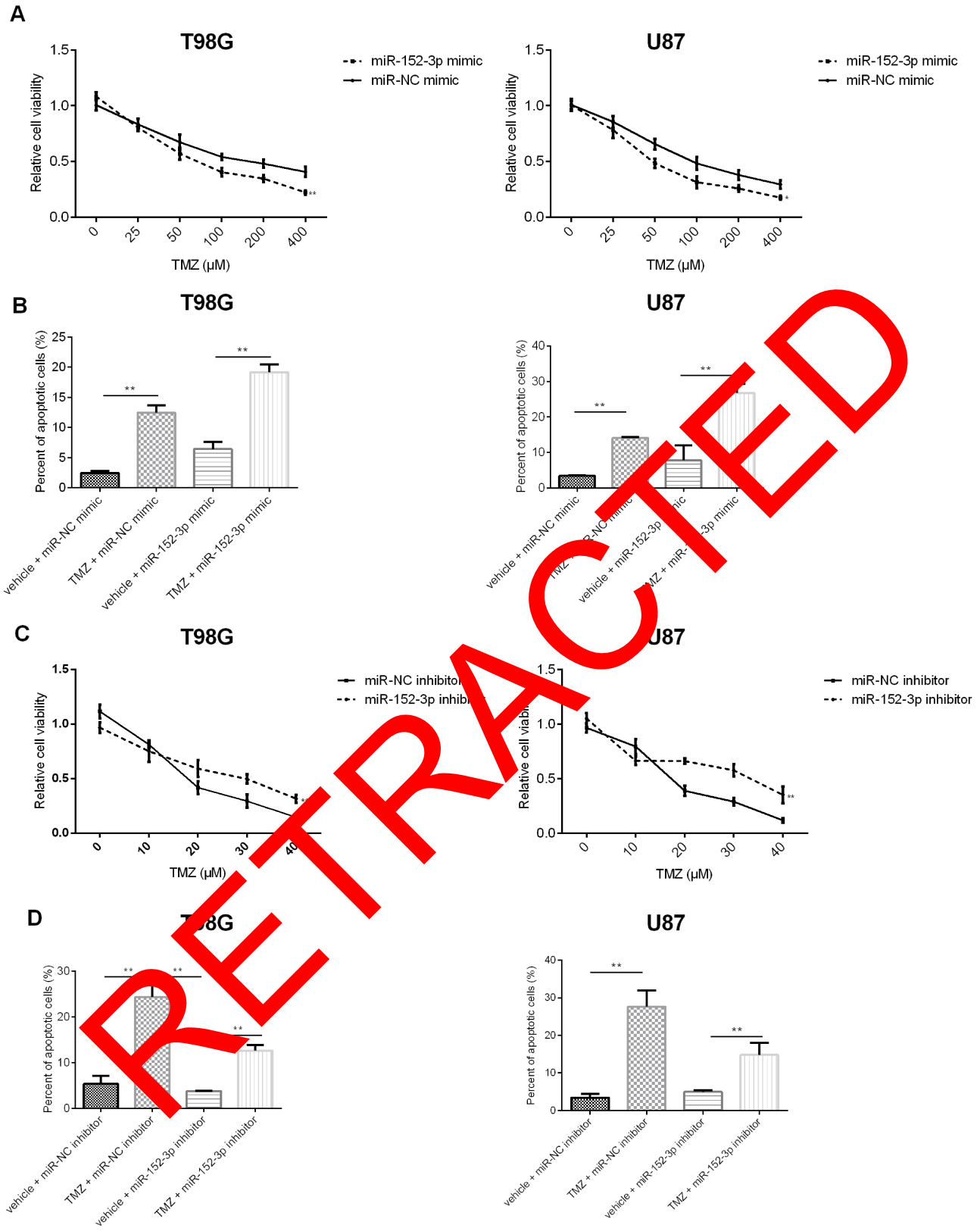




**Figure 4** MiR-152-3p heightened TMZ sensitivity of glioblastoma cells. (**A**) The cell viability assay illustrated that miR-152-3p mimic strengthened the TMZ-induced inhibition of cell viability. (**B**) The role of miR-152-3p in TMZ-induced cell apoptosis was estimated by flow cytometry. (**C**) miR-152-3p inhibitor could remarkably elevate the viability of glioblastoma cells treated with TMZ. (**D**) miR-152-3p inhibitor could significantly reduce cell apoptosis percentage of glioblastoma cells treated with TMZ. *p<0.05, **p<0.01.

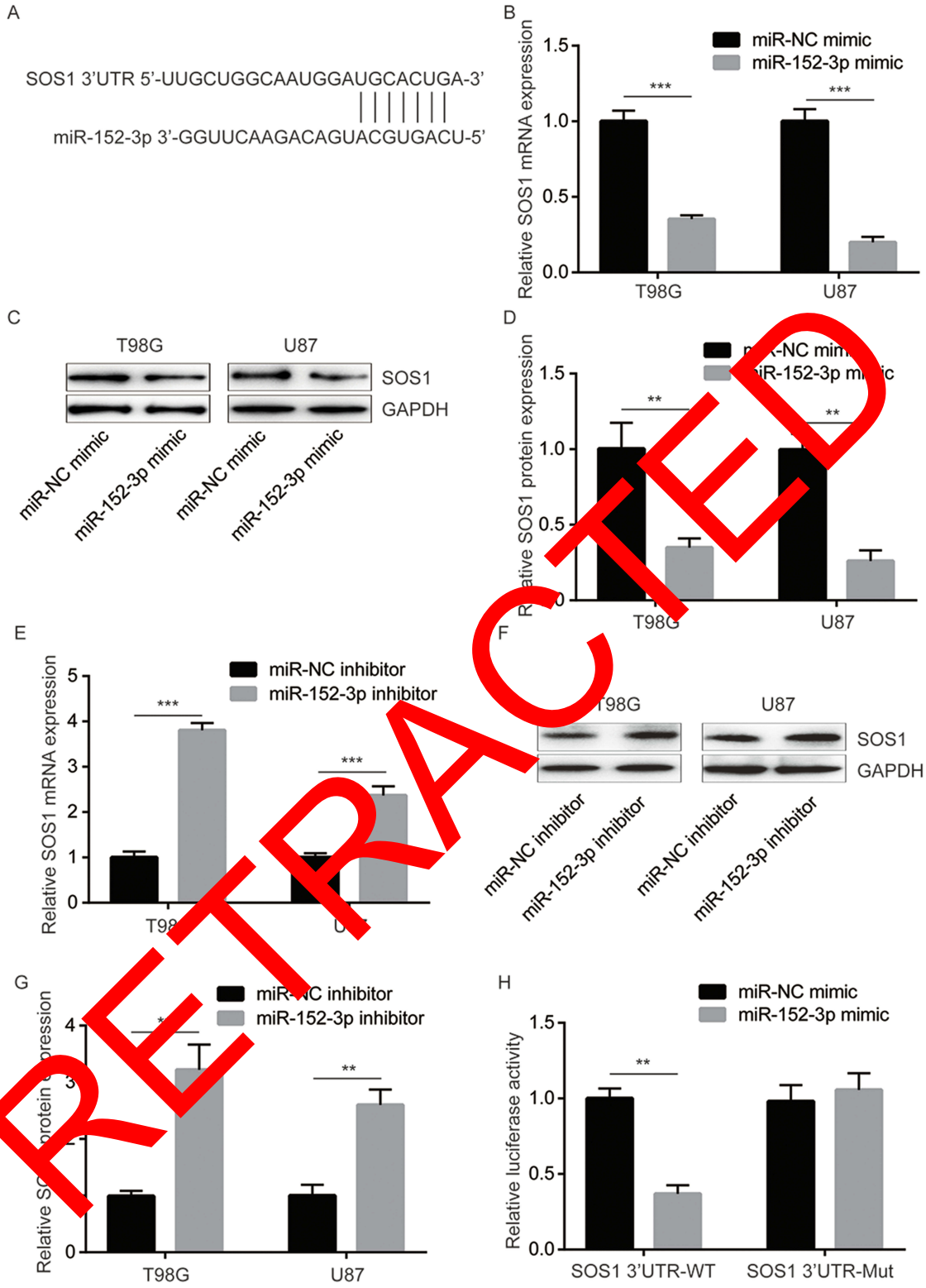




**Figure 5** MiR-152-3p negatively regulated SOS1 and could bind to its 3′-UTR. (**A**) Bioinformatics database prediction of the putative binding site of miR-152-3p on 3′-UTR of SOS1. (**B**) Compared to miR-NC mimic, miR-152-3p mimic significantly decreased SOS1 mRNA expression in T98G and U87 glioblastoma cells. (**C**) Compared to miR-NC mimic, miR-152-3p mimic significantly decreased SOS1 protein expression in T98G and U87 glioblastoma cells. (**D**) Analyzed data from figure C, miR-152-3p prominently reduced SOS1 protein expression in T98G and U87 glioblastoma cells. (**E**) In comparison to miR-NC inhibitor, miR-152-3p inhibitor observably increased SOS1 mRNA expression in T98G and U87 glioblastoma cells. (**F**) In comparison to miR-NC inhibitor, Western blot showed that miR-152-3p inhibitor observably increased SOS1 protein expression in T98G and U87 glioblastoma cells. (**G**) Analyzed data from figure F, miR-152-3p prominently increased SOS1 protein expression in T98G and U87 glioblastoma cells. (**H**) Luciferase activity was significantly decreased when co-transfected with miR-152-3p mimic and SOS1 3′UTR-WT reporter plasmid in T98G. However, luciferase activity was unaltered when co-transfected with miR-152-3p mimic and SOS1 3′UTR-Mut reporter plasmid. **p<0.01, ***p<0.001.

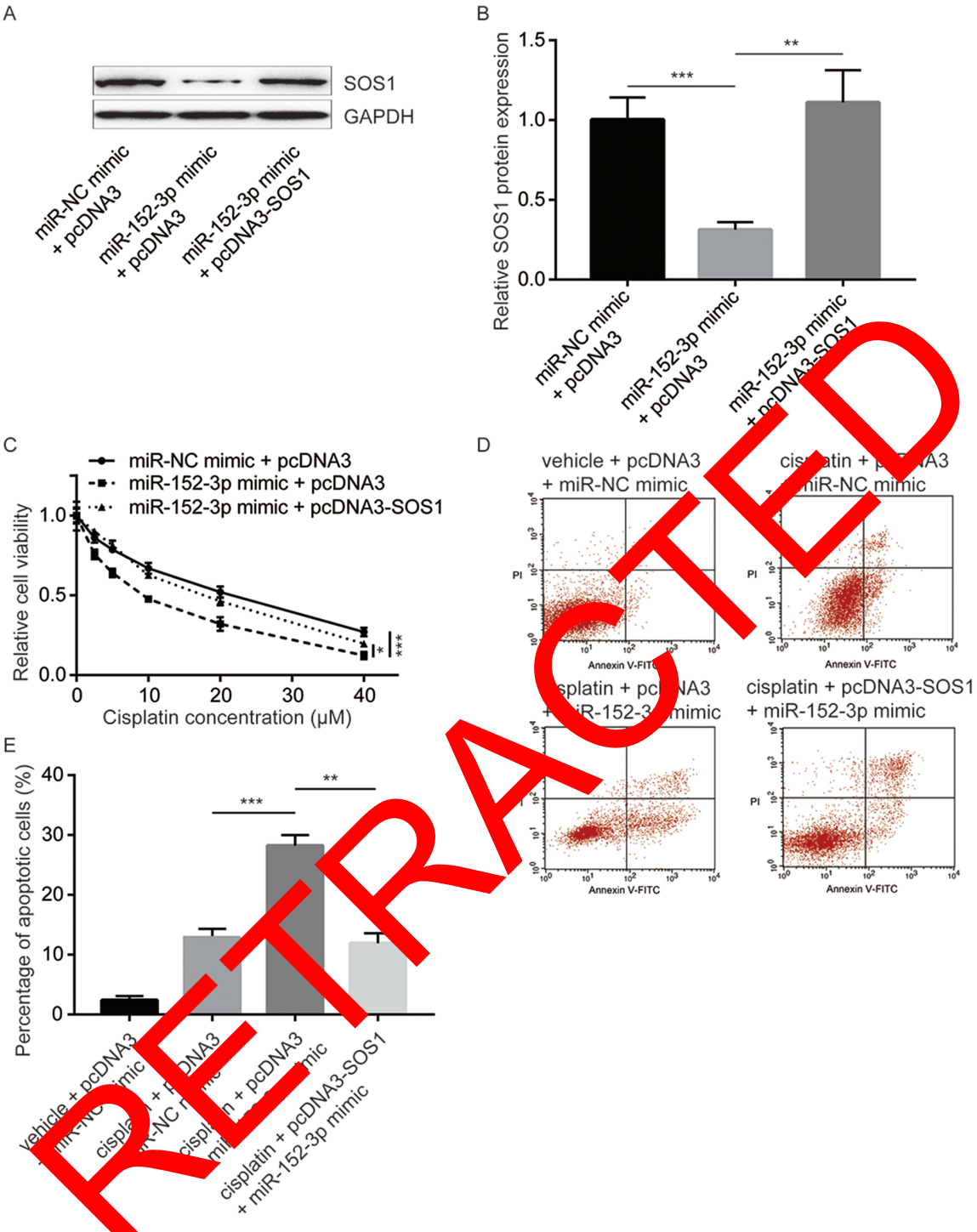




**Figure 6** Overexpression of miR-152-3p augmented cisplatin's anti-tumor effect via repression of SOS1. (**A**) Compared to miR-NC mimic, miR-152-3p mimic dramatically decreased SOS1 protein expression, while SOS1 overexpression could reverse the downregulated SOS1 expression. (**B**) Analyzed data from figure A, in comparison to miR-NC mimic, miR-152-3p mimic notably reduced SOS1 protein expression, but additional SOS1 could reverse the downregulated SOS1 expression. (**C**) Compared to control group, miR-152-3p mimic co-transfected with pcDNA3 plasmid significantly weaken cell viability with increasing concentration of cisplatin (0, 2.5, 5, 10, 20, 40 μM), co-transfection of recombinant SOS1 plasmid and miR-152-3p reversed miR-152-3p mimic-induced decline of cell viability in T98G cells. (**D**) Compared to control group, miR-152-3p mimic co-transfected with pcDNA3 plasmid significantly enhanced cisplatin (5 μM) induced cell apoptosis, but transfection of recombinant SOS1 plasmid reversed miR-152-3p mimic-induced augment of cell apoptosis in T98G cells. (**E**) Analyzed data from figure D, compared to control group, miR-152-3p mimic co-transfected with pcDNA3 plasmid statistical significance increased cisplatin (5 μM) induced cell apoptosis, but transfection of recombinant SOS1 plasmid statistical significance reversed miR-152-3p mimic-induced augment of cell apoptosis in T98G cells. *p<0.05, **p<0.01, ***p<0.001.

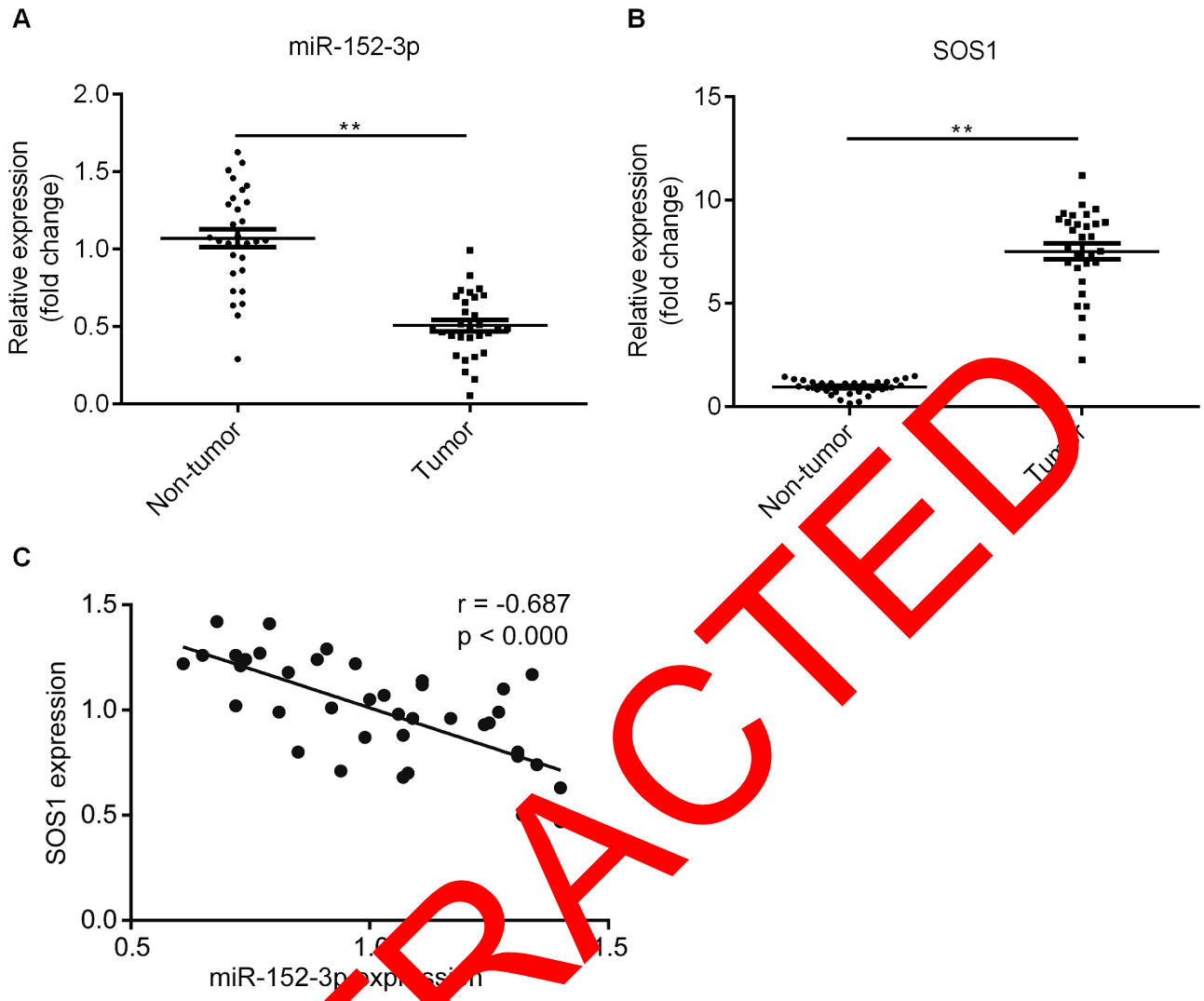




**Figure 7** MiR-152-3p expression negativ lated with SOS RNA expression in glioblastoma tissues. (**A, B**) RT-qPCR analysis was applied to explore the expression of miR-152-3p and SOS1 in clinical sa ples. (**C**) arson correlat n analysis showed that SOS1 mRNA expression levels were negatively correlated with miR-152-3p expression levels in tumor tissues om 40 patients h glioblastoma (r = −0.687). **p<0.01.

treatment of pati s with ioblastoma remains a major challenge.[31] As is kn wn to all, ch notherapy resistance is a major pr em in glio stom treatment.[32] Jia et al have found t miR-7 5p coul nhance temozolomide sensitivity of ug esistant g ioblastoma cells by targeting Yin Yang 1.[33] Z g et al have reported that exosomal transfer of miR-151a en nces chemosensitivity to temozolomide in drug-resistant glioblastoma.[34] Up to now, only several studies explored the role of miRNAs in cisplatin resistance of glioblastoma.[35,36] Li et al have explored that miR-186 reverses cisplatin resistance and inhibits the formation of the glioblastoma-initiating cell phenotype by degrading Yin Yang 1 in glioblastoma.[35] Yang et al have discovered that miR-29a overexpression improves sensitivity of cisplatin in $CD133^+$ glioblastoma cells (T98G and U87MG) and significantly suppresses tumor growth in $CD133^+$ glioblastoma tumor-bearing mice in response to cisplatin treatment.[36] In this research, we creatively explored the function of miR-152-3p in cisplatin sensitivity of glioblastoma, and found that miR-152-3p could increase cisplatin-induced cytotoxicity in U87 and T98G glioblastoma cells.

Since the discovery of miR-152-3p, it has been considered as a tumor suppressor in multiple solid tumors and has been shown to be abnormally down-regulated in glioblastoma.[14,37] Previous studies have shown that, miR-152-3p overexpression prevents tumor cell growth in endometrial cancer,[38] miR-152-3p suppressed cell proliferation, migration, colony formation and invasion in non-small cell lung cancer,[39] and miR-152-3p inhibited glioblastoma cell invasion and proliferation activities via decreasing DNA

Methyltransferase 1 (DNMT1) and the restoration of miR-152-3p had therapeutic significance in glioblastoma treatment.[14] Additionally, miR-152-3p contributed to ovarian cancer cisplatin resistance through direct target DNMT1.[40] In this research, according to TargetScan database prediction, we found that miR-152-3p could negatively regulate SOS1 and influence glioblastoma cisplatin sensitivity. SOS1 is known to be overexpressed in various cancers and plays an important role in many cascade signaling pathways.[41] Lv et al have reported that miR−124 could regulate SOS1 to affect glioblastoma growth[24] Our study innovatively explored the regulatory effect of miR-152-3p on SOS1 in glioblastoma, and our results suggested that SOS1 inhibited the sensitivity of glioblastoma cells to cisplatin and miR-152-3p executed its function in cisplatin resistance of glioblastoma cells via targeting SOS1.

In conclusion, our research demonstrated that miR-152-3p negatively regulated SOS1 to increase cytotoxicity of cisplatin in glioblastoma. Our findings provided new insights on the targeted delivery of miR-152-3p to glioblastoma cells as a potential therapeutic treatment for glioblastoma.

## Acknowledgements

This work is supported by Medical Scientific Program of Shanghai Health and Family Planning Commission (201540163), Scientific Program of Jiangsu Province (BE2018643), Scientific Program of Changzhou (CJ20180029; CE20175025), 333 projects of Jiangsu Scientific Research Program (BRA2018408),and Scientific Program of Shandong Provincial Department of Education(J18KA254).

## Disclosure

The authors report no conflicts of interest in this work.



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