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LncRNA WARS2-IT1 Functions as an Oncogene and is Associated with Poor Outcomes from Glioblastoma

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1. Abstract

1.1. Purpose: Glioblastoma multiforme (GBM) is one of the most common malignant brain tumors in adults and has high mortality and relapse rates. Over the past few years, great advances have been made in the diagnosis and treatment of GBM, but unfortunately, the five-year overall survival rate of GBM patients is approximately 5.1%. Our study aimed to investigate the new mechanism of Long noncoding RNAs (lncRNAs) WARS2-IT1 regulate the malignant progression of Glioblastoma.

1.2. Methods: The expression levels, survival prognosis and biological functions of WARS2-IT1 in glioma were obtained from the online databases. Western blot and Quantitative RT-PCR experiments were used to detect the quality of proteins and RNA after after silencing or overexpressing WARS2-IT1 in glioma cells. Edu and migration assays were used to detect malignant progression of glioma cells after si-WARS2-IT1.

1.3. Results: Our results showed that WARS2-IT1 is a novel oncogenic lncRNA, and the expression of WARS2-IT1 was positively correlated with glioma malignancy and poor prognosis. Downregulating WARS2-IT1 with siRNA significantly suppressed glioblastoma cell proliferation and migration. In glioblastoma cells, WARS2-IT1 can regulate the Hippo signaling pathway and interact with miR-107.

1.4. Conclusion: Our study showed a novel mechanism through which WARS2-IT1 regulates glioblastoma cell malignant progression via the Hippo signaling pathway and the WARS2-IT1/miR-107/CDK6 axis.; thus, WARS2-IT1 may be a potential target for the treatment of glioblastoma.

2. Introduction

Glioblastoma (GBM) is the most malignant primary brain tumor and has an extremely poor prognosis [1]. GBM is known for its inherent ability to acquire resistance to all available therapies; so, despite the continuous updates and developments in clinical techniques, the prognosis of GBM patients has not improved significantly [2, 3]. Thus, exploring new mechanisms underlying the development of GBM and finding novel treatments are urgently needed.

Long noncoding RNAs (lncRNAs) are a family of transcripts with more than 200 nucleotides that lack protein-coding ability and are extensively associated with a variety of cancer-related biological activities. Recent advances in genome sequencing suggest that lncRNA molecules are extensively transcribed and may play an important role in regulating cell function [4-6]. In terms

of transcription, dysregulation of lncRNA expression and the lncRNA-microRNA and lncRNA-protein interactions indicate the importance of lncRNA in cells [7]. It has been proposed that lncRNAs are involved in cell growth, apoptosis, stem cell self-renewal/differentiation and mRNA stability in biological processes [8-10]. LncRNA WARS2-intronic transcript 1 (WARS2-IT1) is significantly associated with hepatocellular carcinoma patient prognoses, and higher expression levels of WARS2-IT1 were related to better recurrence-free survival (RFS) rates [11, 12]. However, there has been no report on WARS2-IT1 in glioma, and whether WARS2-IT1 is involved in the progression of glioma needs to be explored.

The Hippo signalling pathway is composed of a group of conserved kinases that regulate tissue and organ growth and is involved in many biological processes, such as cell proliferation, differentiation, migration and apoptosis, by regulating the effector proteins Yes-associated protein (YAP) and transcriptional coactivator with a PDZ-binding motif (TAZ) [13-16]. YAP1 is an important downstream protein of the Hippo signaling pathway that regulates the nuclear translocation of YAP1(14). YAP1 has been reported to be an oncoprotein and promotes tumor growth, especially in glioma [17]. Bioinformatics analysis found that lncRNA WARS2-IT1-targeted genes are primarily enriched in the Hippo signalling pathway. These findings suggest that WARS2-IT1 may have important association with the Hippo signalling pathway. In the present study, we demonstrated that lncRNA WARS2-IT1 regulated the proliferation and migration of GBM cells via the Hippo signalling pathway and target miR-107. The oncogenic function of WARS2-IT1 indicates that it may be a potential treatment target for GBM in the future.

3. Materials and Methods

3.1. Online Databases

The RNA sequence data for glioma and glioma patient data were downloaded from TCGA datasets (https://portal.gdc.cancer.gov/); GEPIA website (http://gepia.cancer-pku.cn/); LncBook website (https://bigd.big.ac.cn/lncbook/index); miRcode website (http://www.mircode.org/); starBase website (http://starbase.sysu.edu.cn/index.php).

3.2. Cell Culture and Transfection

Human GBM cell lines (U118, LN229, LN18, U308, SNB19, U87 and U251) were purchased from the Chinese Academy of Sciences Cell Bank. The cell lines were cultured in Dulbecco's modified Eagle's medium (DMEM) (Gibco, Waltham, MA, USA) supplemented with 10% fetal bovine serum (Gibco) and incubated at 37°C with 5% CO2. When the cells reached 60-70% confluency in dishes, siRNA was transfected using Lipofectamine 3000 (Invitrogen, Waltham, MA, USA) according to the manufacturer's instructions. After 48-72 h of transfection, we extracted RNA for the next experiment. The WARS2-IT1 siRNA target sequences in this study were as follows:

si-WARS2-IT1-1 target sequence: GAAGAAGAUAGGAAGAUAAdTdT

si-WARS2-IT1-2 target sequence: GACUAAUACAGUAAGUUGAdTdT

si-WARS2-IT1-3 target sequence: GAGCAAAGGUGACUUGUUAdTdT

si-NC target sequence: UUCUCCGAACGUGUCACGUdTdT.

The siRNA kit was designed and purchased from HANBIO (Shanghai, China).

3.3. RNA Isolation and Quantitative RT-PCR Assays

Total RNA was isolated from cells using Trizol reagent (Invitrogen, Waltham, MA, USA) following the manufacturer's protocol. RNA was reverse transcribed into cDNA using a reverse transcription system (Promega, Madison, USA). LncRNA was measured on an ABI QuantStudio 3 using GoTaq qRT-PCR master mix (Promega, Madison, USA) according to the manufacturer's protocol. GAPDH was used as an internal control. The primers for GAPDH, WARS2-IT1, YAP1, CDK6 and miR-107 were designed and purchased from RIBOBIO (Guangzhou, China).

3.4. Cell Proliferation Assays

U87 and SNB19 cells were seeded onto glass inserts in a 12-well plate at a density of 3x104 cells per well. After 12 hours of incubation, the cells were transfected with si-WARS2-IT1 for 48-72 hours, with si-NC as a control. Then, the cells were stained with 5-ethynyl-2-deoxyuridine (Edu) (RIBOBIO, Guangzhou, China) according to the instructions of the EdU cell proliferation detection kit. Cell proliferation was analyzed by inverted fluorescence microscopy (Olympus, Japan).

3.5. Cell Migration Assays

Glioblastoma cells were pre-treated with si-WARS2-IT1 or si-NC for 24 h, and the cells were resuspended in free-serum DMEM (25×105 cells per ml). Next, 3-5x105 cells were seeded in transwell filters (8-μm pore size, Corning Costar, USA). Then, 500 μl of 10% FBS DMEM was added to the lower chambers. After incubation for 24 h, the migrating cells were counted under an ortho-microscope (Olympus, Japan) after fixation with paraformaldehyde and staining with crystal violet. Experiments were performed in triplicate wells, and each experiment was performed in triplicate.

3.6. Western Blot

After cells were transfected for 72 hours, cell samples were lysed in RIPA buffer supplemented with protease inhibitor. A BCA protein assay kit was used to evaluate the protein samples. Proteins were separated on 10% SDS-PAGE gels and electrophoretically transferred to PVDF membranes (Millipore, USA). After incubation with antibodies against YAP1 (1:1000 dilution, Cell Signaling Technology, 12395), E-cadherin (1:1000 dilution, Cell Signaling

Technology, 3195), N-cadherin (1:1000 dilution, Cell Signaling Technology, 13116), MMP-9 (1:1000 dilution, Cell Signaling Technology, 13667), MMP-2 (1:1000 dilution, abcam, ab97779), CDK6(1:1000, abcam , ab124821) and GAPDH (1:10000 dilution, abcam, EPR16891), we used G:BOX (Syngene Company, UK) to detect the proteins.

3.7. Statistical Analysis

All data are expressed as the mean ± S.D. Student's t-test and ANOVA were used to compare the experimental groups. Statistical analysis was performed with GraphPad Prism 8 software, and $p<0.05$ was considered to be statistically significant.

4. Results

4.1. The Expression Level of WARS2-IT1 Was Correlated with The Pathological Grade and Prognosis of Glioma Patients

We selected The Cancer Genome Atlas (TCGA) data sets, which included 5 non-tumor brain and 666 primary glioma samples, to investigate the expression level of WARS2-IT1 in glioma. Of the 666 glioma samples, 155 were GBM (WHO IV), 511 were low grade glioma (LGG), 261 were WHO III and 250 were WHO II. WARS2-IT1 expression was significantly higher in GBM tissues than in LGG tissues (Figure 1A). Then, we used Kaplan-Meier survival curves to analyze the association between WARS2-IT1 and overall survival (OS) in the TCGA data sets. We found that glioma patients expressing WARS2-IT1 levels higher than the median were associated with decreased survival compared with those with WARS2-IT1 levels lower than the median (Figure 1B). These findings demonstrated that WARS2-IT1 expression was positively correlated with glioma malignancy and negatively correlated with OS in glioma patients. We investigated WARS2-IT1 expression levels in several GBM cell lines (A172, U308, U118, LN18, U87, U251, LN229, and SNB19) using qRT-PCR (Figure 1C). We used the GEPIA website to analyze the expression of WARS2-IT1 in various types of tumors. We found that the expression of WARS2-IT1 was significantly different in different tumor types, with abnormal expression in both high-grade and low-grade gliomas (Figure 1D).

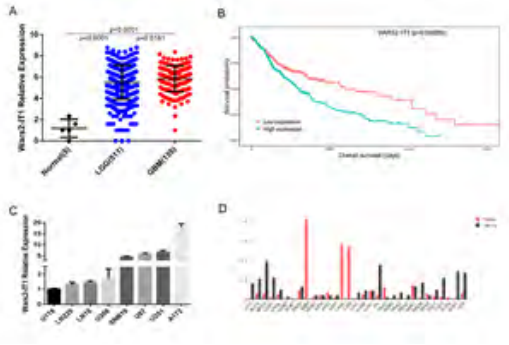

Figure 1: The WARS2-IT1 expression in TCGA database and WARS2-IT1 indicates poor prognosis in glioma patients. (A) The expression levels of WARS2-IT1 were analyzed in non-tumor brain and different grades glioma in TCGA database. (B) The relationship between expression of WARS2-IT1 and survival of glioma patients was analyzed via Kaplan-Meier survival cure. (C) The relative expression of WARS2-IT1 in eight GBM cell lines measured by qRT-PCR. (D) The expression levels of WARS2-IT1 were analyzed in variety of tumor.

4.2. Downregulation of WARS2-IT1 Inhibits the Proliferation and Migration Of GBM cells

Based on the above results, we selected U87 and SNB19 cells as representative cell lines for investigation. Then, we downregulated WARS2-IT1 expression with siRNAs to determine its function in cell proliferation and migration. A qRT-PCR assay was performed, and the results showed that si-3 targeting WARS2-IT1 had the highest knockdown efficiency (Figure 2A). Therefore, we selected si-3 for subsequent analyses. We next used an EdU assay to detect cell proliferation. Compared with U87 and SNB19 cells in the control group, cells with downregulated WARS2-IT1 showed reduced growth (Figure 2B). Next, we used a transwell assay to investigate the cell migration ability after si-WARS2-IT1 transfection in U87 and SNB19 cells. The migration ability of si-WARS2-IT1 cells was significantly lower than that of the control cells (Figure 2C). We detected the expression of E-cadherin, N-cadherin, matrix metalloproteinase (MMP)-9 and matrix metalloproteinase (MMP)-2, which are closely related to tumor metastasis capacity, by western blotting (Figure 2D). These results showed that WARS2-IT1 plays a primary role in GBM cell proliferation and migration.

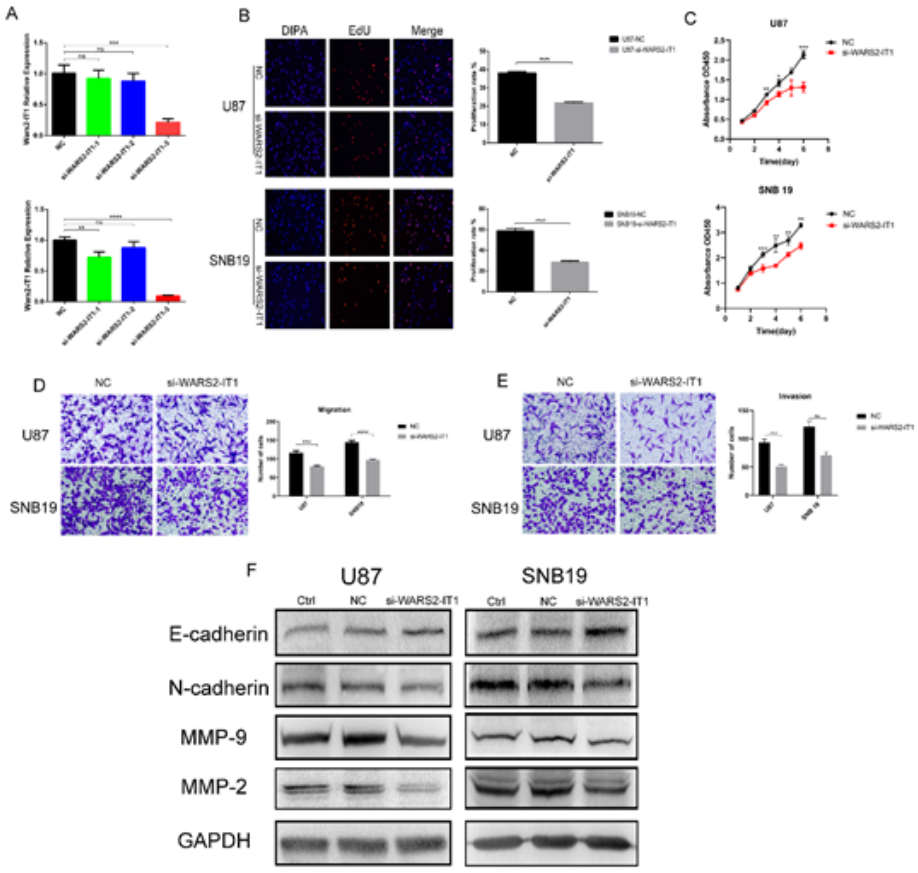

Figure 2: Downregulated expression of WARS2-IT1 suppress proliferation and migration of GBM cells. (A) qRT-PCR was used to analyzed the effect of U87 and SNB19 cell lines transfected with three WARS2-IT1 siRNAs. (B,C) EdU cells proliferation assay and Transwell assay were used to detect cells growth ability and migration ability after infecting si-WARS2-IT1. (D) Western blot analyzed for tumor growth and metastasis molecular markers in GBM cells infected si-WARS2-IT1. Significant results were presented as NS non-significant, *P < 0.05, **P < 0.01, or ***P < 0.001.

4.3. WARS2-IT1-Associated Genes are Enriched Mainly in The Hippo Signaling Pathway

To investigate the potential mechanisms of WARS2-IT1 in GBM cells, a correlation analysis was performed using the LncBook and miRcode websites. The LncBook website predicted 498 potential microRNAs that interact with WARS2-IT1, and the miRcode website predicted 79 potential microRNAs (Supplementary Table.1). The top 8 microRNAs are shown in (Table 1 and Table 2). We obtained consensus microRNAs from the results predicted by the two websites and hypothesized that these microRNAs were most likely to interact directly with WARS2-IT1. Five microRNAs were further evaluated, including miR-216b-5p, miR-107, miR-34-5p, miR-449c-5p and miR-24-3p (Figure 3A).

Then, we used the starBase website to predict the potential target mRNA of the five microRNAs. The results showed that there were 2502 target genes of miR-216b-5p, 4288 target genes of miR-107, 2108 target genes of miR-34-5p, 2437 target genes of miR-449c-5p, and 3018 target genes of miR-24-5p (Supplementary Table. 2). In (Table 3), we show the top 8 mRNAs. We found that 167 mRNAs are shared by these five microRNAs (Figure 3B and Table 4), and the 167 mRNAs were WARS2-IT1-correlated genes. Next, we used 167 mRNAs for GO and pathway analysis. We found that WARS2-IT1-associated genes were associated mainly with the Hippo signaling pathway (Figure 3C). The biological processes identified were involved mainly in the positive regulation of transcription (Figure 3D), while the cellular component chart further showed that the genes were located mainly in the nucleoplasm, cytoplasm and extrinsic component of the cytoplasmic side of the plasma membrane (Figure 3E). Therefore, these results proved that WARS2-IT1 may regulate the malignant progression of GBM cells via the Hippo signaling pathway.

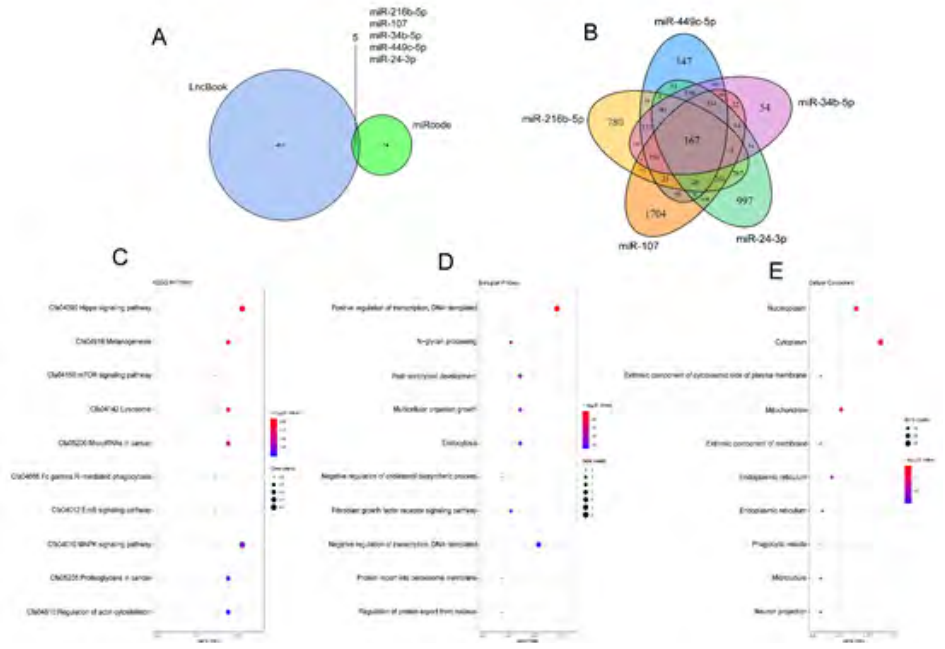

Figure 3: The WAES2-IT1-associated genes are mainly enriched in Hippo signaling pathway. (A) The microRNAs that potential interact with WARS2-IT1 in LncBook website and miRcode website. (B) The common genes among miR-216b-5p, miR-107, miR-34-5p,miR-449c-5p and miR-24-3p. (C) KEGG pathway analyzed was build by WARS2-IT1 related genes. (D,E) Gene ontology analysis of WARS2-IT1-associated genes according to biological process, cellular component.

Supplementary Table 1: WARS2-IT1 potential target microRNAs

LncBook website					
miR-4666a-3p	miR-4660	miR-4659a-3p	miR-4659b-3p	miR-4654	miR-4769-5p
miR-4536-5p	miR-4535	miR-4529-3p	miR-4512	miR-4502	miR-4480
miR-4477a	miR-6876-5p	miR-4474-5p	miR-4474-3p	miR-4469	miR-4460
miR-4447	miR-4472	miR-4448	miR-4437	miR-4436b-5p	miR-4436b-3p
miR-4632-5p	miR-6735-5p	miR-6879-5p	miR-7843-5p	miR-4434	miR-4422
miR-4329	miR-4326	miR-4324	miR-4314	miR-4310	miR-7157-5p
miR-4303	miR-4299	miR-4292	miR-6791-5p	miR-4290	miR-4286
miR-4287	miR-4685-3p	miR-4284	miR-4283	miR-4277	miR-4275
miR-4272	miR-4264	miR-4261	miR-4260	miR-4257	miR-4254
miR-4252	miR-3978	miR-3941	miR-3938	miR-3936	miR-3928-5p
miR-3927-5p	miR-4653-5p	miR-3915	miR-3912-5p	miR-3910	miR-3692-3p
miR-3691-3p	miR-3689d	miR-3686	miR-3680-3p	miR-3675-3p	miR-3672
miR-6864-3p	miR-3671	miR-3662	miR-3653-5p	miR-3617-3p	miR-3611
miR-3607-3p	miR-3605-5p	miR-3529-3p	miR-3200-5p	miR-3194-3p	miR-3192-5p
miR-3191-3p	miR-3185	miR-3184-3p	miR-3182	miR-3175	miR-3168
miR-3165	miR-3159	miR-3158-5p	miR-3158-3p	miR-3157-5p	miR-3156-5p
miR-3156-3p	miR-3153	miR-6733-5p	miR-6739-5p	miR-3151-5p	miR-3150a-3p
miR-3145-5p	miR-3143	miR-3135b	miR-3130-5p	miR-4482-5p	miR-3123
miR-3121-5p	miR-3119	miR-3065-3p	miR-3064-5p	miR-2682-3p	miR-6781-3p
miR-2681-3p	miR-2467-3p	miR-2392	miR-2355-5p	miR-2355-3p	miR-2276-3p
miR-2115-5p	miR-2115-3p	miR-2114-5p	miR-2110	miR-1976	miR-1914-5p
miR-1909-3p	miR-6722-3p	miR-1468-3p	miR-1343-3p	miR-1323	miR-1322
miR-1299	miR-1296-3p	miR-1295b-5p	miR-1912	miR-1273g-3p	miR-1270
miR-1264	miR-1255b-2-3p	miR-1254	miR-3116	miR-1252-3p	miR-1251-3p
miR-1250-3p	miR-1245b-5p	miR-1238-3p	miR-1237-3p	miR-1236-3p	miR-1233-5p

miR-6778-5p	miR-1233-3p	miR-1231	miR-1226-3p	miR-1225-5p	miR-1207-5p
miR-4763-3p	miR-1207-3p	miR-1205	miR-1199-5p	miR-6751-3p	miR-1197
miR-1193	miR-1184	miR-942-5p	miR-942-3p	miR-6893-5p	miR-939-3p
miR-892a	miR-877-3p	miR-770-5p	miR-766-5p	miR-766-3p	miR-671-5p
miR-670-3p	miR-661	miR-660-3p	miR-658	miR-655-5p	miR-651-3p
miR-647	miR-646	miR-645	miR-644a	miR-642b-5p	miR-642a-5p
miR-641	miR-3617-5p	miR-638	miR-6774-5p	miR-629-3p	miR-627-3p
miR-624-5p	miR-622	miR-619-5p	miR-6506-5p	miR-617	miR-612
miR-1285-3p	miR-3187-5p	miR-5189-5p	miR-6860	miR-607	miR-597-3p
miR-593-3p	miR-587	miR-584-3p	miR-583	miR-580-5p	miR-561-3p
miR-552-3p	miR-551b-5p	miR-548q	miR-548p	miR-548g-3p	miR-548e-5p
miR-548az-5p	miR-548t-5p	miR-548a-3p	miR-548ar-3p	miR-548az-3p	miR-548e-3p
miR-548f-3p	miR-548ay-3p	miR-548at-5p	miR-548aa	miR-548ap-3p	miR-548t-3p
miR-548ao-3p	miR-548an	miR-548aj-5p	miR-548f-5p	miR-548g-5p	miR-548x-5p
miR-3609	miR-545-3p	miR-544a	miR-539-5p	miR-539-3p	miR-532-5p
miR-532-3p	miR-526b-5p	miR-522-3p	miR-520g-3p	miR-520f-5p	miR-519d-5p
miR-518c-5p	miR-516b-5p	miR-515-5p	miR-513c-5p	miR-514b-5p	miR-513a-5p
miR-513a-3p	miR-513c-3p	miR-3606-3p	miR-512-5p	miR-510-5p	miR-508-5p
miR-505-3p.2	miR-502-5p	miR-500a-3p	miR-497-3p	miR-494-3p	miR-493-5p
miR-493-3p	miR-492	miR-491-5p	miR-491-3p	miR-488-5p	miR-486-3p
miR-484	miR-483-3p.2	miR-455-3p.1	miR-450b-3p	miR-769-3p	miR-450a-2-3p
miR-449b-3p	miR-433-3p	miR-423-5p	miR-383-5p.2	miR-382-5p	miR-382-3p
miR-381-3p	miR-371b-3p	miR-361-5p	miR-342-3p	miR-335-3p	miR-331-3p
miR-330-3p	miR-329-3p	miR-362-3p	miR-324-3p	miR-1913	miR-302c-5p
miR-302b-5p	miR-302d-5p	miR-301a-5p	miR-301b-5p	miR-299-3p	miR-224-5p
miR-216b-3p	miR-216b-5p	miR-216a-5p	miR-210-5p	miR-205-3p	miR-202-3p
miR-200c-5p	miR-550a-3p	miR-194-3p	miR-188-3p	miR-186-3p	miR-185-3p
miR-153-5p	miR-153-3p	miR-149-5p	miR-149-3p	miR-4728-5p	miR-6785-5p
miR-6883-5p	miR-6874-3p	miR-147a	miR-144-3p	miR-141-5p	miR-134-3p
miR-133a-3p.2	miR-133b	miR-132-3p	miR-212-3p	miR-128-3p	miR-124-3p.1
miR-105-5p	miR-103b	miR-107	miR-96-3p	miR-34c-3p	miR-34b-5p
miR-449c-5p	miR-33b-3p	miR-515-3p	miR-519e-3p	miR-31-5p	miR-6788-5p
miR-30b-3p	miR-1273h-5p	miR-6779-5p	miR-6780a-5p	miR-29b-2-5p	miR-25-5p
miR-24-3p	miR-22-3p	miR-21-3p	miR-3591-3p	miR-18a-3p	miR-17-5p
miR-93-5p	miR-17-3p	miR-16-1-3p	miR-497-5p	miR-9-3p	let-7a-2-3p
miR-8075	miR-8063	miR-8056	miR-8055	miR-7855-5p	miR-7848-3p
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miR-6875-3p	miR-6871-5p	miR-6867-3p	miR-6865-3p	miR-6861-5p	miR-6857-5p
miR-6846-5p	miR-6848-5p	miR-6843-3p	miR-6841-5p	miR-6830-5p	miR-6829-5p
miR-6828-5p	miR-6825-5p	miR-6823-5p	miR-6877-3p	miR-6815-3p	miR-6810-5p
miR-6809-5p	miR-6809-3p	miR-6807-5p	miR-6804-3p	miR-6799-5p	miR-6796-3p
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miR-6719-3p	miR-6715a-3p	miR-6515-5p	miR-6514-3p	miR-6507-3p	miR-6505-3p
miR-6134	miR-6085	miR-6813-5p	miR-6079	miR-6072	miR-6891-3p
miR-6069	miR-5701	miR-5695	miR-5584-3p	miR-5582-3p	miR-5187-3p
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miR-5009-5p	miR-8058	miR-5009-3p	miR-5006-5p	miR-5003-5p	miR-5003-3p
miR-5001-3p	miR-4999-3p	miR-4803	miR-4802-5p	miR-4802-3p	miR-4797-3p
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miR-4769-3p	miR-4762-5p	miR-4758-3p	miR-4757-5p	miR-6744-3p	miR-4755-5p
miR-5006-3p	miR-4753-3p	miR-4749-3p	miR-5196-5p	miR-4743-3p	miR-4742-3p
miR-4733-3p	miR-4733-5p	miR-4728-3p	miR-4727-3p	miR-4727-5p	miR-4724-3p
miR-4719	miR-4717-3p	miR-4709-5p	miR-4704-5p	miR-4704-3p	miR-4699-5p
miR-4698	miR-4693-5p	miR-6792-3p	miR-4691-3p	miR-6858-5p	miR-4688
miR-6743-5p	miR-4687-5p	miR-4679	miR-4678	miR-4668-3p	miR-4667-3p

miRcode website					
miR-135a	miR-138	miR-140	miR-142-3p	miR-143	miR-144
miR-150	miR-153	miR-155	miR-15a	miR-190	miR-194
miR-1ab	miR-203	miR-208a	miR-216a	miR-216b	miR-24
miR-27a	miR-101	miR-103a	miR-33a-3p	miR-34a	miR-10a
miR-128	miR-499-5p	miR-135a-5p	miR-138a	miR-140-5p	miR-1721
miR-5127	miR-16	miR-190a	miR-206	miR-208a-3p	miR-216b-5p
miR-24a	miR-27a-3p	miR-101a	miR-107	miR-365	miR-34b-5p
miR-10a-5p	miR-128a	miR-876-3p	miR-4770	miR-16a	miR-613
miR-24-3p	miR-107ab	miR-365-3p	miR-449a	miR-1244	miR-195
miR-449c-5p	miR-322	miR-424	miR-497	miR-1907	miR-135b
miR-15b	miR-208b	miR-27b	miR-34c	miR-10b	miR-138b
miR-190b	miR-208b-3p	miR-24b	miR-101b	miR-34c-5p	miR-128b
miR-16b	miR-449b	miR-15c	miR-27c	miR-10c	miR-16c
miR-449c					

Table 1: The top 8 microRNAs in LncBook website that interact with WARS2-IT1

MiRNA ID	Score	Energy	Binding Start	Binding End	Experimental Evidence
hsa-miR-1323	142	-11.66	580	601	no
hsa-miR-1296-3p	155	-19.52	1450	1472	no
hsa-miR-5582-3p	142	-9.96	604	626	no
hsa-miR-1299	145	-22.32	1047	1068	no
hsa-miR-3915	146	-13.11	1933	1955	no
hsa-miR-1322	160	-13.34	138	156	no
hsa-miR-5187-3p	149	-16.09	503	523	no
hsa-miR-519d-5p	156	-14.92	1425	1449	no

Table 2: The top 8 microRNAs in miRcode website that interact with WARS2-IT1

microRNA Family	Seed Position	Seed Type	Repeat	Conservation		
				Primates	Mammals	Other vert
miR-135ab	chr1:119605680	7-mer-m8	yes	44%	0%	0%
miR-138	chr1:119607315	7-mer-m8	yes	11%	0%	0%
miR-140	chr1:119606187	7-mer-m8	yes	33%	0%	0%
miR-142-3p	chr1:119606493	7-mer-m8	yes	22%	0%	0%
miR-143	chr1:119605746	7-mer-m8	yes	33%	0%	0%
miR-144	chr1:119605517	7-mer-A1	yes	33%	0%	0%
miR-150	chr1:119606867	7-mer-A1	yes	22%	0%	0%
miR-153	chr1:119606066	7-mer-A1	yes	22%	0%	0%

Table 3: The top 8 mRNAs in starBase website that interact with the five microRNAs

hsa-miR-449c-5p	hsa-miR-216b-5p	hsa-miR-107	hsa-miR-34b-5p	hsa-miR-24-3p
GDI1	CCNL2	VAMP7	DDX3Y	DDX3Y
C1orf159	CHD5	RNF223	C1orf159	RNF223
ACAP3	ICMT	UBE2J2	FAAP20	CCNL2
NADK	NOL9	ACAP3	PLEKHG5	CDK11A
FAAP20	RERE	DVL1	ERRFI1	NPHP4
PLEKHG5	LZIC	MXRA8	RERE	PLEKHG5
NOL9	MTHFR	SSU72	CTNNBIP1	NOL9
ERRFI1	FBXO42	NADK	FBXO42	DNAJC11

Table 4: The 167 common genes among the five microRNAs target genes based on starBase website

The Common Genes					
STK40	EFCAB14	ADAR	RC3H1	STX6	PLEKHA6
TEAD1	CRY2	ATG13	NAA40	RNF169	ARCN1
MAPK1IP1L	UBR1	ARPP19	CSNK1G1	RAB8B	CEMIP
METTL16	DHX33	SSH2	ATXN7L3	ZNF652	TRIM25
ATL2	XPO1	MGAT4A	MAP3K2	FZD5	PER2
CRKL	ZNRF3	NF2	XPNPEP3	MAP4	CDC25A
TMEM161B	SH3PXD2B	SNX18	PCBD2	SLC26A2	NDST1
CREB3L2	KMT2C	SUN1	CLDN12	UBN2	CTSB
RPS6KA3	AMMECR1	FAM122B	MECP2	CDK16	CLCN5
ERLIN1	HELLS	WBP1L	PLEKHA1	ARHGAP1	SELENON
MLEC	KATNAL1	VPS36	TMED8	BCL2L2	SLC11A2
PAPD5	ADGRG1	HAS3	NFAT5	CALB2	IQGAP1
KDSR	DSEL	MBP	ZNF100	ATAD2B	PNPO
MAPK1	GUCD1	RBFOX2	TOB2	PEX26	GPD2
DVL3	NAA15	SPCS3	PDE4D	MTX3	ZMAT3
ALDH5A1	RPL7L1	QKI	C6orf120	PURB	CNOT6
MED22	SNAPC3	PTPDC1	SNX30	ZNF618	ERLIN2
AGO1	POU2F1	QSOX1	RRP15	IBA57	PIP4K2A
RNF41	ANKRD52	GNS	NT5DC3	GOLGA3	SRGAP1
USP31	SHCBP1	CMTM4	PHLPP2	AP1G1	TMEM170A
RPS6KB1	DCAF7	PRKCA	SOX9	CBX2	SMAD2
PMEPA1	STK35	KIF3B	YWHAB	RAB22A	RTL10
DCUN1D1	EDEM1	SETD5	ZNF621	RBM15B	MME
GFOD1	ATXN1	C6orf106	XPO5	REV3L	FRK
KIF1B	KDM5A	ZNF592	HELZ	BCL2L11	USF 3
SLC36A1	JRK	SLC6A14	DNAJC16	M6PR	AKAP13
TAOK1	SFT2D3	ZNF148	GALNT10	C8orf58	TACC1
ZBTB10	CHRAC1	PTAR1	TRIM14	ANKS6	

Supplementary Table 2: Starbase predicts miRNA target genes

miR-34b-5p									
DDX3Y	C1orf159	FAAP20	PLEKHG5	ERRFI1	RERE	CTNNBIP1	FBXO42	RCC2	MUL1
HSPG2	LUZP1	E2F2	IFNLR1	SLC9A1	WASF2	EYA3	MECR	PUM1	PTP4A2
MARCKSL1	SYNC	RNF19B	TRIM62	SMIM12	STK40	SNIP1	RRAGC	HEYL	MED8
CCDC163	PIK3R3	LRRC41	MOB3C	ATPAF1	EFCAB14	FAF1	EPS15	NDC1	CYB5RL
AL357673.1	MYSM1	SLC35D1	PIGK	AC118549.1	EVI5	GCLM	FRRS1	SLC25A24	CLCC1
SORT1	WDR77	TRIM33	SIKE1	IGSF3	VTCN1	NOTCH2	PRKAB2	NBPF24	NBPF20
SV2A	APH1A	CERS2	LYSMD1	VPS72	TDRKH	GATAD2B	CRTC2	TPM3	UBE2Q1
ADAR	YY1AP1	RIT1	UBQLN4	ISG20L2	SH2D2A	SLAMF6	CD48	ARHGAP30	NECTIN4
RGS5	CREG1	SLC19A2	TNFSF4	RC3H1	KIAA0040	STX6	ARPC5	UCHL5	DENND1B

CSRP1	KDM5B	RABIF	PLEKHA6	PPP1R15B	RBBP5	SLC45A3	NUCKS1	SLC26A9	PLXNA2
PTPN14	SLC30A10	TAF1A	TP53BP2	WDR26	ITPKB	JMJD4	C1orf198	ARID4B	LYST
HEATR1	ESPN	VAMP3	PER3	H6PD	TMEM201	PIK3CD	KIF1B	PEX14	UBIAD1
AGTRAP	PDPN	DNAJC16	SPEN	ARHGEF10L	NBL1	OTUD3	CDA	NBPF3	ZBTB40
PITHD1	SELENON	ARID1A	PIGV	TRNP1	WDTC1	GPR3	KHDRBS1	KPNA6	TXLNA
HDAC1	RBBP4	S100PBP	NCDN	AGO1	CDCA8	MACF1	CAP1	ZMPSTE24	ATP6V0B
B4GALT2	OSBPL9	SCP2	PRKAA2	RAVER2	PDE4B	ST6GALNAC3	HS2ST1	LRRC8B	FNBP1L
ABCD3	TRMT13	SLC30A7	CYB561D1	ATP5PB	CAPZA1	MOV10	VANGL1	PTGFRN	MAN1A2
WDR3	NBPF8	NBPF9	AC239799.1	NOTCH2NL	LIX1L	PEX11B	RNF115	NBPF12	NBPF15
NBPF16	SETDB1	PRUNE1	PIP5K1A	TUFT1	INTS3	ZBTB7B	ATF6	UHMK1	RGS4
POGK	POU2F1	C1orf112	SUCO	RALGPS2	TOR3A	TOR1AIP1	CEP350	QSOX1	XPR1
MR1	C1orf21	NR5A2	CAMSAP2	GPR25	NAV1	BTG2	MDM4	CR1	DTL
PPP2R5A	FLVCR1	PROX1	RRP15	MARK1	C1orf115	BROX	DISP1	CNIH4	IBA57
RHOU	GALNT2	NTPCR	EDARADD	MTR	CNST	ZNF672	TUBB8	ASB13	SFMBT2
FRMD4A	FAM171A1	NEBL	SKIDA1	PIP4K2A	SVIL	MTPAP	CXCL12	43532	ERCC6
ANK3	RUFY2	VSIR	DNAJB12	MSS51	AP3M1	ANXA11	CPEB3	MYOF	NOC3L
MMS19	MORN4	ERLIN1	C10orf76	LDB1	ACTR1A	BBIP1	GFRA1	RAB11FIP2	FAM204A
ATE1	CHST15	FAM53B	ZMYND11	PFKFB3	TAF3	GATA3	ECHDC3	DHTKD1	ARL5B
GPR158	RET	NCOA4	DKK1	SIRT1	DDX50	DDX21	VPS26A	HK1	EIF4EBP2
ZSWIM8	VCL	NUTM2E	NUTM2A	PAPSS2	PTEN	IFIT3	43529	CEP55	TBC1D12
HELLS	PGAM1	PI4K2A	BTRC	NOLC1	SUFU	WBP1L	SLK	VTI1A	CASP7
NHLRC2	FAM45A	PLEKHA1	PTPRE	ZNF511	BET1L	IRF7	GATD1	PIDD1	DUSP8
IFITM10	AC068580.4	NUP98	RHOG	OR51E2	HBB	HBD	RRP8	TPP1	TRIM66
TMEM41B	MRVI1	RRAS2	SOX6	PIK3C2A	LGR4	ABTB2	ARHGAP1	CELF1	MTCH2
MED19	MPEG1	OSBP	DDB1	CYB561A3	AHNAK	B3GAT3	PLA2G16	CDC42BPG	CDCA5
SYVN1	EIF1AD	SPTBN2	KMT5B	CPT1A	SHANK2	ARAP1	PGM2L1	XRRA1	THAP12
PAK1	MTMR2	CASP5	EXPH5	RDX	SIK3	PCSK7	BACE1	MPZL3	BCL9L
DPAGT1	NECTIN1	ETS1	ARHGAP32	NCAPD3	RASSF7	TMEM80	BRSK2	STIM1	SYT9
EIF3F	TUB	SWAP70	PARVA	TEAD1	TMEM86A	EIF3M	PRRG4	KIAA1549L	TRIM44
PRR5L	API5	CRY2	MAPK8IP1	MDK	ATG13	MADD	FAM111B	TMEM109	TKFC
SDHAF2	AP003108.2	DAGLA	INCENP	POLR2G	NAA40	DNAJC4	ESRRA	RPS6KA4	DPF2
PCNX3	PACS1	KDM2A	RAD9A	PPP6R3	ARHGEF17	RELT	PPME1	POLD3	RNF169
GVQW3	ACER3	AQP11	PCF11	ANKRD42	ARHGAP42	AASDHPPT	ELMOD1	CUL5	ATM
DDX10	ZC3H12C	FDX1	SIK2	DLAT	TMEM25	ARCN1	PHLDB1	HMBS	CBL
SORL1	SPA17	FOXRED1	JAM3	KDM5A	RAD52	TNFRSF1A	SCNN1A	VAMP1	ZNF384
LPCAT3	M6PR	LRP6	SOX5	BHLHE41	CAPRIN2	SINHCAF	GXYLT1	YAF2	VDR
SENP1	ZNF641	KANSL2	CCNT1	ADCY6	PRKAG1	CERS5	SLC11A2	CSRNP2	KRT85
KRT5	CBX5	SARNP	AC023055.1	RNF41	ANKRD52	CS	DCTN2	CDK4	CTDSP2
GNS	KRR1	OSBPL8	PPP1R12A	LIN7A	EEA1	TMCC3	NT5DC3	SLC41A2	ALDH1L2
NUAK1	PRDM4	GIT2	PPP1CC	WSB2	GCN1	TRIAP1	STX2	POLE	GOLGA3
B4GALNT3	ERC1	WNT5B	TULP3	TSPAN9	CCND2	FOXJ2	STRAP	MED21	STK38L
FAR2	DNM1L	IRAK4	ARID2	SMARCD1	DIP2B	METTL7A	TNS2	SP1	TARBP2
HOXC13	HOXC11	RAB5B	MYL6	COQ10A	NXPH4	SHMT2	43533	USP15	SRGAP1
TBC1D30	RAP1B	MDM2	ATXN7L3B	GLIPR1	SYT1	C12orf29	TMTC3	SOCS2	APAF1
FICD	UBE3B	SH2B3	ERP29	TRAFD1	OAS2	PLBD2	RNFT2	RFC5	SUDS3
MLEC	TMEM120B	BCL7A	TMED2	ATP6V0A2	ZNF664	AC068790.8	ULK1	EP400	SACS
MTMR6	SHISA2	UBL3	KATNAL1	ZC3H13	LCP1	VPS36	PCDH9	DIS3	KLF12
KCTD12	MYCBP2	EDNRB	POU4F1	RBM26	DZIP1	DOCK9	ARGLU1	ANKRD10	MPHOSPH8
SPATA13	NUP58	PAN3	COG3	TRIM13	CKAP2	UBAC2	TM9SF2	TEP1	HNRNPC

RAB2B	RBM23	SLC7A8	ZFHX2	IPO4	CHMP4A	STRN3	EGLN3	SNX6	NFKBIA
TRAPPC6B	SOS2	SAV1	ERO1A	TMEM30B	SGPP1	VTI1B	ZFYVE26	SYNJ2BP	MAP3K9
NUMB	ELMSAN1	ENTPD5	ALDH6A1	RPS6KL1	NEK9	ANGEL1	IRF2BPL	POMT2	TMED8
SPTLC2	TC2N	TRIP11	PRIMA1	SERPINA1	ATP5MPL	IGHA1	ABHD4	MRPL52	BCL2L2
NGDN	THTPA	NFATC4	G2E3	KLHDC2	GNG2	SAMD4A	MAPK1IP1L	KTN1	NAA30
DAAM1	PPM1A	FNTB	EIF2S1	PLEKHH1	RDH12	SLC39A9	TTC9	PCNX1	YLPM1
GPATCH2L	CPSF2	OTUB2	PAPOLA	DYNC1H1	RCOR1	TNFAIP2	ZBTB42	PACS2	NIPA2
TJP1	DPH6	BMF	EHD4	VPS39	TMEM87A	CDAN1	UBR1	ZSCAN29	SLC30A4
FBN1	ARPP19	PRTG	NEDD4	MNS1	ICE2	RORA	CSNK1G1	RBPMS2	SPG21
IGDCC4	DPP8	LARP6	GRAMD2A	SCAMP2	FAM219B	PEAK1	TBC1D2B	ACSBG1	HOMER2
HAPLN3	PLIN1	MESP1	TARSL2	KLF13	FAM98B	THBS1	CHP1	RTF1	TUBGCP4
MAP1A	SERF2	CASC4	CTDSPL2	TMOD2	TCF12	RAB8B	USP3	SNX1	ZNF609
PIAS1	FEM1B	GLCE	PAQR5	THSD4	NEO1	CD276	CSK	SCAMP5	C15orf39
SNX33	FBXO22	HMG20A	ARNT2	CEMIP	ZNF592	PDE8A	AKAP13	IQGAP1	CRTC3
BLM	MAN2A2	ST8SIA2	ALDH1A3	PGP	ZNF200	CDIP1	ZNF500	USP7	EMP2
LITAF	ZC3H7A	PARN	XYLT1	KNOP1	GPR139	DCUN1D3	USP31	GGA2	BOLA2
BOLA2-SMG1P6	ZNF785	SHCBP1	C16orf87	NETO2	SNX20	AKTIP	AMFR	FAM192A	CSNK2A2
SLC38A7	CMTM4	RANBP10	SLC7A6OS	MTSS1L	VAC14	PHLPP2	AP1G1	TXNL4B	GLG1
TMEM170A	ADAT1	CBFA2T3	ANKRD11	DBNDD1	FAM234A	RAB11FIP3	PIGQ	CRAMP1	AL031708.1
JPT2	TBC1D24	NAA60	NUDT16L1	ALG1	PMM2	RMI2	SNX29	SHISA9	ERCC4
ABCC1	TNRC6A	SPN	ZNF48	CTF1	PAPD5	MMP2	OGFOD1	CCL22	ADGRG1
EDC4	PLA2G15	HAS3	VPS4A	NFAT5	WWP2	DDX19B	CALB2	ATMIN	KIAA0513
JPH3	SPATA33	SPIRE2	GAS8	VPS53	ABR	CRK	SLC43A2	PRPF8	METTL16
CLUH	TRPV1	SHPK	AC027796.3	CXCL16	ZNF232	DHX33	NEURL4	VAMP2	AC135178.1
NCOR1	ZNF624	TOM1L2	AKAP10	LGALS9B	C17orf51	FAM222B	FLOT2	NUFIP2	SSH2
MYO1D	RAD51D	ACACA	SYNRG	SRCIN1	PCGF2	CWC25	STAT5B	RETREG3	PLEKHH3
VAT1	DUSP3	ATXN7L3	GJC1	EFTUD2	KIF18B	PLCD3	MRPL10	CBX1	HOXB3
HOXB8	ZNF652	ANKRD40	SPAG9	MMD	TRIM25	SRSF1	SUPT4H1	43712	BRIP1
MED13	HELZ	SDK2	JPT1	GRB2	CASKIN2	ACOX1	SOCS3	CYTH1	TBC1D16
NPTX1	FAAP100	NPLOC4	MAFG	CENPX	TIMM22	SGSM2	RAP1GAP2	KIF1C	ZFP3
MIS12	ACADVL	SENP3	CYB5D1	PFAS	NTN1	SHISA6	HS3ST3B1	ADORA2B	KSR1
AC015688.5	TMEM97	TMEM199	SUPT6H	TAOK1	GOSR1	ATAD5	NF1	ZNF207	DHRS11
TADA2A	LASP1	CDK12	ERBB2	CASC3	WIPF2	IGFBP4	ATP6V0A1	NAGLU	MLX
CNTNAP1	NBR1	DBF4B	ADAM11	NMT1	ACBD4	MAPT	PNPO	NFE2L1	CALCOCO2
HLF	PCTP	PRR11	YPEL2	DHX40	RPS6KB1	TBX2	DCAF7	MAP3K3	PRKCA
PITPNC1	PRKAR1A	KCNJ2	SOX9	TTYH2	SLC9A3R1	LLGL2	SMIM5	METTL23	AC005837.2
SEC14L1	BIRC5	C1QTNF1	CBX2	PIEZO2	FAM210A	ESCO1	CDH2	ZNF24	SYT4
EPG5	PIAS2	SMAD2	SMAD7	MBD2	CCDC68	TCF4	LMAN1	KDSR	DSEL
ZNF516	MBP	USP14	MTCL1	ANKRD12	RAB31	VAPA	LDLRAD4	HRH4	C18orf25
RNF165	MAPK4	SMAD4	C18orf54	NEDD4L	MALT1	SERPINB5	SERPINB2	TIMM21	MIER2
UQCR11	TCF3	ATP8B3	SGTA	PIP5K1C	PRR22	SLC25A23	ELAVL1	ZNF561	ZNF562
OLFM2	CDC37	YIPF2	AC008758.1	ZNF709	ZNF490	AC010422.6	PALM3	PRKACA	DNAJB1
BRD4	EPS15L1	SMIM7	JAK3	BORCS8	TM6SF2	ZNF100	ZNF599	DMKN	HSPB6
ZNF850	ZNF585B	SARS2	AC011455.2	DYRK1B	PRX	C19orf54	CYP2A7	ERF	CNFN
LYPD3	IRGQ	PPP1R13L	ERCC1	SIX5	AP2S1	MAMSTR	TMEM86B	ZNF784	ZNF772
ZNF154	ZNF418	FSTL3	MUM1	MPND	HDGFL2	FEM1A	KDM4B	ZNF358	EVI5L
RAB11B	ZNF317	NFIX	ADGRE5	DDA1	SLC27A1	FCHO1	RPL18A	MAST3	KLHL26
GATAD2A	YJEFN3	GPATCH1	KIAA0355	WTIP	LRFN3	CAPNS1	FAM98C	PLEKHG2	ZNF546
CYP2B6	HNRNPUL1	CCDC97	MEGF8	ZNF284	NECTIN2	BLOC1S3	ARHGAP35	SPHK2	FUT2

BAX	RPL13A	FCGRT	RCN3	PRMT1	ZNF480	MYADM	NLRP2	RPL28	ZNF580
ZNF530	ZNF776	ZNF587	ZSCAN22	PXDN	ADI1	NOL10	WDR35	SDC1	ATAD2B
KIF3C	PREB	GTF3C2	STRN	EIF2AK2	CDC42EP3	ATL2	SOS1	COX7A2L	PREPL
XPO1	GFPT1	PAIP2B	POLR1A	ADRA2B	TMEM127	LMAN2L	MGAT4A	AFF3	LONRF2
TGFBRAP1	IL1B	CLASP1	ERCC3	MAP3K2	IWS1	CCDC115	ZRANB3	ZEB2	NR4A2
ITGB6	COBLL1	STK39	TLK1	SP3	LNPK	TTN	CWC22	TFPI	STAT1
CAVIN2	PGAP1	FAM126B	ALS2	KLF7	METTL21A	FZD5	IDH1	LANCL1	ERBB4
IKZF2	TNS1	EPHA4	TRIP12	SLC16A14	USP40	RAB17	PER2	KIF1A	ASAP2
GRHL1	KLF11	HPCAL1	LPIN1	TRIB2	MYCN	KLHL29	FKBP1B	RAB10	SLC35F6
TMEM214	SNX17	MRPL33	BABAM2	CLIP4	SPAST	SLC30A6	RASGRP3	CEBPZOS	PKDCC
EPAS1	SOCS5	EPCAM	PAPOLG	REL	ANTXR1	DYSF	TET3	MTHFD2	MAT2A
PTCD3	MRPL35	THNSL2	PROM2	CNNM3	INPP4A	C2orf15	MRPL30	RPL31	MAP4K4
RANBP2	BCL2L11	FBLN7	ZC3H6	IL1RN	PTPN4	EPB41L5	RALB	SFT2D3	UGGT1
PTPN18	UBXN4	GPD2	PPIG	UBR3	SSFA2	DUSP19	ZC3H15	FAM171B	BMPR2
ADAM23	CREB1	PIKFYVE	RPL37A	CTDSP1	CNOT9	RETREG2	DNAJB2	RHBDD1	COPS7B
EIF4E2	TRAF3IP1	ASB1	FKBP1A	NSFL1C	CENPB	JAG1	CST4	CST1	CST2
NOL4L	PXMP4	NCOA6	RBM12	SOGA1	FITM2	SULF2	ADNP	BMP7	PMEPA1
CABLES2	DIDO1	YTHDF1	GMEB2	ZBTB46	ZNF512B	ZCCHC3	SIRPA	STK35	AP5S1
MAVS	PANK2	SMOX	PRND	MCM8	SYNDIG1	ENTPD6	PYGB	KIF3B	ASXL1
CBFA2T2	ITCH	SRC	LBP	RALGAPB	LPIN3	HNF4A	TTPAL	WISP2	YWHAB
PABPC1L	STK4	SYS1	SLC12A5	SLC9A8	PARD6B	RAB22A	NPEPL1	FAM217B	TPD52L2
DNAJC5	ADAMTS5	C21orf59	PAXBP1	C2CD2	CBS	CBSL	ICOSLG	SUMO3	PTTG1IP
LSS	USP25	HUNK	IFNAR2	AP000295.1	SON	SLC37A1	PDXK	PFKL	COL18A1
C22orf39	RTL10	MAPK1	ZNF280B	GUCD1	HPS4	TPST2	XBP1	AP1B1	LIF
RNF215	PISD	RTCB	RBFOX2	MYH9	FOXRED2	C1QTNF6	DDX17	CBX6	TOB2
DESI1	SHISAL1	RTL6	KIAA0930	ALG12	PEX26	CRKL	RAB36	GRK3	ZNRF3
NF2	SFI1	YWHAH	TIMP3	H1F0	KDELR3	APOBEC3C	SYNGR1	TAB1	MIEF1
XPNPEP3	EP300	L3MBTL2	XRCC6	SREBF2	NUP50	PPP6R2	SRGAP3	IQSEC1	XPC
TBC1D5	SATB1	SGO1	SLC4A7	OSBPL10	DYNC1LI1	CLASP2	TRANK1	EPM2AIP1	LRRFIP2
CYP8B1	ZNF445	SCAP	MAP4	CDC25A	CELSR3	QARS	RHOA	NICN1	AC104452.1
GMPPB	PCBP4	SELENOK	FAM208A	IL17RD	ADAMTS9	MAGI1	TMF1	FOXP1	EIF4E3
PDZRN3	CGGBP1	CBLB	CD47	USF3	NAA50	LSAMP	GSK3B	LRRC58	FSTL1
GOLGB1	KPNA1	HACD2	CCDC14	ZNF148	RAB43	ISY1-RAB43	TMCC1	SLCO2A1	AMOTL2
NMNAT3	TFDP2	GK5	XRN1	TM4SF1	PLCH1	SSR3	KPNA4	SPTSSB	PDCD10
GOLIM4	TNIK	PLD1	NCEH1	ZMAT3	PEX5L	DCUN1D1	ABCC5	TMEM41A	LIPH
ETV5	CPN2	LRRC15	ATP13A3	TFRC	NCBP2	PIGZ	DLG1	RUBCN	ARL8B
EDEM1	SETD5	BRPF1	ARPC4	FANCD2	VHL	IRAK2	HRH1	HDAC11	OXNAD1
NR1D2	TGFBR2	CCR4	CRTAP	FBXL2	GOLGA4	CTDSPL	ZNF621	LIMD1	SACM1L
ARIH2	TCTA	RBM5	CYB561D2	MAPKAPK3	RBM15B	APPL1	PTPRG	MITF	PPP4R2
ALCAM	BBX	NDUFB4	IFT122	NUDT16	DNAJC13	UBA5	SLC25A36	ZBTB38	ATP1B3
ARHGEF26	MME	GFM1	PPM1L	MYNN	SEC62	FNDC3B	ZNF639	ACTL6A	NDUFB5
USP13	EIF2B5	DVL3	EIF4G1	MAP3K13	ST6GAL1	CCDC50	OPA1	FBXO45	SENP5
LRCH3	HAUS3	TNIP2	STX18	GRPEL1	QDPR	SEPSECS	ARAP2	PDS5A	RBM47
APBB2	SGCB	USP46	LNX1	CLOCK	YTHDC1	GRSF1	COX18	CXCL2	SDAD1
CNOT6L	ANTXR2	WDFY3	FAM13A	GPRIN3	SLC9B2	PLA2G12A	ANXA5	ANKRD50	MFSD8
PGRMC2	SCLT1	PABPC4L	ELF2	NR3C2	SH3D19	NAF1	ACSL1	ZNF595	PCGF3
FGFRL1	RNF4	MSANTD1	EVC	TBC1D14	TRMT44	RBPJ	FAM114A1	RHOH	LIMCH1
SLC30A9	GUF1	ATP10D	DCUN1D4	PDGFRA	AMTN	MOB1B	SLC4A4	CXCL1	PARM1
STBD1	FAM47E-STBD1	SHROOM3	FRAS1	MRPS18C	HERC6	SMARCAD1	TET2	SGMS2	SEC24B

ANK2	INTU	LARP1B	NAA15	SMARCA5	EDNRA	TRIM2	RAPGEF2	PALLD	SPCS3
LPCAT1	FAM173B	CTNND2	MYO10	ZFR	ADAMTS12	PRLR	LIFR	RICTOR	EMB
PDE4D	SGTB	FAM169A	F2RL2	ARSB	MTX3	SSBP2	TMEM167A	EDIL3	TMEM161B
ARRDC3	FAM172A	STARD4	NREP	REEP5	MCC	ATG12	RAPGEF6	AC008695.1	AFF4
FSTL4	SAR1B	FAM13B	ETF1	DNAJC18	TMEM173	DIAPH1	SPARC	ATOX1	CCNJL
PANK3	SH3PXD2B	RNF44	PDLIM7	DOK3	MRNIP	TBC1D9B	MGAT1	CEP72	CCT5
43530	C5orf22	NPR3	OSMR	PTGER4	SNX18	GPX8	IPO11	ERBIN	RAD17
MARVELD2	SERF1B	BDP1	MCCC2	TNPO1	IQGAP2	MSH3	LNPEP	PRRC1	RAD50
AC116366.3	JADE2	PCBD2	TGFBI	FAM53C	WDR55	ZMAT2	PCDHB12	PCDHB13	KCTD16
GRPEL2	PPARGC1B	SLC26A2	NDST1	SYNPO	GPX3	SLC36A1	G3BP1	GALNT10	LARP1
CYFIP2	PTTG1	CCNG1	RANBP17	NEURL1B	ERGIC1	ATP6V0E1	CREBRF	ARL10	TSPAN17
ZNF346	NSD1	SQSTM1	CNOT6	LYRM4	NRN1	SSR1	BLOC1S5	GFOD1	ATXN1
NUP153	TPMT	ZNF184	TRIM26	PPP1R10	LSM2	NEU1	VPS52	TAPBP	BAK1
RPS10-NUDT3	NUDT3	C6orf106	TRERF1	C6orf226	XPO5	TRAM2	LGSN	SLC17A5	COL12A1
FAM46A	UBE3D	ME1	SYNCRIP	CGA	LYRM2	BACH2	MAP3K7	MMS22L	SEC63
SESN1	WASF1	DDO	CDK19	REV3L	FRK	TSPYL4	TSPYL1	SLC2A12	NHSL1
PLAGL1	EPM2A	SHPRH	NUP43	RAET1L	IPCEF1	DLL1	NQO2	PSMG4	DSP
BMP6	JARID2	RBM24	RNF144B	SOX4	ALDH5A1	BTN2A1	PPP1R11	PRR3	ABCF1
TUBB	MICB	BRD2	ZNF76	C6orf89	PIM1	TBC1D22B	RNF8	DAAM2	NFYA
FOXP4	MDFI	TAF8	RPL7L1	VEGFA	SLC29A1	CDC5L	RUNX2	ENPP4	ZNF451
SH3BGRL2	TPBG	NT5E	SMIM8	MANEA	MARCKS	NCOA7	MYB	PEX7	ARFGEF3
HECA	UST	TAB2	GINM1	ULBP2	ARID1B	GTF2H5	TMEM181	MAP3K4	QKI
C6orf120	TNRC18	RNF216	EIF2AK1	DAGLB	THSD7A	SNX13	RAPGEF5	IGF2BP3	CYCS
JAZF1	NOD1	CAMK2B	TMED4	PURB	IGFBP3	GRB10	RCC1L	POMZP3	HGF
ABCB1	CDK6	SLC25A13	BAIAP2L1	NAPEPLD	DNAJC2	ORC5	PNPLA8	THAP5	CTTNBP2
WASL	ZNF800	GCC1	IMPDH1	CNOT4	MTPN	CREB3L2	EZH2	ZNF746	KMT2C
SUN1	CHST12	C1GALT1	SNX10	CREB5	AVL9	EEPD1	RALA	HECW1	DBNL
YKT6	ADCY1	IGFBP1	IKZF1	VKORC1L1	POM121	PHTF2	STEAP2	CLDN12	CDK14
ANKIB1	RBM48	PPP1R9A	ARPC1A	AC004922.1	ZNF789	ZKSCAN5	GNB2	MUC3A	TRIM56
PRKRIP1	ZNF277	CAV1	MET	ING3	ARF5	AHCYL2	NRF1	CPA4	MKLN1
CALD1	UBN2	LUC7L2	C7orf55-LUC7L2	ARHGEF5	ZNF398	ZNF282	DPP6	RNF32	NOM1
UBE3C	CTSB	PSD3	TNFRSF10D	ENTPD4	STC1	TRIM35	GSR	FUT10	RNF122
NSD3	FGFR1	IMPAD1	PDE7A	NCOA2	EYA1	RPL7	STAU2	UBE2W	JPH1
HEY1	RMDN1	NBN	RBM12B	VIRMA	C8orf37	TSPYL5	RNF19A	YWHAZ	TRPS1
HAS2	ATAD2	WASHC5	FAM49B	ASAP1	NDRG1	SLC45A4	JRK	LYNX1	LYNX1-SLURP2
FAM83H	NRBP2	ZNF16	CLN8	MCPH1	ERI1	TNKS	MTMR9	FDFT1	TUSC3
INTS10	SLC39A14	C8orf58	RHOBTB2	CHMP7	SLC25A37	FP15737	DOCK5	ELP3	FZD3
HMBOX1	MAK16	ERLIN2	ASH2L	TACC1	GINS4	AP3M2	HGSNAT	ZBTB10	E2F5
PTDSS1	MTDH	VPS13B	DCAF13	EBAG9	DEPTOR	ZHX2	MYC	CHRAC1	THEM6
RHPN1	C8orf33	RFX3	GLIS3	AK3	KIAA2026	NFIB	MLLT3	KLHL9	CDKN2A
ELAVL2	MOB3B	SMU1	FAM219A	RPP25L	SIGMAR1	FAM221B	RNF38	FBXO10	PTAR1
KLF9	ZFAND5	GAS1	FAM120AOS	FBP1	TRIM14	TBC1D2	ANKS6	TMEM246	ELP1
C9orf152	ALAD	POLE3	PSMD5	PHF19	RAB14	RC3H2	STRBP	NR6A1	FAM129B
FAM102A	CIZ1	ZER1	SPOUT1	SH3GLB2	TOR1A	SURF6	MED22	SURF1	SURF4
INPP5E	NOTCH1	SAPCD2	TPRN	PNPLA7	CDC37L1	UHRF2	SNAPC3	DENND4C	ACER2
IL11RA	AL162231.3	TESK1	RGP1	FAM122A	PCSK5	VPS13A	WNK2	PTPDC1	TGFBR1
PALM2	PALM2-AKAP2	AKAP2	KIAA1958	SNX30	ZNF618	COL27A1	PAPPA	DAB2IP	RABGAP1
OLFML2A	GAPVD1	LMX1B	ZBTB34	SLC2A8	URM1	TBC1D13	PHYHD1	MIGA2	NCS1
LAMC3	AIF1L	NUP214	NTNG2	GTF3C4	CACFD1	SSNA1	NELFB	CTPS2	MAP3K15

RPS6KA3	ELK1	SMC1A	HSD17B10	PHF8	FAM120C	WNK3	FGD1	ARHGEF9	AMER1
ZC4H2	SNX12	ZMYM3	HDAC8	RLIM	AL035425.2	AMMECR1	TENM1	APLN	GPC4
FAM122B	MOSPD1	MAP7D3	IDS	HCFC1	MECP2	FAM3A	WWC3	TMSB4X	MOSPD2
CA5B	SYAP1	TXLNG	REPS2	ZFX	UBA1	CDK16	RBM3	SUV39H1	CCDC120
CLCN5	APEX2	MSN	AR	YIPF6	FAM155B	UPRT	DRP2	SLC6A14	ATP1B4
XIAP	OCRL	PHF6	ZNF449	SLC9A6	MTMR1	EMD	PLXNA3		

4.4. WARS2-IT1 Positively Regulates Oncogenic YAP1 Expression in GBM Cells

The Hippo signaling pathway is an evolutionarily conserved signaling pathway that controls organ size and regulates cell proliferation, apoptosis and carcinogenesis [13]. The Hippo signaling pathway regulates the nuclear translocation of YAP1, which is a core downstream effector and regulates the transcriptional program of many genes (such as CTGF, CYR61 and AXL) to promote cell proliferation and migration [18-20]. Based on the above results, we hypothesized that WARS2-IT1 is involved in the biological processes of glioma cells by regulating the expression of YAP1. To validate our hypothesis, first we analyzed the correlation between WARS2-IT1 and YAP1 in glioma tissues based on TCGA database, the result shown that the expression of WRAS2-IT1 was positive correlation with YAP1(Figure 4A). Then we tested changes in YAP1 by western blotting and qRT-PCR after knockdown of WARS2-IT1 in U87 and SNB19 cells. As shown in (Figure 4B and Figure 4C), the YAP1 mRNA and protein levels were significantly reduced. After over-expression of WARS2-IT1, the YAP1 mRNA and protein levels were significantly increased (Figure 4D-F). Together, these results suggested that WARS2-IT1 can participate in the Hippo signaling pathway through mediating the expression of YAP1.

4.5. WARS2-IT1 Positively Regulated CDK6 Expression Via miR-107

LncRNAs can also regulate the expression of target genes by serving as competing endogenous RNAs (ceRNAs) in cells [6, 21, 22]. Based on the the LncBook and miRcode databases, miR-107 was predicted to interact with LncRNA WARS2-IT1(Figure 3A). Then we analyzed the correlation between them in TCGA database. The result shown that WARS2-IT1 was negatively correlated with miR-107 (Figure 5A). Furthermore, the dual luciferase reporter assays revealed that miR-107 directly bound to the lnc-WARS2-IT1 regions (Figure 5B,C). Then, we used qRT-PCR to detect the miR-107 expression after si/oe-WARS2-IT1 in U87 and SNB19 cells. The results shown that miR-107 levels were significantly reduced after over-express WARS2-IT1, and knock-down WARS2-IT1 can up-regulate miR-107 expression (Figure 5D). CDK6 is known to be an important regulator of cell cycle progression, modulating cell cycle G1 phase progression and G1/S transition [23, 24]. Based on the starbase, we found that CDK6 is a potential target of miR-107, and had been reported miR-107 directly modulate CDK6 expression by binding miR-107 seed complementary site located in 3`UTR of CDK6 [24]. Then we used western blotting to detect the levels of CDK6 protein and mRNA after knockdown or overexpression miR-107 (Figure 5E). We speculated that there is a regulatory loop among CDK6, miR-107 and WARS2-IT1 in glioma cells. To confirm the relationship between the CDK6 and WARS2-IT1, we tested changes in CDK6 by western blotting and qRT-PCR after knockdown or over-express of WARS2-IT1 in U87 and SNB19 cells (Figures 5F and 5H). Then, we analyzed the correlation between the WARS2-IT1 and CDK6 by using glioma samples from the TCGA database. The result shown that WARS2-IT1 was positive correlated with miR-107 (Figure 5G). Moreover, CDK6 was restored after miR-107 was inhibited in WARS2-IT1 knockdown cells (Figure 5H).

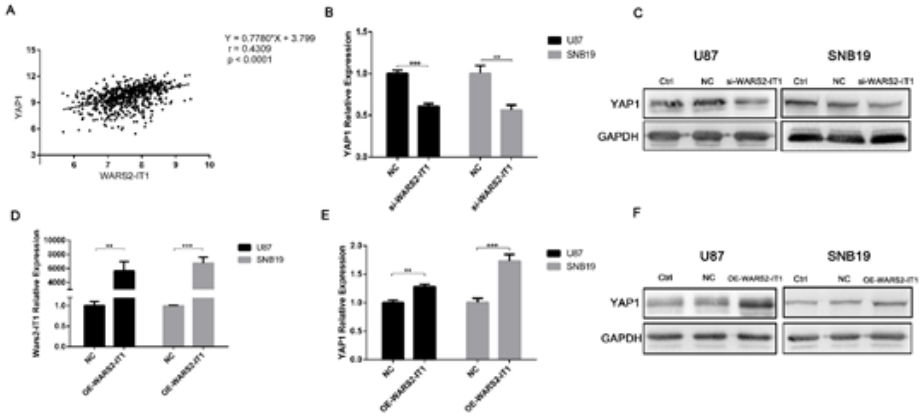

Figure 4: WARS2-IT1 regulates YAP1 expression in GBM cells. (A) Association analysis of the relationship between WARS2-IT1 and YAP1 predicted in glioma tissues based on TCGA database. (B,C) qRT-PCR assay and western blotting assay represent YAP1 mRNA and protein expression in U87 and SNB19 cells infected with si-WARS2-IT1. (D,E,F) qRT-PCR assay and western blotting assay represent YAP1 mRNA and protein expression in U87 and SNB19 cells infected with OE-WARS2-IT1. Significant results
were presented as NS non-significant, *P < 0.05, **P < 0.01, or ***P < 0.001.

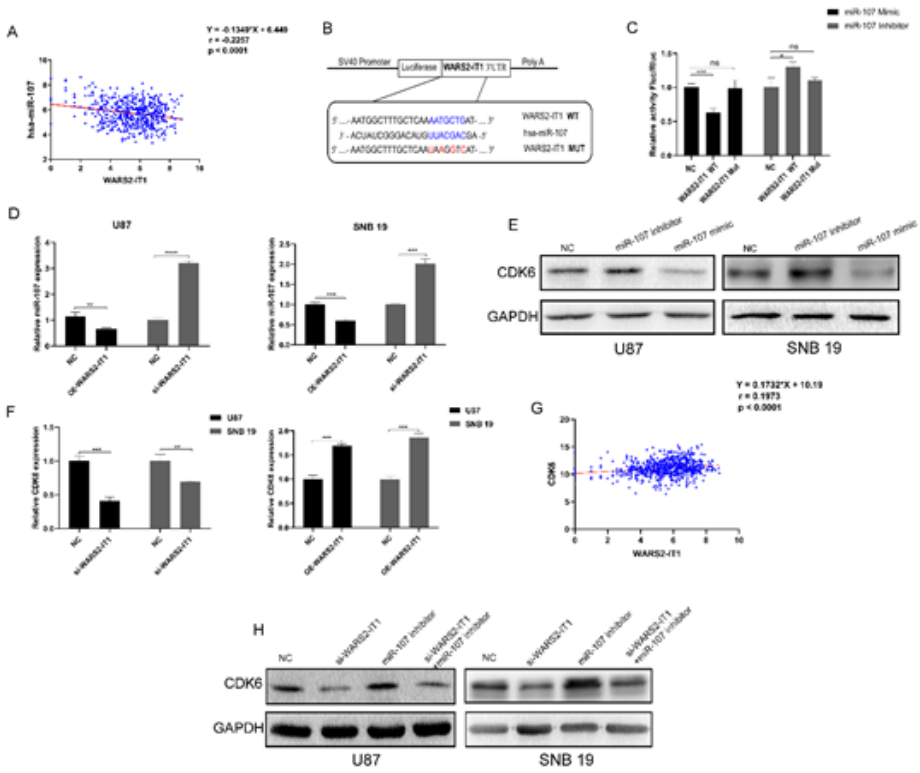

Figure 5: WARS2-IT1 positively regulated CDK6 expression via miR-107. (A)Association analysis of the relationship between WARS2-IT1 and miR-107 predicted in glioma tissues based on TCGA database.(B) Schematic outline of predicted and mutant binding sites for miR-107 on WARS2-IT1.(C) Luciferase activity of wild-type/mutant WARS2-IT1 upon transfection of control mimics/inhibitor and miR-107 in 293T cells (n =3). (D)qRT-PCR assay represent miR-107 expression in U87 and SNB19 cells infected with si-WARS2-IT1/OE-WARS2-IT1. (E) Western blotting assay represent CDK6 protein expression in U87 and SNB19 cells infected with mimics/inhibitor and miR-107. (F)qRT-PCR assay represent CDK6 mRNA expression in U87 and SNB19 cells infected with si-WARS2-IT1/OE-WARS2-IT1. (G) Association analysis of the relationship between WARS2-IT1 and CDK6 predicted in glioma tissues based on TCGA database. (H) Western blot analysis of CDK6 in U87 and SNB19 cells transfected with si-WARS2-IT1 or a miR-107 inhibitor. Significant results were presented as NS non-significant, *P < 0.05, **P < 0.01, or ***P < 0.001.

5. Discussion

Glioblastoma (GBM) is the most malignant central nervous tumor. The standard treatment for malignant brain tumors is maximum safe margin resection, adjuvant radiotherapy and temozolomide chemotherapy [25-27]. However, the overall survival of GBM patients remains poor, with an average of fewer than two years [27, 28]. This frustrating result makes GBM an urgent research subject. In recent years, with the continuous development of RNA sequencing technology, thousands of lncRNAs have been discovered [22]. As a result, lncRNA functions are constantly being discovered, especially in tumors [29]. In the present study, we first found that lncRNA WARS2-IT1 was overexpressed in glioma patient tissues and glioma cell lines. Downregulating WARS2-IT1 expression by siRNA suppressed cell growth and migration in GBM cells. Thus, these findings indicated that WARS2-IT1 participated in the regulation of GBM progression and could be a potential therapeutic target in GBM.

In hepatocellular carcinoma (HCC), lncRNA WARS2-IT1 is an independent prognostic indicator of recurrence-free survival (RFS), and higher WARS2-IT1 expression levels are related to better RFS and OS rates [11, 12]. However, there is no report that WARS2-IT1 is involved in regulating the pathological process of glioma. In our study, we found that WARS2-IT1 was overexpressed in glioma cells and glioma tissues. Using pubic data sets, we discovered that WARS2-IT1 expression was positively correlated with glioma malignancy and patient prognosis. After knocking down WARS2-IT1 by siRNA, we found that cell proliferation and migration were obviously suppressed in glioblastoma cells. These data show that WARS2-IT1 plays a vital role in glioblastoma.

The molecular mechanisms of lncRNA are complex. A number of studies have suggested that lncRNAs, miRNAs and target mRNAs can form ceRNA regulatory networks in tumors [30-32]. In the present study, we used LncBook and miRcode to predict the potential target miRNA, and we considered miRNAs that overlapped in the two databases as the most likely to interact with WARS2-IT1. Five miRNAs, miR-216b-5p, miR-107, miR-34-5p, miR-449c-5p and miR-24-3p, were shared by both databases. Then, we used starBase to predict the potential target genes of these five miRNAs, and 167 mRNAs were shared among them. The KEGG pathway analyses indicate that these WARS2-IT1-associated genes are in-

volved in the Hippo signaling pathway. In vitro analysis showed that the downregulation of WARS2-IT1 significantly reduced GBM cell proliferation and decreased the expression of YAP1, which is the core effector protein of the Hippo signaling pathway. These results show that WARS2-IT1 regulates cell proliferation and migration via the Hippo signaling pathway.

Based on LncBook and miRcode we found that WARS2-IT1 can direct interaction with miR-107, and functions as a miRNA "sponge" modulating miR-107 level in glioma cells. Furthermore, we also identified the negative correlation between WARS2-IT1 level and miR-107in glioma tissue sample and cells, which may provide an answer of how WARS2-IT1 modulate the level of miR-107 in glioma. Previous study shown that NEAT1 can modulate CDK6 through miR-107, which in turn regulates the cell cycle in human GSCs (24, 33). We found decreased CDK6 protein levels and increased miR-107 RNA levels in the WARS2-IT1 knockdown cells. These results shown that existence a regulatory loop among CDK6, miR-107 and WARS2-IT1 in glioma cells. This may explain that si-WARS2-IT1 could reduce the proliferation of glioma cells. These results show that WARS2-IT1 also regulates cell proliferation and migration via the WARS2-IT1/miR-107/CDK6 axis. In conclusion, we first demonstrated that lncRNA WARS2-IT1 was overexpressed in glioma and positively correlated with malignancy. Knocking down WARS2-IT1 impaired the proliferation and migration ability of glioma cells via the Hippo signaling pathway and the WARS2-IT1/miR-107/CDK6 axis (Figure 6). Our research suggests that WARS2-IT1 has potential applications in the treatment and prognosis of glioma.

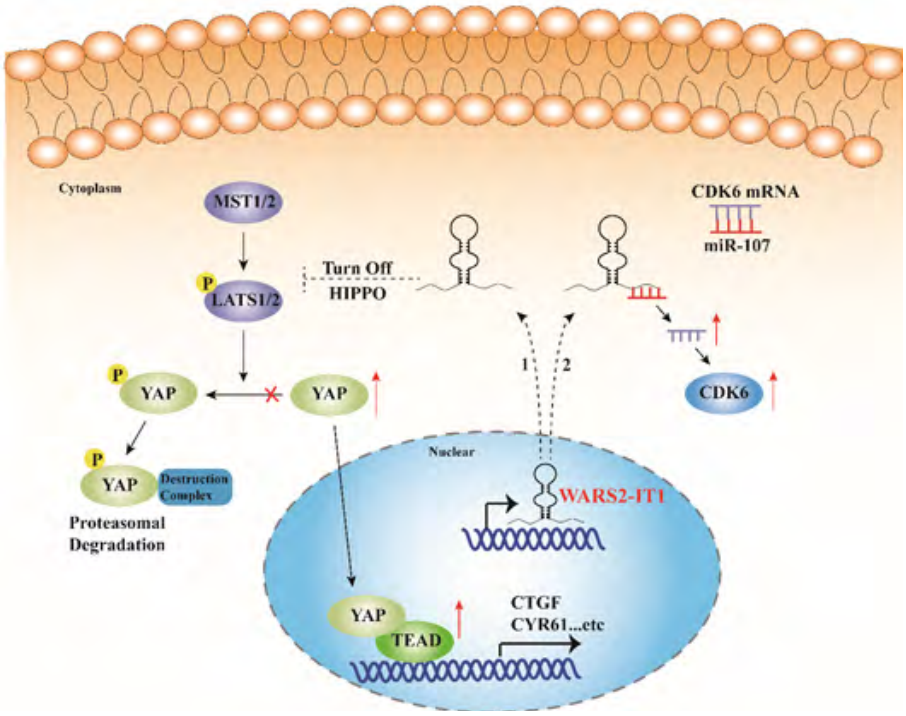

Figure 6: The mechanism diagram of WARS2-IT1.(1)WARS2-IT1 participate in the Hippo signaling pathway;(2) WARS2-IT1 functions as a ceRNA by competitively binding to miR-107 to upregulate CDK6 expression.

6. Acknowledgement

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7. Conflict of Interest

The authors declare that they have no conflict of interest.

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