European Review for Medical and Pharmacological Sciences 2020; 24: 12748-12756

Long non-coding RNA NR2F1-AS1 promoted neuroblastoma progression through miR-493-5p/TRIM2 axis

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Abstract. – OBJECTIVE: Long noncoding RNA (lncRNA) plays a vital role in the progression of various cancers. However, the potential mechanisms of NR2F1-AS1 in the tumorigenesis of neuroblastoma (NB) have not been determined.

PATIENTS AND METHODS: The expression levels of NR2F1-AS1, miR-493 and TRIM2 were detected by RT-qPCR. The downstream target genes of NR2F1-AS1 or miR-493 were predicted by bioinformatics analysis (http://starbase.sysu.edu.cn/), which was further indicated by Luciferase reporter and RNA immunoprecipitation (RIP) assays. CCK-8, transwell, and TUNEL assays were performed to determine the viability, migration, invasion and apoptosis of NB cells.

RESULTS: NR2F1-AS1 was highly expressed and miR-493 was lowly expressed in NB tissues and cell lines. The high expression of NR2F1-AS1 was associated with poor prognosis in NB. NR2F1-AS1 knockdown inhibited proliferation, migration, and invasion, and accelerated apoptosis of NB cells. MiR-493 was a downstream target of NR2F1-AS1, and the silencing of miR-493 reversed NR2F1-AS1 knockdown-attenuated progression of NB. Moreover, TRIM2 was demonstrated to be directly targeted by miR-493, and the upregulation of TRIM2 could abolish the inhibitory effect of miR-493 overexpression on the progression of NB. Finally, it was found that NR2F1-AS1 regulated TRIM2 expression by sponging miR-493.

CONCLUSIONS: The present study demonstrated that NR2F1-AS1 promoted the progression of NB through the miR-493/TRIM2 axis. This finding may provide new insight into the treatment of NB.

Key Words:
NR2F1-AS1, Neuroblastoma (NB), MiR-493, TRIM2.

Introduction

Neuroblastoma (NB) is a common extracranial tumor in children, accounting for 15% of childhood cancer deaths, threatening the life and health safety of children worldwide[1]. Neuroblastoma is characterized by rapid development, strong cell proliferation and invasion, and a high degree of malignancy[2]. Although great progress has been made in NB treatment, such as clinical radiotherapy, chemotherapy, surgical treatment and multidisciplinary treatment, the overall survival rate of NB patients is not significantly improved[3]. Therefore, it is urgent to understand the molecular mechanism of NB.

Long noncoding RNAs (lncRNAs) are a group of transcripts with a length longer than 200 nucleotides, which have no protein-coding ability[4]. They are involved in multiple biological processes of tumor development[5]. Li et al[6] indicated that lncRNA NR2F1-AS1 served as an oncogene in osteosarcoma *via* miR-483-3p/FOXA1 axis. Guo et al[7] reported that NR2F1-AS1 enhanced the migration of thyroid cancer cells by targeting miRNA-338-3p. Wang et al[8] demonstrated that NR2F1-AS1 contributed to the proliferation of endometrial cancer cells by targeting miR-363. However, the exact mechanisms of NR2F1-AS1 in NB progression have not been elucidated.

MicroRNAs (miRNAs), a class of endogenous non-coding RNA, play a regulatory role in tumorigenesis by regulating the expression of downstream genes[9-11]. Jiao et al[12] indicated that miR-493 inhibited the development of oral cavity cancer

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by targeting HMGA2. Wang et al[13] illustrated that miR-493 acted as a tumor suppressor to inhibit the proliferation of liver cancer cells *via* targeting VAMP2. Similarly, Zhao et al[14] indicated that miR-493 promoted apoptosis and inhibited proliferation in hepatocellular carcinoma cells by targeting GP73. In this study, we identified TRIM2 as the downstream target gene of miR-493. However, the expression and biological role of miR-493 and TRIM2, especially the function of the combined axis in NB, have not been reported.

In this research, we found that NR2F1-AS1 functioned as a miR-493 sponge to promote NB development by increasing TRIM2 expression. This study might provide a promising treatment target for NB patients.

Patients and Methods

Clinical Specimens and Cell Culture

32 pairs of NB tissues and adjacent normal tissues were obtained from the Children's Hospital of Soochow University. NB cells (SK-N-SH, SK-N-AS, NB-1643, and NB-1691) and human umbilical vein endothelial cells (HUVEC) were obtained from American Type Culture Collection (ATCC; Manassas, VA, USA). Cells were cultured in RPMI-1640 medium (Thermo Fisher Scientific, Rockford, IL, USA) containing 10% fetal bovine serum (Gibco; Thermo Fisher Scientific, USA) and 5% CO_2. This investigation was approved by the Ethics Committee of the Children's Hospital of Soochow University.

Cell Transfection

Short hairpin RNAs (shRNAs) targeting NR2F1-AS1 with non-targeting shRNAs as a negative control (sh-NC), miR-493 mimics with and miR-493 inhibitor with their negative controls and TRIM2 overexpression vectors (pcDNA3.1/TRIM2) with its negative control (pcDNA3.1) were purchased from Genechem (Shanghai, China). The transfection was performed using Lipofectamine 2000 reagent (Invitrogen, Carlsbad, CA, USA).

RT-qPCR

Total RNA was extracted from tissues and cells using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). Reverse Transcription Kit (TaKaRa, Dalian, China) was used to reverse the RNAs to cDNA. RT-qPCR was performed using the SYBR-Green PCR Master Mix kit (Takara, Dalian, China). The relative expression level of genes was calculated by the $2^{-\Delta\Delta Ct}$ method. GAPDH and U6 were used as endogenous controls.

Transwell Assay

For invasion assay, free-medium contained cells were added into the upper chamber coated with Matrigel, and 600 ul Dulbecco's Modified Eagle's Medium (DMEM; Thermo Fisher Scientific, Waltham, MA, USA) contained 10% FBS (Gibco; Thermo Fisher Scientific, USA) was added into the bottom chamber. After 24 h, cells in the upper chamber were removed, and cells in the lower membrane were fixed in 4% paraformaldehyde and dyed with 0.1% crystal violet (Beyotime, Jiangsu, China). For migration assay, the insert membranes were coated without Matrigel but were cultured under the same conditions. Invaded and migrated cells were counted under a microscope (ZEISS, Jena, Germany).

Luciferase Reporter Assay

Wild-type and mutant fragments of NR2F1-AS1 (or TRIM2) were subcloned into the pGL3 vectors (GenePharma, Shanghai, China) to establish NR2F1-AS1-WT (or TRIM2-WT) or NR2F1-AS1- Mut (or TRIM2-Mut) plasmid. Subsequently, miR-493 mimics or NC mimics were transfected into 293T cells that were transfected with wild-type or mutant fragments. The Luciferase activity was measured by a Dual-Luciferase reporter assay system (Promega, Madison, WI, USA).

Cell Counting Kit-8 (CCK-8)

Cells (1×10^4 cells/well) were seeded into the 96-well plates. After being incubated for 0 h, 24 h, 48 h, or 72 h, CCK-8 solution (10 µL; Dojindo, Laboratories, Kumamoto, Japan) was added and incubated for 4 h. The absorbance at 450 nm was detected using a microplate reader (Thermo Fisher Scientific, Inc. Waltham, MA, USA).

RNA Immunoprecipitation (RIP)

SK-N-SH and SK-SY5Y cells were lysed using a RIP lysis buffer and incubated with magnetic beads conjugated with the Ago2 antibody (Anti-Ago2, Abcam) or IgG antibody (Anti-IgG, Abcam). Subsequently, RT-qPCR was used to measured NR2F1-AS1, miR-493, or TRIM2 expression enriched on beads.

TUNEL Assay

TUNEL Apoptosis Kit (Roche, Mannheim, Germany) was employed to assess cell apop-

tosis. After dehydrating by ethanol, SK-N-SH and SK-SY5Y cells were dyed and cultured with TUNEL reaction mixture (Roche, Basel, Switzerland). The nuclear staining was performed with DAPI. A microscope (Olympus, Tokyo, Japan) was utilized to observe TUNEL-positive cells.

Statistical Analysis

SPSS 16.0 (SPSS Inc., Chicago, IL, USA) and GraphPad Prism (La Jolla, CA, USA) were used for data analysis in this study. All experiments were conducted at least three times and the results were expressed as mean ± SD. Kaplan-Meier methods was used to assess the overall survival rate. The comparisons between two groups were performed using a Student's *t*-test and comparisons among multiple groups were analyzed using one-way ANOVA. $p < 0.05$ was considered statistically significant.

Results

NR2F1-AS1 and MiR-493 Were Dysregulated in NB Tissues and Cells

RT-qPCR showed that NR2F1-AS1 was highly expressed in NB tissues compared with that in normal tissues (Figure 1A). Similarly, NR2F1-AS1 was significantly upregulated in NB cells (SK-N-SH, SK-N-AS, NB-1643, and NB-1691) compared with that in HUVEC (Figure 1B). Kaplan-Meier assay showed that patients with high NR2F1-AS1 expression had a shorter overall survival time than those patients with low NR2F1-AS1 expression (Figure 1C). By contrast, miR-493 expression was remarkably decreased in NB tissues and cells (Figure 1D and E). The prognosis of NB patients with a low level of miR-493 was worse than that of patients with a high level of miR-493 (Figure 1F). Moreover, miR-493 expression was inversely

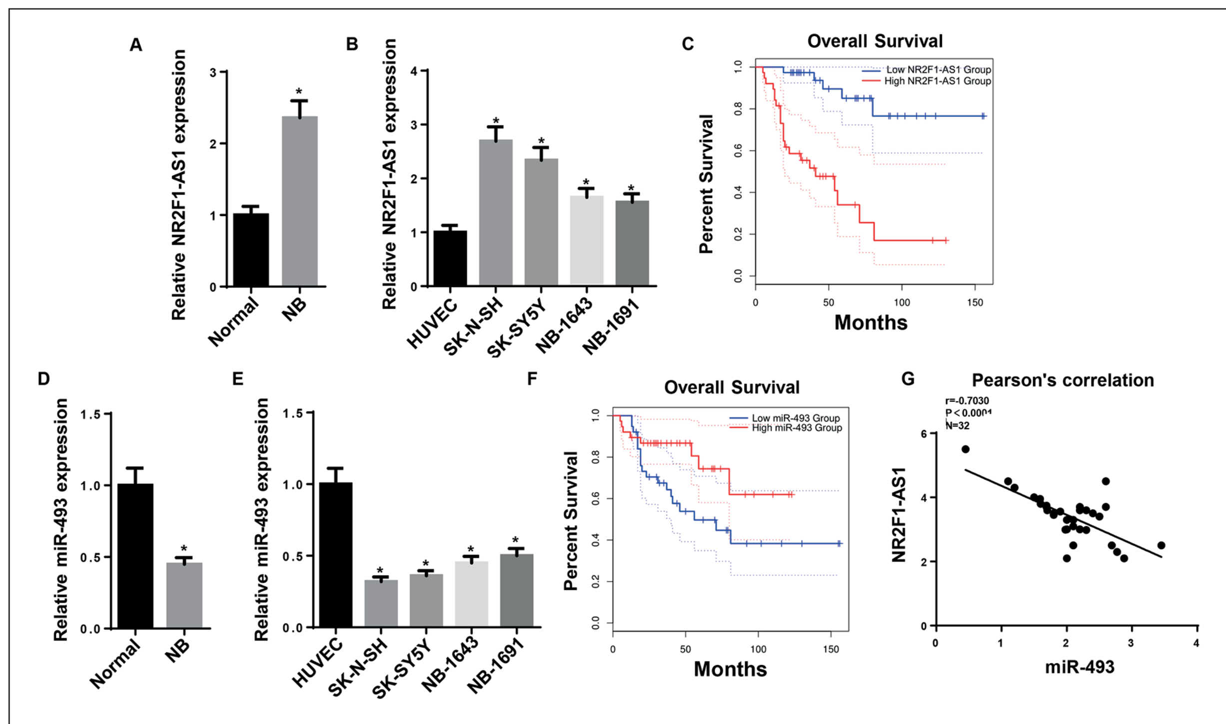

Figure 1. NR2F1-AS1 and miR-493 were dysregulated in NB tissues and cells. **A,** RT-qPCR showed the relative expression of NR2F1-AS1 in neuroblastoma tissues (n=32) and adjacent normal tissues (n=32). **B,** RT-qPCR showed the relative expression of NR2F1-AS1 in NB cells (SK-N-SH, SK-N-AS, NB-1643 and NB-1691) and HUVEC. **C,** Kaplan-Meier survival analysis shows the correlation between NR2F1-AS1 expression and prognosis of NB patients. **D,** RT-qPCR showed the relative expression of miR-493 in neuroblastoma tissues (n=32) and adjacent normal tissues (n=32). **E,** RT-qPCR showed the relative expression of miR-493 in NB cells (SK-N-SH, SK-N-AS, NB-1643 and NB-1691) and HUVEC. **F,** Kaplan-Meier survival analysis shows the correlation between miR-493 expression and prognosis of NB patients (**G**) NR2F1-AS1 expression was negatively correlated with miR-493 in NB tissues. *$p < 0.05$.

correlated with NR2F1-AS1 expression in NB tissues (Figure 1G). The results suggested that NR2F1-AS1 and miR-493 might be involved in the development of NB.

NR2F1-AS1 Knockdown Inhibited Tumorigenesis of NB

To explore the biological role of NR2F1-AS1 in NB, SK-N-SH, and SK-SY5Y cells were transfected with sh-NC, sh-NR2F1-AS1#1 or sh-NR2F1-AS1#2. RT-qPCR results showed that NR2F1-AS1 expression was significantly downregulated in SK-N-SH and SK-SY5Y cells transfected with sh-NR2F1-AS1#1 and sh-NR2F1-AS1#2 (Figure 2A). Moreover, knockdown of NR2F1-AS1 suppressed the proliferation, migration, and invasion, and promoted apoptosis of NB cells (Figure 2B-E). These results suggested that NR2F1-AS1 played an oncogenic role in the pathogenesis of NB.

Silencing of MiR-493 Reversed NR2F1-AS1 Knockdown-Attenuated Progression of NB

Through StarBase (http://starbase.sysu.edu.cn/), miR-493 was predicted as a potential downstream target of NR2F1-AS1 (Figure 3A). Luciferase reporter assay indicated that miR-493 mimics weakened the Luciferase activity of NR2F1-AS1-WT, but no significant change was observed in the NR2F1-AS1-Mut group (Figure 3B). RIP assay showed that NR2F1-AS1 and miR-493 were markedly enriched in Anti-Ago2 (Figure 3C). Moreover, RT-qPCR showed that miR-493 expression was enhanced by NR2F1-AS1 knockdown in SK-N-SH cells (Figure 3D). Furthermore, NR2F1-AS1 knockdown suppressed proliferation, migration, and invasion of SK-N-SH cells, which was partially counteracted by the silencing of miR-493 (Figure 3E and G).

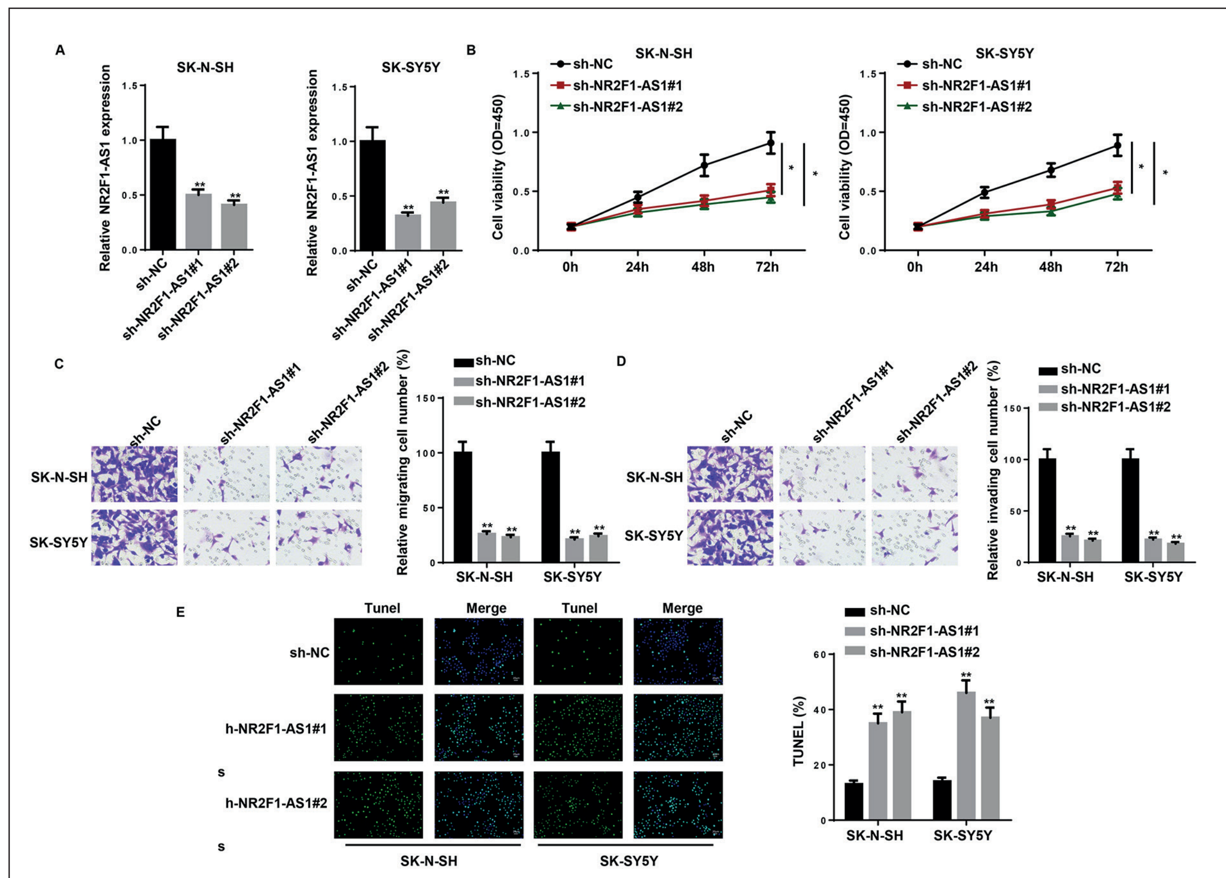

Figure 2. NR2F1-AS1 knockdown inhibited tumorigenesis of NB. **A,** RT-qPCR showed the relative expression of NR2F1-AS1 in SK-N-SH and SK-SY5Y cells transfected with sh-NC or sh-NR2F1-AS1. **B-D,** CCK-8 and transwell assays showed the proliferation, migration and invasion of SK-N-SH and SK-SY5Y cells transfected with sh-NC or sh-NR2F1-AS1 (magnification, ×100). **E,** TUNEL assays showed the apoptosis of SK-N-SH and SK-SY5Y cells transfected with sh-NC or sh-NR2F1-AS1 (magnification, ×40). *$p < 0.05$.

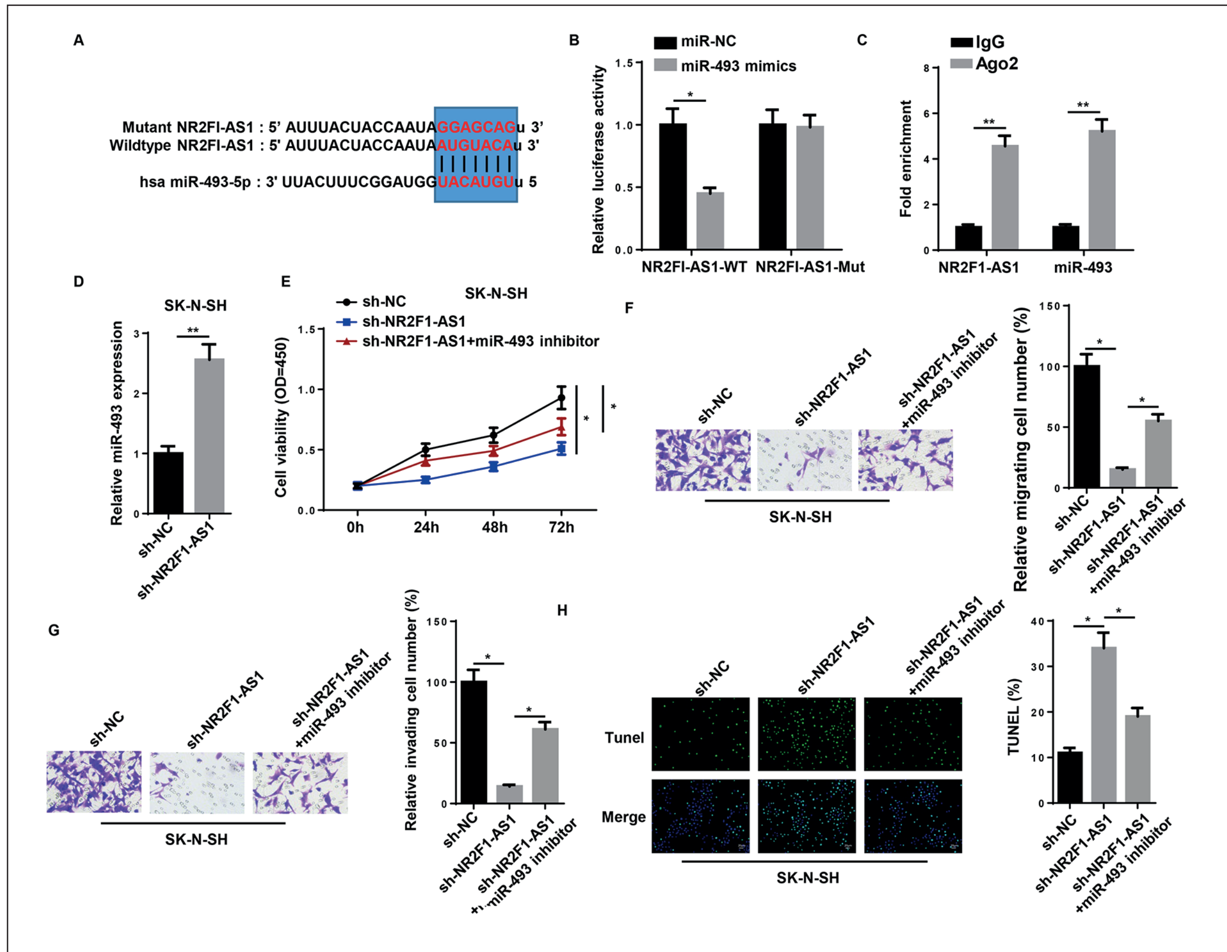

Figure 3. Silencing of miR-493 reversed NR2F1-AS1 knockdown-attenuated progression of NB. **A,** The binding sites and mutant binding sites between NR2F1-AS1 and miR-493 were presented. **B,** and **C,** Luciferase reporter and RIP assays were utilized to detect the interaction between NR2F1-AS1 with miR-493 in SK-N-SH cells. **D,** RT-qPCR showed the relative expression of miR-493 in SK-N-SH cells transfected with sh-NC or sh-NR2F1-AS1. **E-G,** CCK-8 and transwell assays showed the proliferation, migration and invasion of SK-N-SH cells transfected with sh-NC, sh-NR2F1-AS1 and sh-NR2F1-AS1+miR-493 inhibitor (magnification,×100). **H,** TUNEL assays showed the apoptosis of SK-N-SH cells transfected with sh-NC, sh-NR2F1-AS1 and sh-NR2F1-AS1+miR-493 inhibitor (magnification, ×40). *$p < 0.05$.

Besides, TUNEL assay revealed that the inhibition of miR-493 reversed the stimulative effects of NR2F1-AS1 depletion on apoptosis of NB cells (Figure 3H). Taken together, our data indicated that NR2F1-AS1 served as a molecular sponge for miR-493 to promote the tumorigenesis of NB.

TRIM2 Served as a Direct Target of MiR-493

To investigate the mechanism of miR-493 in NB, we searched the downstream target of miR-493. As shown in Figure 4A, StarBase predicted the complementary binding sites between tripartite motif-containing protein 2 (TRIM2) and miR-493, suggesting that TRIM2 might be a target of miR-493 (Figure 4A). Luciferase reporter assay indicated that miR-493 mimics significantly decreased the Luciferase activity of the TRIM2-WT group in 293T cells, while the NR2F1-AS1-Mut group showed no distinct change (Figure 4B). Meanwhile, RIP assay showed that the enrichment of NR2F1-AS1 and miR-493 in SK-N-SH cells was increased in anti-Ago2 group (Figure 4C). As shown in Figure 4D, we found that TRIM2 was enhanced by miR-439 inhibitor. Moreover, there was a negative correlation between miR-493 and TRIM2 (Figure 4E). In summary, these data revealed that TRIM2 was a target of miR-493.

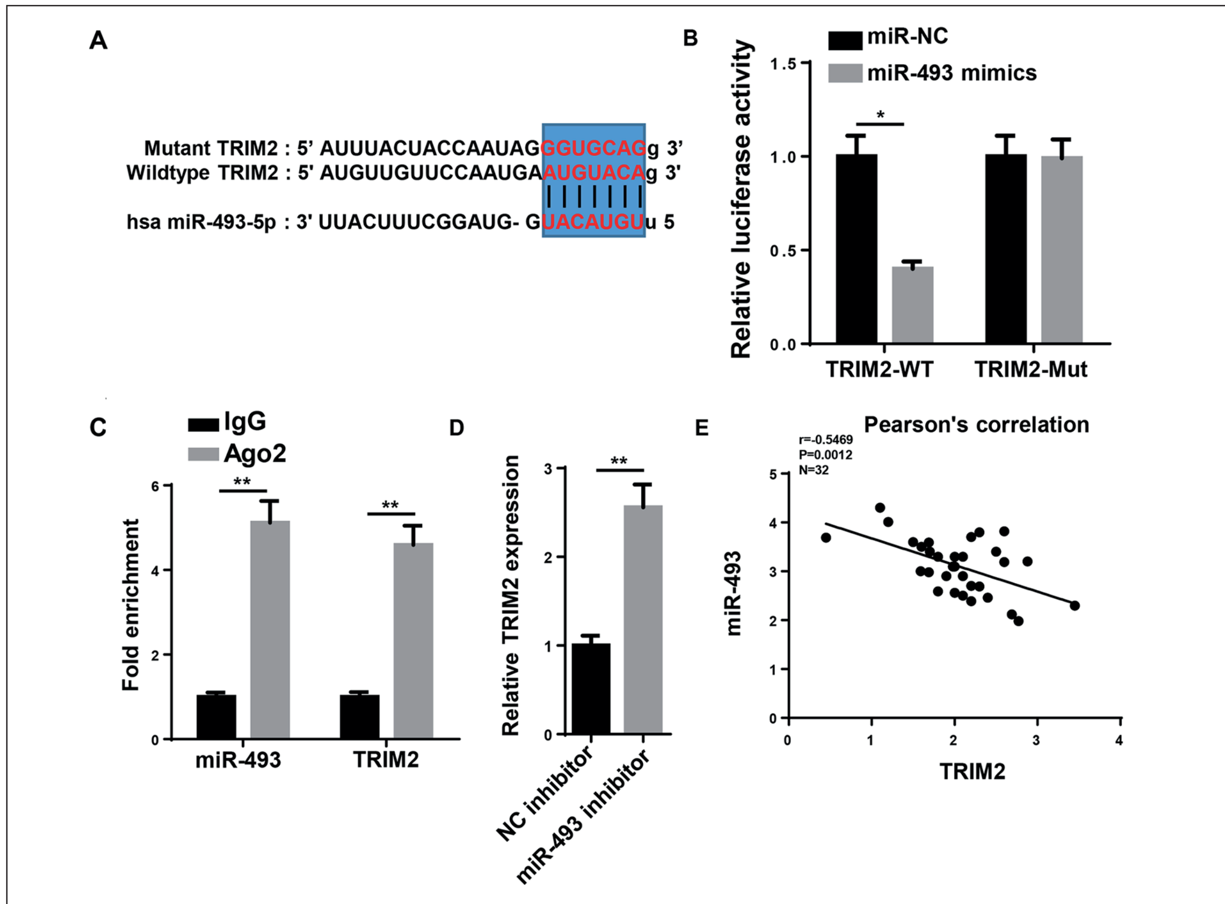

Figure 4. TRIM2 served as a direct target of miR-493. **A,** The binding sites and mutant binding sites between TRIM2 and miR-493 were described. **B,** and **C,** The interaction between TRIM2 and miR-493 was affirmed by luciferase reporter and RIP assays in SK-N-SH cells. **D,** RT-qPCR showed the relative expression of TRIM2 in SK-N-SH cells transfected with NC inhibitor and miR-493 inhibitor. **E,** TRIM2 expression was negatively correlated with miR-493 in NB tissues. *$p < 0.05$.

NR2F1-AS1/MiR-493 Axis Promoted NB Progression by Regulating TRIM2

To confirm whether the NR2F1-AS1/miR-493 axis promoted the development of NB by regulating TRIM2, SK-N-SH cells were transfected with NC mimics, miR-493 mimics, miR-493 mimics + pcDNA3.1, miR-493 mimics + TRIM2. As shown in Figure 5B, the upregulation of TRIM2 restored the inhibitory effect of miR-493 mimics on TRIM2 expression in SK-N-SH cells (Figure 5A). Functional analyses revealed that the suppressed effects of miR-493 overexpression on proliferation, migration, and invasion could be partially abrogated by the upregulation of TRIM2 (Figure 5B-D). TUNEL assays demonstrated that TRIM2 overexpression inverted the promoting effect of miR-493 mimics on apoptosis of SK-N-SH cells (Figure 5E). Besides, miR-493 expression was negatively correlated with TRIM2 in NB tissues (Figure 5F). RT-qPCR further revealed that interference of NR2F1-AS1 decreased TRIM2 expression, which was inverted following miR-493 inhibitor transfection (Figure 5G). Therefore, these results demonstrated that NR2F1-AS1 facilitated NB progression *via* the miR-493/TRIM2 axis.

Discussion

Previous studies[15-18] have shown that lncRNA was involved in the pathogenesis of NB. In particular, lncRNA XIST promoted the development and progression of NB by regulating H3 histone methylation of DKK1[19]. LncRNA SNHG1 facilitated the tumorigenesis of NB by sponging the miR-15b-5p/SIAH1 axis[20]. NBAT-1 suppressed cell proliferation of NB by downregulating miR-

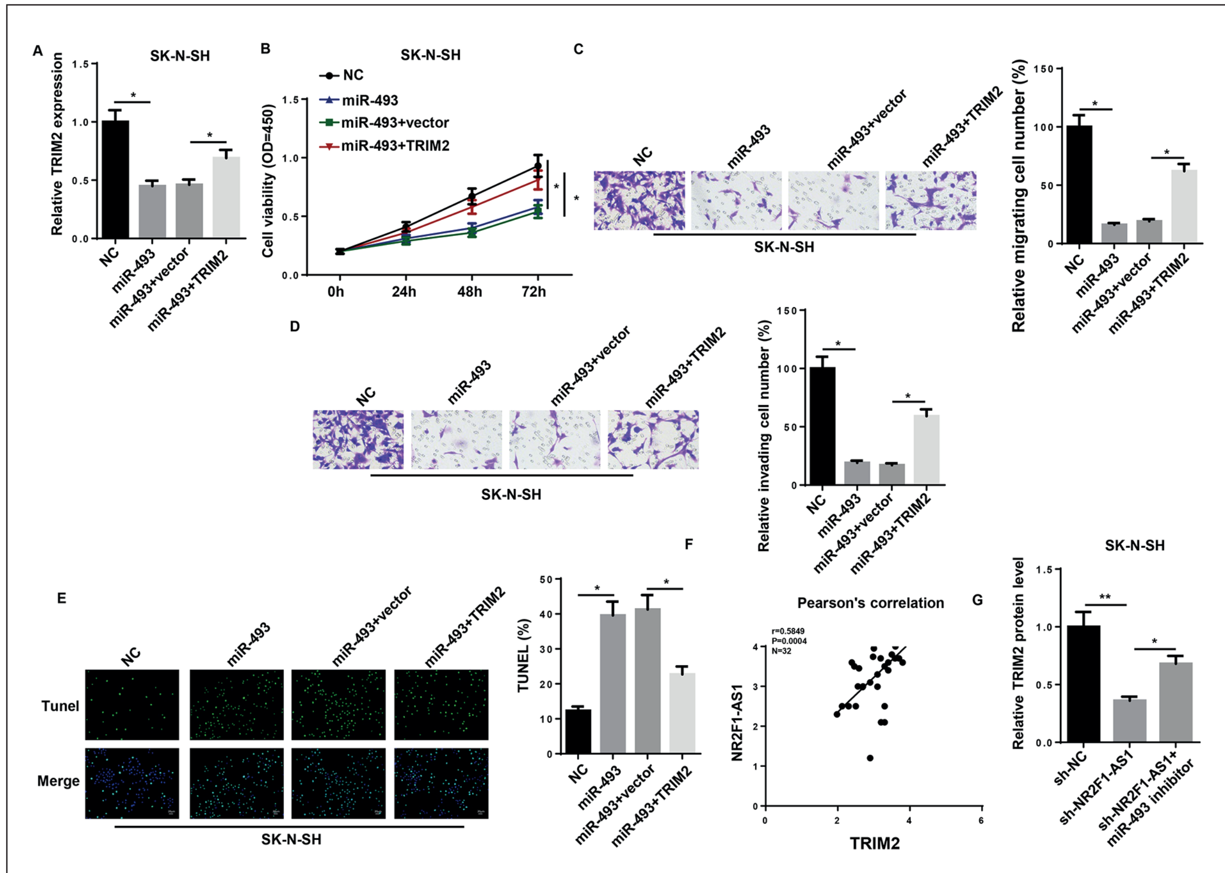

Figure 5. NR2F1-AS1/miR-493 axis promoted NB progression by regulating TRIM2. **A,** RT-qPCR showed the relative expression of TRIM2 in SK-N-SH cells transfected with NC mimics, miR-493 mimics, miR-493 mimics+pcDNA3.1 and miR-493 mimics+TRIM2. **B,** CCK-8 assays revealed the proliferation of SK-N-SH cells in different groups. **C,** and **D,** Transwell assays indicated the migration and invasion of SK-N-SH cells in different groups (magnification, x100). **E,** TUNEL assay showed the apoptosis of SK-N-SH cells in different groups (magnification, ×40). **F,** TRIM2 expression was positively correlated with NR2F1-AS1 in NB tissues. **G,** RT-qPCR showed the relative TRIM2 expression in SK-N-SH transfected with sh-NC, sh-NR2F1-AS1 and sh-NR2F1-AS1+miR-493 inhibitor. *$p < 0.05$.

10a[21]. Nevertheless, it is still necessary to further clarify the roles of lncRNAs in NB development. Considering that NR2F1-AS1 has not been studied in NB, we detected the level of NR2F1-AS1 in NB tissues and cells and carried out functional experiments. We found that NR2F1-AS1 was highly expressed in NB tissues and cells, and the high of NR2F1-AS1 was correlated with poor prognosis in NB. Loss-of-function assays demonstrated that the knockdown of NR2F1-AS1 inhibited proliferation, migration, and invasion, and accelerated apoptosis of NB cells.

LncRNA might regulate cancer progress by acting as miRNA sponges under competitive endogenous RNAs (ceRNA) network[22]. Su et al[23] uncovered that DCST1-AS1 acted as ceRNA to promote cell proliferation in gastric cancer by targeting miR-605-3p. Liang et al[24] indicated that LINC00467 accelerated cell proliferation and suppressed cell apoptosis in glioblastoma cells by targeting miR-339-3p. Wang et al[25] showed that RHPN1-AS1 knockdown inhibited the tumorigenesis and metastasis of ovarian cancer by acting as a ceRNA against miR-596. In our study, we demonstrated that miR-493 was a downstream target of NR2F1-AS1, and miR-493 expression was negatively correlated with NR2F1-AS1 expression. Functional analyses further demonstrated that NR2F1-AS1 promoted the tumorigenesis of NB by sponging miR-493.

TRIM2, an 81kDa multidomain protein, also known as CMT2R or RNF86, is located at 4q31.3[26]. TRIM2 has been identified as an oncogene in various cancers, such as osteosar-

coma, breast cancer, and clear cell renal cell carcinoma[27-29]. MiRNA was involved in cancer progression by regulating mRNA[30]. Chen et al[31] reported that miR-145 acted as a tumor suppressor in epithelial ovarian cancer by negatively regulating TRIM2. Wang et al[32] indicated that miR-15b promoted the progression of lung adenocarcinoma by sponging BCL2. In our study, we observed that miR-493 inhibited TRIM2 expression by direct interaction. Moreover, the addition of TRIM2 abolished the repressive effects of miR-493 overexpression on the proliferation, migration, and invasion of NB cells. The inhibition of miR-493 abolished the inhibitory effect of NR2F1-AS1 knockdown on TRIM2 expression, indicating that NR2F1-AS1 regulating TRIM2 expression by sponging miR-493 in NB.

Conclusions

Our study demonstrated that NR2F1-AS1 promoted the progression of NB *via* regulating the miR-493/TRIM2 axis. These findings indicated that NR2F1-AS1 might be an effective target for NB.

Conflict of Interest

The Authors declare that they have no conflict of interests.

Funding

This work was supported by the Suzhou Clinical Medicine Innovation Team Introduction Project (SZYJTD201706) and the Suzhou Municipal Science and Technology Project (SYS201759).

References

1) Ke XX, Zhang D, Zhao H, Hu R, Dong Z, Yang R, Zhu S, Xia Q, Ding HF, Cui H. Phox2B correlates with MYCN and is a prognostic marker for neuroblastoma development. Oncol Lett 2015; 9: 2507-2514.
2) Li Z, Chen H. miR-34a inhibits proliferation, migration and invasion of paediatric neuroblastoma cells via targeting HNF4alpha. Artif Cells Nanomed Biotechnol 2019; 47: 3072-3078.
3) Pinto NR, Applebaum MA, Volchenboum SL, Matthay KK, London WB, Ambros PF, Nakagawara A, Berthold F, Schleiermacher G, Park JR, Valteau-Couanet D, Pearson AD, Cohn SL. Advances in risk classification and treatment strategies for neuroblastoma. J Clin Oncol 2015; 33: 3008-17.
4) Dang HX, White NM, Rozycki EB, Felsheim BM, Watson MA, Govindan R, Luo J, Maher CA. Long non-coding RNA LCAL62/LINC00261 is associated with lung adenocarcinoma prognosis. Heliyon 2020; 6: e03521.
5) Zhang HY, Xing MQ, Guo J, Zhao JC, Chen X, Jiang Z, Zhang H, Dong Q. Long noncoding RNA DLX6-AS1 promotes neuroblastoma progression by regulating miR-107/BDNF pathway. Cancer Cell Int 2019; 19: 313.
6) Li S, Zheng K, Pei Y, Wang W, Zhang X. Long noncoding RNA NR2F1-AS1 enhances the malignant properties of osteosarcoma by increasing forkhead box A1 expression via sponging of microRNA-483-3p. Aging (Albany NY) 2019; 11: 11609-11623.
7) Guo F, Fu Q, Wang Y, Sui G. Long non-coding RNA NR2F1-AS1 promoted proliferation and migration yet suppressed apoptosis of thyroid cancer cells through regulating miRNA-338-3p/CCND1 axis. J Cell Mol Med 2019; 23: 5907-5919.
8) Wang L, Zhao S, Mingxin YU. LncRNA NR2F1-AS1 is involved in the progression of endometrial cancer by sponging miR-363 to target SOX4. Pharmazie 2019; 74: 295-300.
9) Yang J, Chen S, Dou W, Xie R, Gao J. MiR-654-3p predicts the prognosis of hepatocellular carcinoma and inhibits the proliferation, migration, and invasion of cancer cells. Cancer Biomark 2020; 28: 73-79.
10) Wu B, Liu G, Jin Y, Yang T, Zhang D, Ding L, Zhou F, Pan Y, Wei Y. MiR-15b-5p promotes growth and metastasis in breast cancer by targeting HPSE2. Front Oncol 2020; 10: 108.
11) Chen Y, Gao DY, Huang L. In vivo delivery of miRNAs for cancer therapy: challenges and strategies. Adv Drug Deliv Rev 2015; 81: 128-141.
12) Jiao D, Liu Y, Tian Z. microRNA-493 inhibits tongue squamous cell carcinoma oncogenicity via directly targeting HMGA2. Onco Targets Ther 2019; 12: 6947-6959.
13) Wang G, Fang X, Han M, Wang X, Huang Q. MicroRNA-493-5p promotes apoptosis and suppresses proliferation and invasion in liver cancer cells by targeting VAMP2. Int J Mol Med 2018; 41: 1740-1748.
14) Zhao J, Xu T, Wang F, Cai W, Chen L. MiR-493-5p suppresses hepatocellular carcinoma cell proliferation through targeting GP73. Biomed Pharmacother 2017; 90: 744-751.
15) Bao J, Zhang S, Meng Q, Qin T. SNHG16 silencing inhibits neuroblastoma progression by downregulating HOXA7 via sponging miR-128-3p. Neurochem Res 2020; 45: 825-836.
16) Zhao X, Li D, Yang F, Lian H, Wang J, Wang X, Fang E, Song H, Hu A, Guo Y, Liu Y, Li H, Chen Y, Huang K, Zheng L, Tong Q. Long noncoding RNA NHEG1 drives beta-catenin transactivation and neuroblastoma progression through interacting with DDX5. Mol Ther 2020; 28: 946-962.
17) Zhang N, Liu FL, Ma TS, Zhang ZZJ. LncRNA SNHG1 contributes to tumorigenesis and mecha-

nism by targeting miR-338-3p to regulate PLK4 in human neuroblastoma. Eur Rev Med Pharmacol Sci 2019; 23: 8971-8983.

18) Yu Y, Chen F, Yang Y, Jin Y, Shi J, Han S, Chu P, Lu J, Tai J, Wang S, Yang W, Wang H, Guo Y, Ni X. LncRNA SNHG16 is associated with proliferation and poor prognosis of pediatric neuroblastoma. Int J Oncol 2019; 55: 93-102.

19) Zhang J, Li WY, Yang Y, Yan LZ, Zhang SY, He J, Wang JX. LncRNA XIST facilitates cell growth, migration and invasion via modulating H3 histone methylation of DKK1 in neuroblastoma. Cell Cycle 2019; 18: 1882-1892.

20) Chen Y, Lian YJ, Ma YQ, Wu CJ, Zheng YK, Xie NC. LncRNA SNHG1 promotes alpha-synuclein aggregation and toxicity by targeting miR-15b-5p to activate SIAH1 in human neuroblastoma SH-SY5Y cells. Neurotoxicology 2018; 68: 212-221.

21) Zhou X, Lu H, Li F, Han L, Zhang H, Jiang Z, Dong Q, Chen X. LncRNA cancer susceptibility candidate (CASC7) upregulates phosphatase and tensin homolog by downregulating miR-10a to inhibit neuroblastoma cell proliferation. Neuroreport 2020; 31: 381-386.

22) Yang J, Yu D, Liu X, Changyong E, Yu S. LncRNA PCED1B-AS1 activates the proliferation and restricts the apoptosis of glioma through cooperating with miR-194-5p/PCED1B axis. J Cell Biochem 2019.

23) Su YZ, Cui MF, Du J, Song B. LncRNA DCST1-AS1 regulated cell proliferation, migration, invasion and apoptosis in gastric cancer by targeting miR-605-3p. Eur Rev Med Pharmacol Sci 2020; 24: 1158-1167.

24) Liang R, Tang Y. LINC00467 knockdown repressed cell proliferation but stimulated cell apoptosis in glioblastoma via miR-339-3p/IP6K2 axis. Cancer Biomark 2020; 28: 169-180.

25) Wang J, Ding W, Xu Y, Tao E, Mo M, Xu W, Cai X, Chen X, Yuan J, Wu X. Long non-coding RNA RHPN1-AS1 promotes tumorigenesis and metastasis of ovarian cancer by acting as a ceRNA against miR-596 and upregulating LETM1. Aging (Albany NY) 2020; 12: 4558-4572..

26) Cao H, Fang Y, Liang Q, Wang J, Luo B, Zeng G, Zhang T, Jing X, Wang X. TRIM2 is a novel promoter of human colorectal cancer. Scand J Gastroenterol 2019; 54: 210-218.

27) Qin Y, Ye J, Zhao F, Hu S, Wang S. TRIM2 regulates the development and metastasis of tumorous cells of osteosarcoma. Int J Oncol 2018; 53: 1643-1656.

28) Yin H, Zhu Q, Liu M, Tu G, Li Q, Yuan J, Wen S, Yang G. GPER promotes tamoxifen-resistance in ER+ breast cancer cells by reduced Bim proteins through MAPK/Erk-TRIM2 signaling axis. Int J Oncol 2017; 51: 1191-1198.

29) Xiao W, Wang X, Wang T, Xing J. TRIM2 downregulation in clear cell renal cell carcinoma affects cell proliferation, migration, and invasion and predicts poor patients' survival. Cancer Manag Res 2018; 10: 5951-5964.

30) Tan Y, Chen L, Li S, Hao H, Zhang D. MiR-384 inhibits malignant biological behavior such as proliferation and invasion of osteosarcoma by regulating IGFBP3. Technol Cancer Res Treat 2020; 19: 1533033820909125.

31) Chen X, Dong C, Law PT, Chan MT, Su Z, Wang S, Wu WK, Xu H. MicroRNA-145 targets TRIM2 and exerts tumor-suppressing functions in epithelial ovarian cancer. Gynecol Oncol 2015; 139: 513-9.

32) Wang J, Yao S, Diao Y, Geng Y, Bi Y, Liu G. MiR-15b enhances the proliferation and migration of lung adenocarcinoma by targeting BCL2. Thorac Cancer 2020; 11: 1396-1405.