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LncRNA TRG-AS1 promotes glioblastoma cell proliferation by competitively binding with miR-877-5p to regulate SUZ12 expression

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ABSTRACT

Glioblastoma is one of the most fatal diseases in human central nerve system. However, the prognosis and treatment of glioblastoma still call for steady improvement. In recent years, increasing studies have revealed that the abnormal expression of long non-coding RNA (lncRNA) is closely related to carcinogenesis and prognosis. Unfortunately, many lncRNAs still need further research in their function and molecule mechanism. LncRNA TRG-AS1 hasn't been detected in any types of cancers before. TRG-AS1 is associated with poor prognosis and is upregulated in glioblastoma tissues and cells. TRG-AS1 can also accelerate glioblastoma cell proliferation in return. On the other hand, miRNA-877-5p expresses low in glioblastoma and contains binding sites with both TRG-AS1 and SUZ12. Furthermore, TRG-AS1 suppresses the expression of miR-877-5p while miR-877-5p suppresses SUZ12 expression. Overexpression of TRG-AS1 could promote the expression of SUZ12.Rescue assays demonstrates that overexpression of SUZ12 can counteract the decline of glioblastoma cell proliferation induced by knockdown of TRG-AS1. Based on all these assays, TRG-AS1 promotes glioblastoma cell proliferation by acting as a ceRNA of miR-877-5p to regulate SUZ12 expression. TRG-AS1 might serve as a new target in glioblastoma treatment.

1. Introduction

Glioblastoma (GBM), one of the deadliest diseases in central nerve system, has received increasing attention in cancer research [5,25]. GBM holds a surprisingly high morbidity rate and death rate, especially in elder males [30]. Patients only have a life expectancy of less than two years after being diagnosis [16]. Despite the rapid development in tumor biology, efficient treatment against or even the molecule mechanism of GBM is still not satisfactory. This situation calls for new methods in GBM treatment.

Recent studies on long non-coding RNAs (lncRNAs) might offer a new path in GBM research. Studies have proved that only less than 2% of genomes can be translated into proteins, leaving more than 98% of transcripts to become non-coding RNAs [7]. Non-coding RNAs can be divided into small interfering RNAs (siRNAs), microRNAs (miRNAs), long non-coding RNAs (lncRNAs) and so on by their variation in length [23]. Non-coding RNAs with the length of more than 200 nucleotides are defined as lncRNAs. LncRNAs have been found to regulate tumorigenesis and cancer cell function in a large amount of studies [11,20,22]. In the field of GBM research, the mediating function of lncRNAs has also been proved. For example, six lncRNAs were identified to be highly associated with prognosis of GBM patients [35]. Another example is that TP73-AS1 promotes the drug resistance of GBM cells and is associated with GBM aggressiveness [18]. T cell receptor gamma locus antisense RNA 1 (TRG-AS1) occurred in this study is a lncRNA which has not been researched in any types of cancers. The function and mechanism of TRG-AS1 are still yet to explore. In the meantime, non-coding RNAs with the length of 20–25 nucleotides were categorized as miRNAs. MiRNAs have also been proved to regulate various functions of cancer cells [6]. MiR-877-5p is a miRNA which has already been studied in liver injury and hepatocellular cancer [19,29]. However, its function and mechanism in GBM are still unclear.

Based on data collected from GEPIA database (http://gepia.cancer-pku.cn/), TRG-AS1 was associated with poor prognosis. In this research, we found that TRG-AS1 could promote glioblastoma cells proliferation. MiR-877-5p was downregulated in GBM cells and contained binding sites with both TRG-AS1 and SUZ12. SUZ12 is an mRNA which is overexpressed in GBM tissues. TRG-AS1 could suppress the expression of miR-877-5p while miR-877-5p would suppress the expression of SUZ12. More importantly, TRG-AS1 positively regulated the expression of SUZ12. SUZ12 expression decreased with overexpression of miR-877-5p, but was rescued by overexpression of TRG-AS1. TRG-AS1, miR-877-5p and SUZ12 could all be detected from RNA-induced silencing complex and its attachment. So, TRG-AS1 acted as a competing

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endogenous RNA (ceRNA) of miR-877-5p to regulate SUZ12 expression. This mechanism was further verified through rescue assay on GBM cell proliferation.

2. Material and methods

2.1. Tissue samples

51 samples of glioma tissues and 51 samples of normal tissues were applied in this research. All tissues were collected from patients of Fujian Medical University Union Hospital, who had all signed consents to this research. None of these patients have received radiotherapy or chemotherapy before collection. This study has gained the approval of the Ethics Committee of Fujian Medical University Union Hospital.

2.2. Cell culture

Human GBM cell lines U251 and U87, with the normal human astrocytes (NHAs) were obtained from American Type Culture Collection (ATCC; Manassas, VA, USA). Another two human glioblastoma cell lines A172 and LN229 were purchased from the Chinese Academy of Sciences (Shanghai, China). All cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM; Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA), complemented with 10% fetal bovine serum (FBS; Gibco). All cell lines were cultured in a moist atmosphere at 37℃, containing 5% CO_2.

2.3. Cell transfection

Glioblastoma cell lines were placed in a 24-well plate until cell amount confluency reached 70%. Sh-NC and shRNAs that specifically target TRG-AS1 (sh-TRG-AS1#1: ATCCCCGGTTGTCTCTGTCTAGATATT CAAGAGATATCTAGACAGAGACAACCTTTTTGGAAA, sh-TRG-AS1#2: GATCCCCAGGTGTTACAGTACTTAAATTCAAGAGATTTAAGTACTGTAA CACCTTTTTTGGAAA, sh-TRG-AS1#3: GATCCCCCATACTTACCAATGTG TTATTCAAGAGATAACACATTGGTAAGTATGTTTTTGGAAA) were purchased from GenePharma (Shanghai, China). NC mimics (UCACAACCU CCUAGAAAGAGUAGA), miR-877-5p mimics (GUAGAGGAGAUGGCGCA GGG), NC inhibitor (UCUACUCUUUCUAGGAGGUUGUGA) and miR-877-5p inhibitor (CCCUGCGCCAUCUCCUCUAC) were also obtained from GenePharma. TRG-AS1 and SUZ12 were cloned into pcDNA3.1 (Invitrogen, Carlsbad, CA, USA) for overexpression. All plasmids were transfected respectively into A172 and LN229 cells with Lipofectamine 2000 (Invitrogen, Shanghai, China).

2.4. RT-qPCR

RT-qPCR was carried out to measure the relative expression of TRG-AS1, miR-877-5p and SUZ12. Under manufacturer-recommended protocol, total RNAs were extracted from glioblastoma cell lines with TRIzol reagent (Invitrogen). Reverse Transcription Kit (Takara, Otsu, Japan) and Taqman Advanced miRNA cDNA Synthesis Kit (Thermo Fisher Scientific) were used to reverse transcribe RNAs into cDNAs. RT-qPCR was carried out with the RNA-direct™ SYBR Green Real-time PCR Master Mix (Toyobo, Osaka, Japan) on Roche LightCycler 480 Real-Time PCR System (Applied Biosystems). All experiments were repeated in triplicate. The relative expressions were calculated with $2^{-\Delta\Delta Ct}$ method. GAPDH and U6 were used as internal standards for quantification. The primer sequences were as follows: TRG-AS1, forward: 5′-GGAGTCTGCTCTAAGAGCTG-3′, reverse: 5′-CAGAGCAAAGATGCTCTGC-3′; miR-877-5p, forward: 5′-TAGAGGAGATGGCGCAG-3′, reverse: 5′- GAACATGTCTGCGTATCTC-3′; SUZ12, forward: 5′−CCATGCAGGAAATGGAAGAATGTC-3′, reverse: 5′-CTGTCCAACGAAGAGTGAACTGC-3′; GAPDH, forward: 5′-TATGATG ATATCAAGAGGGTAGT-3′, reverse: 5′-TGTATCCAAACTCATTGTCA TAC-3′; U6, forward: 5′-GCTTCGGCAGCACATATACTAAAAT-3′, reverse: 5′-CGCTTCACGAATTTGCGTGTCAT-3′.

2.5. CCK-8 assay

After transfection, 1×10^3A172 and LN229 cells were seeded onto 96-well plates. After 24, 48, 72 and 96 h of incubation, CCK-8 solution was added into each well. All cells were incubated for 4 more hours. The optical density was measured with a plate reader (ELx808, Bio-Tek Instruments, ST, USA) at 450 nm.

2.6. 5-ethynyl-2′-deoxyuridine (EdU) assay

Cells were placed into 24-well plates (1×10^5 cells/well) overnight. After synchronization, all cells were treated with EdU for 24 h. According to the manufacturer's instructions, EdU assay was carried out with Cell Light EdU DNA imaging kit (Invitrogen, Carlsbad, CA, USA). EdU positive cells were measured.

2.7. Nuclear-cytoplasmic fractionation

To locate TRG-AS1 in GBM cells, nuclear and cytoplasmic were separated with PARIS Kit (Life Technologies, MA, USA) under manufacturer's instructions. The relative expression of TRG-AS1 in nucleus and cytoplasm were respectively calculated with RT-qPCR. GAPDH and U6 were selected as internal references of RT-qPCR.

2.8. Fluorescence in situ hybridization (FISH)

FISH was conducted to locate TRG-AS1 in GBM cells. Fluorophore probes targeting TRG-AS1 were designed by Bioresearch Technologies (Novato, CA, USA). Cells were washed with PBS two times and permeabilized for 1 h. After dilution, probes were added into cells. Cells were then cultured in darkness at 37 ℃ overnight. After washing cells with saline sodium citrate, DAPI was added for staining for 10 min. Fluorescence was detected with laser scanning microscope (Leica Microsystems, Shanghai, China).

2.9. Luciferase reporter assay

Luciferase reporter assay was carried out to confirm the binding capacity between miR-877-5p and TRG-AS1 or SUZ12. TRG-AS1 and SUZ12 containing potential binding sites with miR-877-5p were amplified through PCR and cloned into pGL3 vectors (Promega, Madison, USA). MiR-877-5p mimics or NC mimics were co-transfected into A172 or LN229 cells with pGL3-TRG-AS1-WT, pGL3-TRG-AS1-Mut, pGL3-SUZ12-WT or pGL3-SUZ12-Mut. 48 h after transfection, luciferase activities were measured through Dual-Luciferase Reporter Assay System (Promega, USA).

2.10. RNA pull down assay

MiR-877-5p-WT, miR-877-5p-Mut and miR-NC were biotinylated through Biotin RNA Labeling Mix (Roche, Mannheim, Germany). Bio-miR-877-5p-WT, bio-miR-877-5p-Mut or bio-NC was co-incubated with cell lysates and streptavidin beads. After 1 h of incubation, proteins were eluted and expressions of miR-877-5p were measured with RT-qPCR.

2.11. RNA immunoprecipitation (RIP) assay

According to the manufacturer's protocol, Magna RIP RNA-binding protein immunoprecipitation kit (Merck Millipore, Darmstadt, Germany) was applied for RIP assay. All cells were lysed with RIP lysis buffer. Afterwards, cell lysates were cultured with RIP buffer containing magnetic beads conjugated with human anti-Argonaute 2 (Ago2) or negative control IgG antibodies. Input was applied as positive control. Immunoprecipitated RNA was obtained. The expression levels of TRG-AS1, miR-877-5p and SUZ12 were detected with RT-qPCR.

2.12. Western blot

Western blot was performed to detect SUZ12 protein level. NHA and GBM cells were lysed with RIPA buffer containing protease inhibitor. The total proteins were extracted with 10% SDS-PAGE and transferred to PVDF membranes. The membranes were then incubated with antibodies against SUZ12 (Abcam, ab12073) or GADDH (Abcam, ab8245) at 4 ℃ overnight. Afterwards the membranes were added with secondary antibodies at 37 ℃ for 1 h. The relative protein level was then calculated.

2.13. Statistical analysis

To analyze the statistics, SPSS (version 15.0; SPSS, Inc., Chicago, IL, USA) and GraphPad Prism (version 6.0; GraphPad Software, Inc., La Jolla, CA, USA) were applied. All data were presented as mean ± standard deviation (SD). Student's *t*-test and one-way analysis of variance (ANOVA) were applied for comparison between groups. $P < 0.05$ was considered as statistically significant. All experiments were repeated three times.

3. Results

3.1. TRG-AS1 is upregulated in GBM and promotes GBM cell proliferation

According to statistics collected from GEPIA database, we found that lncRNA TRG-AS1 was highly associated with poor prognosis in patients with GBM (Fig. 1A). RT-qPCR revealed a much higher expression of TRG-AS1 in GBM tissues (Fig. 1B). RT-qPCR demonstrated that TRG-AS1 also expressed high in GBM cells (A172, LN229, U251

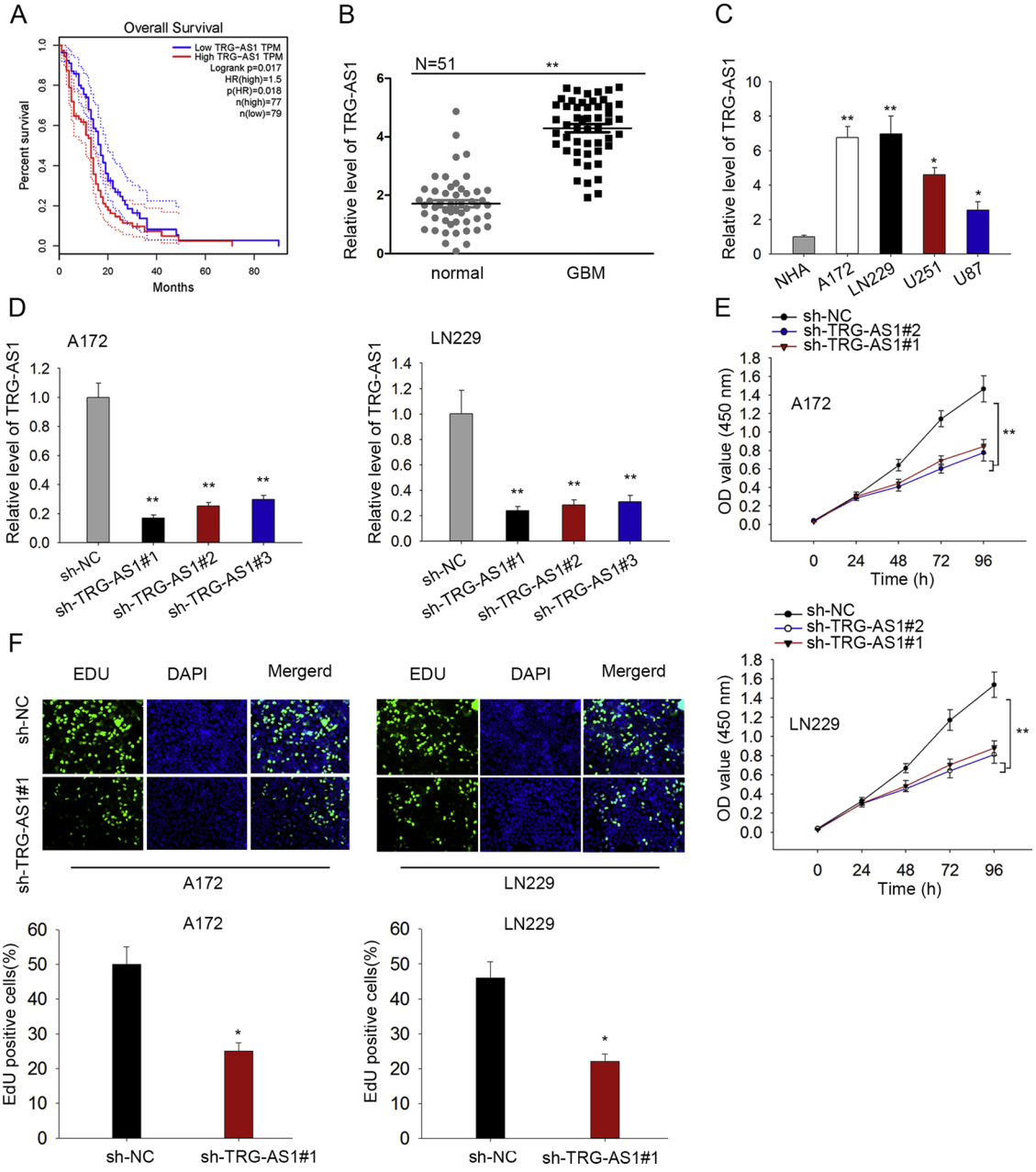

Fig. 1. TRG-AS1 was upregulated and promoted cell proliferation in GBM. (A) Kaplan-Meier analysis of overall survival rate of low and high TRG-AS1 expression patients, data collected from GEPIA database. (B) RT-qPCR of TRG-AS1 in GBM tissues and normal tissues (n = 51). (C) RT-qPCR of TRG-AS1 expression in NHAs and GBM cells. (D) RT-qPCR of TRG-AS1 expression in GBM cells transfected with sh-TRG-AS1. (E) CCK-8 assay in GBM cells transfected with sh-TRG-AS1. (F) EdU assay in GBM cells transfected with sh-TRG-AS1.All assays were performed in triplicate. *$p < 0.05$; **$p < 0.01$.

and U87), compared to NHAs (Fig. 1C). Cell lines A172 and LN229 were chosen for further experiments for their relatively high expression of TRG-AS1. Then we performed a series of assays to see if knockdown of TRG-AS1 would affect GBM cell proliferation in return. To begin with, the expression of TRG-AS1 significantly decreased in GBM cells transfected with sh-TRG-AS1 (Fig. 1D). In CCK-8 assay, cell proliferation clearly slowed down after TRG-AS1 was downregulated (Fig. 1E). Similar results occurred in EdU assay (Fig. 1F), proving that TRG-AS1 would regulate the proliferation ability of GBM cells. So based on these experiments, TRG-AS1 is upregulated in GBM cells and promotes GBM cell proliferation.

3.2. TRG-AS1 can bind with miR-877-5p and downregulates miR-877-5p

To further investigate the mechanism of TRG-AS1 regulating GBM cell proliferation, the subcellular location of TRG-AS1 was detected. Nuclear-cytoplasmic fractionation demonstrated that the majority of TRG-AS1 concentrated in cytoplasm rather than nucleus (Fig. 2A). FISH assay also indicated that the enrichment of TRG-AS1 occurred in cytoplasm area of GBM cells (Fig. 2B). In ceRNA mechanism, lncRNAs locate in cytoplasm and regulate miRNA expression, thus regulating cancer progression [3,17]. TRG-AS1 was found to be located in cytoplasm and might regulate GBM through ceRNA mechanism. By conducting starBase v3.0 (http://starbase.sysu.edu.cn/index.php), we found that 15 miRNAs could bind with TRG-AS1. One of them, miR-877-5p has been studied in other types of diseases but not GBM. So we decided to select miR-877-5p for further research. RT-qPCR demonstrated a higher expression of miR-877-5p in normal tissues (Fig. 2C). RT-qPCR analysis also showed that miR-877-5p was overexpressed in NHA compared to GBM cells (Fig. 2D). To verify the binding capacity between miR-877-5p and TRG-AS1, luciferase reporter assay was carried out. The potential binding site and mutant site were shown in Fig. 2E. From Fig. 2F, we found that pcDNA3.1/TRG-AS1 could suppress the luciferase activity of wild-type miR-877-5p vectors. RIP assay and RNA pull down were also performed. In RIP assay, both TRG-AS1 and miR-877-5p concentrated in anti-AGO2 group (Fig. 2G). Also, TRG-AS1 could be pulled down by biotinylated wild type miR-877-5p (Fig. 2H). Luciferase reporter assay, RIP assay and RNA pull down all proved that miR-877-5p could bind with TRG-AS1. Meanwhile, knockdown of TRG-AS1 caused upregulation of miR-877-5p (Fig. 2I), indicating that TRG-AS1 negatively regulated miR-877-5p expression. Based on these facts, TRG-AS1 can bind with miR-877-5p and downregulates miR-877-5p.

We also performed a series of rescue assays to detect if TRG-AS1 regulated GBM cell proliferation through miR-877-5p. Both CCK-8 and EdU assay revealed that cell proliferation ability was suppressed by

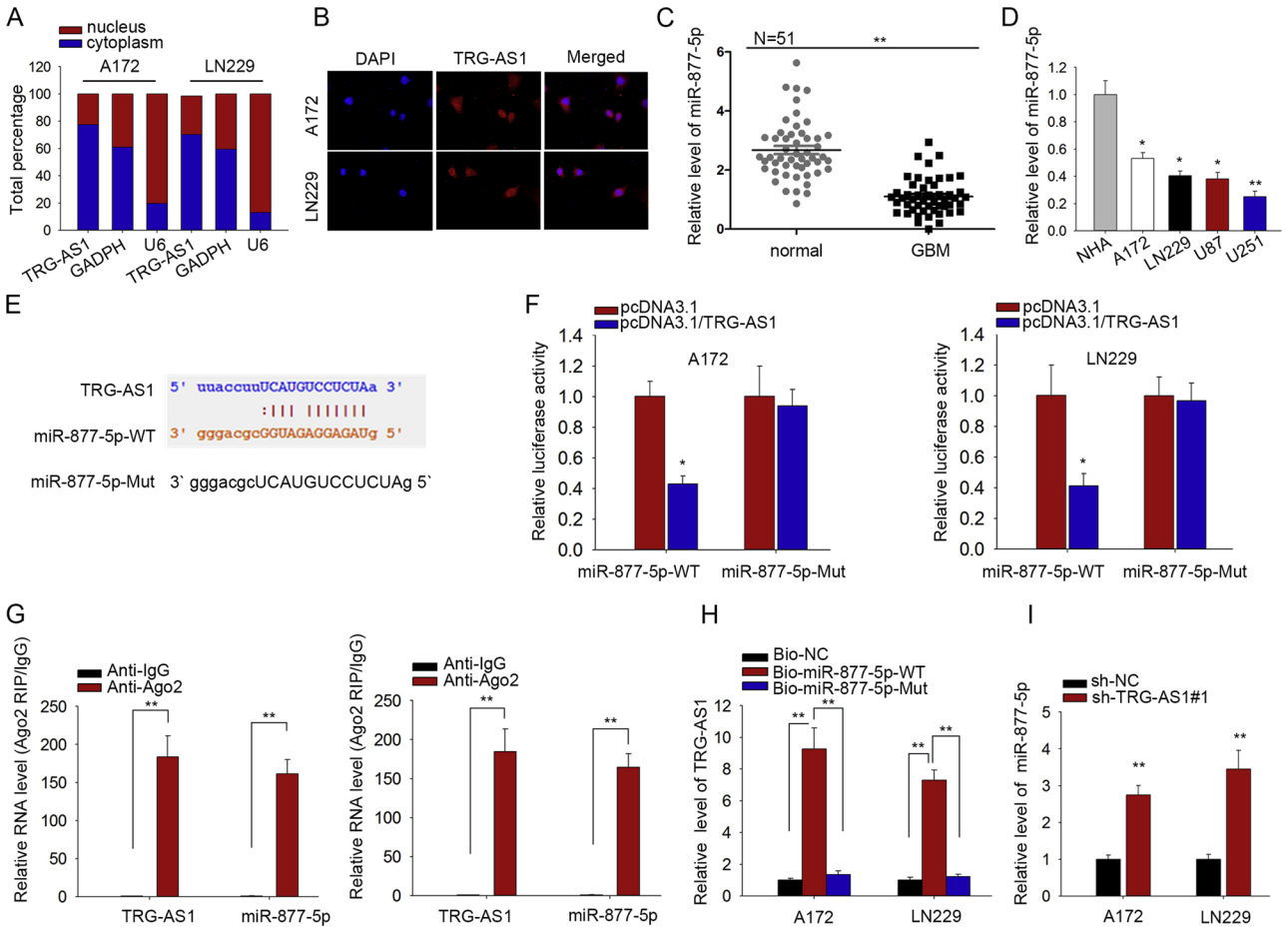

Fig. 2. TRG-AS1 acted as a sponge of miR-877-5p. (A) RT-qPCR of TRG-AS1 expression in cytoplasm and nucleus of GBM cells. (B) FISH assay in GBM cells locating TRG-AS1. (C) RT-qPCR of miR-877-5p in GBM tissues and normal tissues (n = 51). (D) RT-qPCR of miR-877-5p expression in NHA and GBM cells. (E) The potential binding site between TRG-AS1 and miR-877-5p. (F) Luciferase reporter assay verifying miR-877-5p and TRG-AS1 binding at predicted site. (G) RIP assay with miR-877-5p mimics in GBM cells. (H) RNA pull down assay with wild type/mutant type miR-877-5p in GBM cells. (I) RT-qPCR of miR-877-5p in cells transfected with sh-TRG-AS1. All assays were performed in triplicate. *p < 0.05; **p < 0.01.

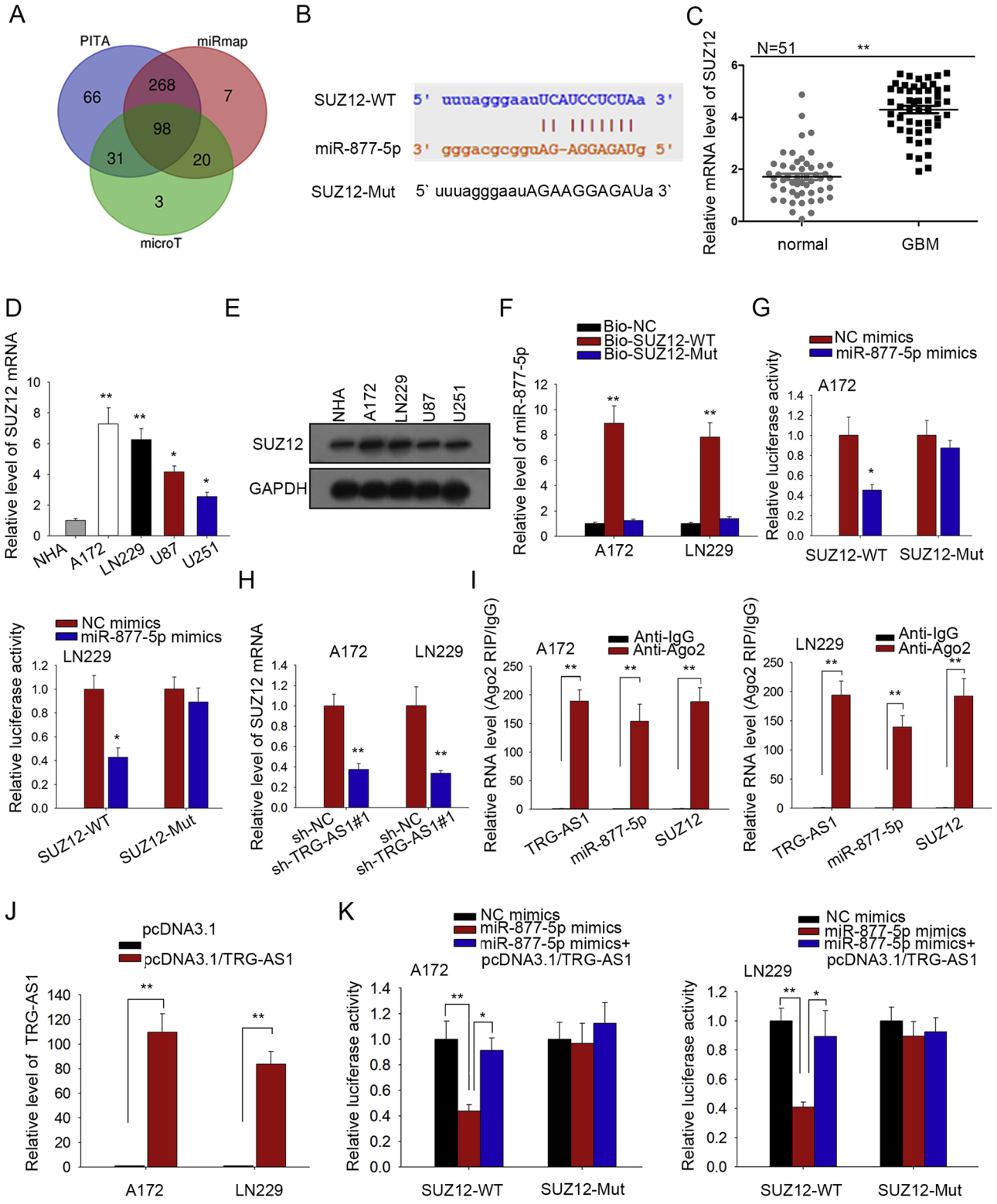

Fig. 3. TRG-AS1 acted as a ceRNA of miR-877-5p to regulate SUZ12 expression. (A) Venn diagram of mRNAs that could bind with miR-877-5p predicted by PITA, miRmap and microT database. (B) Predicted binding site between miR-877-5p and SUZ12. (C) RT-qPCR of SUZ12 expression in GBM tissues and normal tissues (n = 51). (D) RT-qPCR of SUZ12 mRNA in NHA and GBM cells. (E) Western blot of SUZ12 protein expression in NHA and GBN cells. (F) RNA pull down assay with wild type/mutant type biotinylated miR-877-5p in GBM cells. (G) Luciferase reporter assay with miR-877-5p mimics in GBM cells. (H) RT-qPCR of SUZ12 mRNA expression in GBM cells transfected with sh-TRG-AS1. (I) RIP assay with anti-AGO2 antibody in GBM cells. (J) RT-qPCR of TRG-AS1 mRNA in GBM cells transfected with pcDNA3.1/TRG-AS1. (K) Luciferase reporter assay with miR-877-5p mimics and miR-877-5p mimics + pcDNA3.1/TRG-AS1. All assays were performed in triplicate. *$p < 0.05$; **$p < 0.01$.

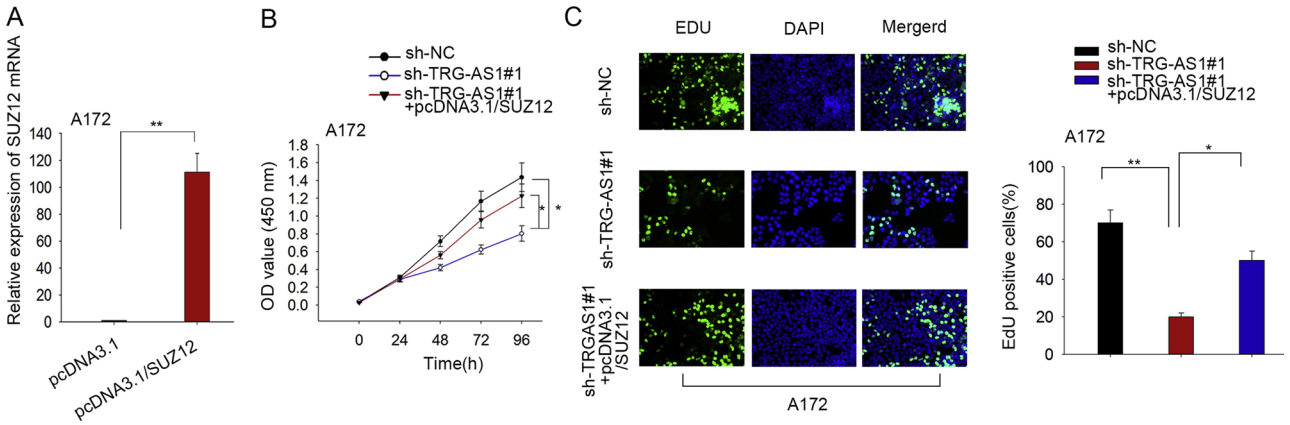

Fig. 4. Overexpression of SUZ12 could rescue the inhibition of GBM cell proliferation ability induced by knockdown of TRG-AS1. (A) RT-qPCR of SUZ12 mRNA in cells transfected with pcDNA3.1/ SUZ12. (B) CCK-8 assay in GBM cells transfected with sh-TRG-AS1 and sh-TRG-AS1 + pcDNA3.1/ SUZ12. (C) EdU assay in cells transfected with sh-TRG-AS1 and sh-TRG-AS1 + pcDNA3.1/ SUZ12. All assays were performed in triplicate. *p < 0.05; **p < 0.01.

knockdown of TRG-AS1, but inhibition of miR-877-5p could partially restore this trend (Fig. S1A, B).

3.3. TRG-AS1 acts as a ceRNA of miR-877-5p to regulate SUZ12 expression

In the previous section, we have confirmed that TRG-AS1 acted as a sponge of miR-877-5p. The target genes of miR-877-5p were then searched through bioinformatics analysis. Based on PITA database (http://genie.weizmann.ac.il/pubs/mir07/mir07_data.html), miRmap database (https://mirmap.ezlab.org/) and microT database (http://diana.cslab.ece.ntua.gr/microT/), we found that 98 mRNAs could bind with miR-877-5p (Fig. 3A). One of them, SUZ12, has been proved to promote cancer progression in various cancers [15,27]. So we chose SUZ12 for further detection. The potential binding sites between miR-877-5p and SUZ12 were presented in Fig. 3B. SUZ12 significantly overexpressed in GBM tissues, compared to normal tissues (Fig. 3C). The expression of SUZ12 was also higher in GBM cells than NHAs (Fig. 3D). Western blot analysis also proved a higher protein level of SUZ12 in GBM cells (Fig. 3E). In Fig. 3F, most of miR-877-5p expression was detected in wild type bio-SUZ12 group. Luciferase reporter assay showed that miR-877-5p mimics decreased the luciferase activity of wild type SUZ12 vectors (Fig. 3G).

As is known to all, lncRNAs could regulate mRNAs expression in ceRNA mechanism [4,9,33]. So we performed experiments to see if expression of SUZ12 would be affected by TRG-AS1. RT-qPCR revealed that knockdown of TRG-AS1 would downregulate the mRNA expression of SUZ12 (Fig. 3H). RIP assay was also performed to verify the ceRNA mechanism. The expressions of TRG-AS1, miR-877-5p and SUZ12 could all be detected from anti-AGO2 group, indicating that TRG-AS1 acted as ceRNA of miR-877-5p to regulate SUZ12 expression (Fig. 3I). Luciferase reporter assay was also performed to verify the ceRNA mechanism. TRG-AS1 was effectively cloned into pcDNA3.1 vectors for further experiments (Fig. 3J). The luciferase activity of wild type SUZ12 vector was significantly weakened by overexpression of miR-877-5p, but was later rescued by pcDNA3.1/TRG-AS1 (Fig. 3K). Based on these assays, TRG-AS1 acts as a ceRNA of miR-877-5p to regulate SUZ12 expression.

3.4. Overexpression of SUZ12 can rescue the decline of cell proliferation induced by knocking down TRG-AS1

Rescue assays were performed to demonstrate if TRG-AS1 regulated GBM cell proliferation through miR-877-5p/SUZ12 axis. The expression of SUZ12 in GBM cells was promoted by transfection of pcDNA3.1/ SUZ12 (Fig. 4A). In CCK-8 assay, cell proliferation was suppressed by knockdown of TRG-AS1, while co-transfection of pcDNA3.1/SUZ12 partly counteracted this trend (Fig. 4B). EdU assay also verified such phenomenon (Fig. 4C). Based on all these assays, overexpression of SUZ12 can rescue the decline of cell proliferation induced by knocking down of TRG-AS1 in GBM.

4. Discussion

GBM is known as one of the most fatal central nerve diseases. The survival rate and life expectancy of GBM have always been pessimistic for patients [8]. There have been plenty of researches carried out on GBM [21,24]. A large portion of them focused on lncRNAs. Plenty of studies have found that dysregulation of lncRNA expression could influence cancer progression via various aspects [1,12]. For example, lncRNA LOWEG acts as a tumor suppressor by inhibiting cell invasion in gastric cancer [34]. Or, lncRNA NKILA regulates hepatocellular carcinoma by regulating NF-κB pathway [31]. LncRNA H19 promotes invasion of GBM cells [13]. There are also plenty of researches demonstrating the influence of lncRNAs on GBM progression. For example, lncRNA AC003092.1 was found to boost temozolomide resistance via miR-195/TFPI-2 pathway [28]. LncRNA TALNEC2 was also proved to mediate the radiation sensitivity of GBM cells [2].

Unfortunately, many lncRNAs have not been thoroughly explored in GBM. LncRNA TRG-AS1 occurred in our research is one of them, the function of which is still unknown in GBM. Through bioinformatics analysis, TRG-AS1 was positively associated with poor prognosis. We discovered that TRG-AS1 could also regulate GBM cell proliferation ability in return. The detailed mechanism of TRG-AS1 regulating GBM cell is the focus of our research.

Competitive endogenous RNA hypothesis has become quite popular in lncRNA research [14]. In ceRNA pattern, lncRNA would act as a sponge of cancer regulating miRNA and further regulate the expression of mRNA binding with miRNA. Many researches have been carried out on ceRNA mechanism. For example, lncRNA KTN1-AS1 was found to promote hepatocellular tumor growth by targeting miR-23c/ERBB2IP axis [32]. Or, CASC9 promotes LIN7A expression by targeting miR-758-3p [10]. CeRNA hypothesis has provided a brand new vision on how lncRNAs regulate cancer progression. In this research, we discovered that both TRG-AS1 and SUZ12 could bind with miR-877-5p. MiR-877-5p has been found to suppress liver injury and hepatocellular cancer as previously mentioned. TRG-AS1 inhibited miR-877-5p expression while miR-877-5p inhibited SUZ12 expression. More importantly, TRG-AS1 positively regulated the expression of SUZ12. SUZ12 mRNA can encode SUZ12 protein in humans, which has been proved to promote various cancer progressions. For example, SUZ12 promotes tumorigenesis in head and neck squamous cell carcinoma [26]. Or, SUZ12 has been found to promote gastric cell proliferation and metastasis [27]. In this

work we found that SUZ12 was also overexpressed in GBM tissues and cells. Through rescue assays, we confirmed that TRG-AS1 acted as ceRNA of SUZ12, thus affecting GBM cell proliferation.

The effect of lncRNA TRG-AS1 in cancer treatment is studied for the first time in our research. The TRG-AS1/miR-877-5p/SUZ12 axis is brand new. LncRNA TRG-AS1 might be a new target in GBM research.

Conflicts of interest

There are no conflicts of interest of any kind in this study.

Acknowledgement

We appreciate all members involved in this study.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.prp.2019.152476.

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