

# MicroRNA-122 acts as tumor suppressor by targeting TRIM29 and blocking the activity of PI3K/AKT signaling in nasopharyngeal carcinoma *in vitro*

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Received July 31, 2017; Accepted January 25, 2018

DOI: 10.3892/mmr.2018.8894

**Abstract.** Nasopharyngeal carcinoma (NPC) is endemic in the southern provinces of China and Southeast Asia. It has been reported that microRNA-122 (miR-122) and tripartite motif-containing protein 29 (TRIM29) serve important roles in many types of tumor. The present study aimed to evaluate the expression of miR-122 and TRIM29, and their clinical significance in NPC, and to examine the associated molecular mechanisms. It was observed that low expression of miR-122 and high expression of TRIM29 led to a low overall survival rate in patients with NPC, which was associated with tumor-node-metastasis (TNM) stage and distant metastasis of NPC. Low expression of miR-122 was correlated reciprocally with high expression of TRIM29 in NPC tissues, and the two were aggravated by radiation treatment in NPC cell lines. Through Cell Counting kit-8 and Transwell assays, miR-122 was demonstrated to be able to inhibit the proliferation, migration and invasion of NPC cells. Through reverse transcription-quantitative polymerase chain reaction (RT-qPCR) and western blot analyses, the expression of metastasis-associated genes, including E-cadherin, metastatic tumor antigen 1, matrix metalloproteinase-2 and metalloproteinase inhibitor 2 were demonstrated to be regulated by miR-122 in NPC cells. Additionally, through a luciferase assay, RT-qPCR and western blot analysis, it was demonstrated that TRIM29 may be a direct target of miR-122. In addition, it was noted that miR-122 decreased the expression of phosphorylated (p) phosphatidylinositol 3-kinase (PI3K) and p-RAC- $\alpha$  serine/threonine-protein kinase (AKT). Collectively, the

results of the present study demonstrated that miR-122 may exert its tumor suppressive role by targeting TRIM29 and inhibiting the activity of PI3K/AKT signaling. It was indicated that miR-122 and TRIM29 may be developed as biomarkers of NPC, and possible molecular targets for the prevention of metastasis in patients with NPC.

## Introduction

Nasopharyngeal carcinoma (NPC) frequently occurs in the nasopharynx pharyngeal fossa and the anterior wall, which originates from the nasopharyngeal mucosal epithelium (1). NPC is endemic in the southern provinces of China and Southeast Asia (2). Due to the small size of the lesion and the nonspecific symptoms in the early stages of NPC, there is a risk of misdiagnosis and eventual distant metastasis. Radiation therapy is the primary treatment for NPC at present (3). Nevertheless, distant metastasis is a frequent cause of treatment failure in patients with NPC following radiotherapy (4), and accounts in a large part for the mortality rate of this type of cancer. In addition, dissemination to distant organs and invasion of tumor cells may be facilitated by epithelial-mesenchymal transition (EMT), which facilitates cytoskeletal transfer into a more migratory state (5). A decrease in E-cadherin expression is a hallmark event in EMT (6). Therefore, there is a need to develop novel strategies for metastasis prevention and to investigate the underlying mechanisms in NPC.

MicroRNAs (miRNAs/miRs), as single-stranded, non-coding small molecule RNAs, have been implicated in various biological progresses, including the development and progression of tumors, and are widely distributed in viruses, plants and higher mammals (7-9). miRNAs bind to the 3'-untranslated region (UTR) of target mRNAs, thereby regulating the expression of target genes (10). miR-122 is dysregulated in a number of types of cancer (10-12). The tripartite motif-containing protein (TRIM) family contains N-terminal RING finger, B-box and coiled-coil domains (13). It has been reported that certain members of the TRIM family participate in multiple physiological events, including cell cycle regulation, signal transduction and oncogenic processes (14). TRIM29, as a member of the TRIM family, serves important

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**Key words:** microRNA-122, tripartite motif-containing protein 29, nasopharyngeal carcinoma, phosphatidylinositol 3-kinase/RAC- $\alpha$  serine/threonine-protein kinase, metastasis

roles in many tumors (15,16). Furthermore, it has been reported that TRIM29 is involved in radioresistance in pancreatic cancer (17). An abnormal activated state of phosphatidylinositol 3-kinase (PI3K)/RAC- $\alpha$  serine/threonine-protein kinase (AKT) is common in a variety of types of cancer (18-20). In addition, it has been reported that the abnormal activation of PI3K/AKT signaling by TRIM29 may promote the progression of thyroid carcinoma (21). Accordingly, it may be speculated that miR-122, TRIM29 and PI3K/AKT may have an unknown association with the progression of NPC.

Therefore, the present study assessed the expression of miR-122 and TRIM29 and their clinical significance in patients with NPC. Furthermore, the potential functions of miR-122 and possible associations with TRIM29 were examined in NPC cells. The associated molecular mechanisms were additionally investigated. The results revealed that miR-122 may exert its tumor suppressive role by targeting TRIM29 and inhibiting PI3K/AKT signaling activity.

## Materials and methods

**Tissue specimens.** The samples were collected from 30 patients with NPC who received standard treatment between June 2014 and August 2015 in Jing Men No. 1 People's Hospital. No patient had a history of cancer and other coexisting tumor. The adjacent tissues were acquired at a site 5 cm from the tumor locus. Written informed consent was obtained from all patients prior to enrolling in the study. The clinical characteristics of the patients are presented in Tables I-III. The protocols associated with the use of patient tissues were approved by the ethics committee of Jing Men No. 1 People's Hospital (Jingmen, China).

**Cell culture and treatment.** Human NPC cell lines 5-8F (CA-89) and 6-10B (CA-91) were obtained from Changsha Axybio Biotechnology Co., Ltd. (Changsha, China). The cells were maintained in RPMI-1640 medium (Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA) with 10% fetal bovine serum (Hyclone; GE Healthcare Life Sciences, Logan, UT, USA) and 50  $\mu$ g/ml streptomycin in a 37°C incubator containing 5% CO<sub>2</sub>. An 8 MV linear accelerator (Elekta Instrument AB, Stockholm, Sweden) was used for one-time exposure at room temperature with the source-skin distance of 100 cm. The irradiation dose was 3 Gy and the dose rate was 400 cGy/min. The control group was not irradiated. The miR-122 mimics (5'-UGGAGUGUGACA AUGGUG UUUG-3') and corresponding negative control (5'-UUC UCCGAACGUGUCACGUTT-3') were purchased from Shanghai GenePharma Co., Ltd. (Shanghai, China). Cells (1x10<sup>4</sup>/well) were seeded into 6-well plates. A total of 12 h post-incubation at 37°C, either miR-122 mimics (75 nM) or corresponding negative control (75 nM) were transfected using Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc.), according to the manufacturer's protocol. Following a further 24 h of incubation at 37°C, the cells were subjected to subsequent analysis.

**Cell proliferation detection.** The survival rate of transfected cells was determined using a Cell Counting kit-8 (CCK-8) assay (MedChemExpress, Monmouth Junction, NJ, USA). The cells (2x10<sup>3</sup>) were incubated in 96-well plates (100  $\mu$ l/well). A

total of 10  $\mu$ l CCK-8 solution was added and incubated for 4 h at 37°C. The absorbance was measured with microplate reader (Bio-Rad Laboratories, Inc., Hercules, CA, USA) at 450 nm.

**Cell migration and invasion assays.** Transwell assays were performed to observe the migratory and invasive abilities of transfected cells. The cells (1x10<sup>4</sup>) in serum-free medium were placed onto the upper Matrigel-coated insert (for the invasion assay) or insert without Matrigel (for the migration assay) of Transwell chambers (Corning Incorporated, Corning, NY, USA). Complete medium was added to the lower chamber. A 24-well plate was used to hold the Transwell inserts. Following 16 h, the cells that stayed on the upper surface were removed, and the cells that had migrated through the pores were collected. Another group was maintained at 37°C for 24 h to allow the cells to invade through the Matrigel into the bottom membrane. Subsequent to fixing with 4% paraformaldehyde for 15 min at room temperature, the collected cells were stained with crystal violet (0.1%) for 20 min at room temperature and quantified using a light microscope (magnification, x100; Nikon Corporation, Tokyo, Japan).

**Luciferase assay.** Bioinformatics analysis was performed using TargetScan ([http://www.targetscan.org/vert\\_71/](http://www.targetscan.org/vert_71/)) and microRNA.org (<http://www.microrna.org/>). The wild type (wt) TRIM29-3'UTR reporter plasmid was purchased from Changsha Axybio Biotechnology Co., Ltd. The mutant type (mut) TRIM29 3'UTR plasmid was generated using a site-directed mutagenesis kit (Takara Bio, Inc., Otsu, Japan) by mutating the site of the target region of miR-122 (CACACUCC to GAGAGAGT), based on the wt TRIM29-3'UTR reporter plasmid. The cells were seeded at a density of 5x10<sup>5</sup>/well into a 6-well plate. After 24 h, the cells were co-transfected with miR-122 mimics or miR-negative control, wt/mut TRIM29 3'UTR reporter plasmid and a *Renilla* luciferase pRL-TK vector (Promega Corporation, Madison, WI, USA) using Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc.). At 48 h, according to the manufacturer's protocol, a dual-luciferase assay system (Promega Corporation) was employed to measure the luciferase activity. The luciferase activity was normalized to *Renilla* luciferase activity.

**Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) analysis.** TRIzol reagent (Invitrogen; Thermo Fisher Scientific, Inc.) was used for the extraction of total RNA and miRNA. The cDNA was synthesized using total RNA, oligodT18 primers (Takara Bio, Inc.) and M-MLV reverse transcriptase (Promega Corporation). The temperature protocol used for RT was as follows: 25°C for 10 min, 42°C for 50 min and 70°C for 10 min. A TaqMan miRNA reverse transcription kit (Applied Biosystems; Thermo Fisher Scientific, Inc.) and a TaqMan human miRNA assay kit (Applied Biosystems; Thermo Fisher Scientific, Inc.) was used for the amplification and quantification of miR-122, respectively. U6 was adopted as the internal control for miR-122. qPCR was performed with Fast SYBR Green Master Mix (Applied Biosystems; Thermo Fisher Scientific, Inc.) on the ABI StepOne Real-Time PCR System (Applied Biosystems; Thermo Fisher Scientific, Inc.). The thermocycling conditions used were as follows: 95°C for 10 min; followed by 40 cycles at 95°C for 15 sec and 60°C

Table I. Correlation analysis between clinical features and the expression of miR-122 in patients with NPC.

Characteristics	No. patients	miR-122 expression		P-value
		Low group	High group	
Age, years				0.358
≤45	12	5	7	
>45	18	10	8	
Sex				0.961
Male	17	9	8	
Female	13	7	6	
TNM stage				0.002 <sup>a</sup>
I+II	10	1	9	
III+IV	20	14	6	
Distant metastasis				<0.001 <sup>a</sup>
No	13	1	12	
Yes	17	14	3	

<sup>a</sup>P<0.05. NPC, nasopharyngeal carcinoma; TNM, tumor-node-metastasis; miR, microRNA.

Table II. Correlation analysis between clinical features and the expression of TRIM29 in patients with NPC.

Characteristics	No. patients	TRIM29 expression		P-value
		Low group	High group	
Age, years				0.713
≤45	17	9	8	
>45	13	6	7	
Sex				0.409
Male	22	12	10	
Female	8	3	5	
TNM stage				0.002 <sup>a</sup>
I+II	10	9	1	
III+IV	20	6	14	
Distant metastasis				0.010 <sup>a</sup>
No	13	10	3	
Yes	17	5	12	

<sup>a</sup>P<0.05. NPC, nasopharyngeal carcinoma; TNM, tumor-node-metastasis; TRIM29, tripartite motif-containing protein 29.

for 60 sec; and a final extension step at 72°C for 7 min. Primers for miR-122 were purchased from Changsha Axybio Biotechnology Co., Ltd. The primer sequences used were as follows: miR-122 forward, 5'-GCCGTGGAGTGTGAT-3'; and reverse, 5'-GTGCAGGGTCCGAGGTCAATGG-3'. The other primer sequences were as follows: U6 forward, 5'-CTCGCT

Table III. Correlation analysis of expression between miR-122 and TRIM29.

miR-122	TRIM29		P-value
	-	+	
-	4	9	-0.522
+	14	3	

miR, microRNA; TRIM29, tripartite motif-containing protein 29.

TCGGCAGCACA-3' and reverse, 5'-AACGCTTCACGAATT TGGCT-3'; TRIM29 forward 5'-ACTCCTTTCCCTGCCTTGT GA-3' and reverse, 5'-GAGAGGCAGGCTGATACCAT-3'; E-cadherin forward 5'-TCACATCCTACACTGCCAG-3' and reverse, 5'-AGTGTCCCTGTTCCAGTAGC-3'; metastatic tumor antigen 1 (MTA1) forward, 5'-ACAGACAAGCAG ATCGACCA-3' and reverse, 5'-GGCCTTGGAGATGTCGTA GA-3'; matrix metalloproteinase-2 (MMP-2) forward, 5'-CGG TGCCCAAGAATAGATGC-3' and reverse, 5'-AGACAG TCTCCATTGGCTCC-3'; metalloproteinase inhibitor (TIMP2) forward, 5'-TGTGTTCCCTCAGTGTGGTT-3' and reverse, 5'-TTCGGTTTCATTGCGTGTGT-3'. The 2<sup>-ΔΔC<sub>q</sub></sup> method was used to analysis the relative expression level of target factors (22).

**Western blot analysis.** The extraction and separation of proteins was performed using radioimmunoprecipitation assay lysis buffer (Thermo Fisher Scientific, Inc.). Protein concentration was determined using a bicinchoninic acid assay protein quantification kit (Thermo Fisher Scientific, Inc.). Protein samples were then separated on 10% SDS-PAGE gels (20 μg/lane). The proteins were transferred onto polyvinylidene fluoride membranes. The blots were blocked with 5% bovine serum albumin (Beijing Solarbio Science & Technology, Co., Ltd., Beijing, China) at room temperature for 2 h and then incubated with primary antibodies at 4°C overnight. The next day, the blots were maintained with horse-radish peroxidase-conjugated secondary antibodies at room temperature for 2 h. The blots were determined with enhanced chemiluminescence reagents (Thermo Fisher Scientific, Inc.). Densitometry was performed using Quantity One software version 4.6.9 (Bio-Rad Laboratories, Inc.).

The primary antibodies used were as follows: Anti-phosphorylated (p)-PI3K p85 (Tyr458)/p55 (Tyr199; 1:1,000; cat. no. 4228), anti-p-AKT (Ser473; 1:2,000; cat. no. 4060), anti-PI3K Class III (1:1,000; cat. no. 3358), anti-AKT (pan; 1:1,000; cat. no. 4685; Cell Signaling Technology, Inc., Danvers, MA, USA), anti-TRIM29 (1:1,000; cat. no. ab108627; Abcam, Cambridge, UK), anti-E-cadherin (1:1,000; cat. no. 3195), anti-MTA1 (1:1,000; cat. no. 5646; both Cell Signaling Technology, Inc.), anti-MMP-2 (1:1,000; cat. no. sc13594; Santa Cruz Biotechnology, Inc., Dallas, TX, USA) and anti-TIMP2 (1:300; cat. no. 89025; R&D Systems, Inc., Minneapolis, MN, USA), and GAPDH (1:5,000; cat. no. ab8245; Abcam). The secondary antibodies were purchased from Beijing CoWin Biotech Co., Ltd. (Beijing, China; cat. nos. Cw0102s and Cw0103s; 1:2,000).

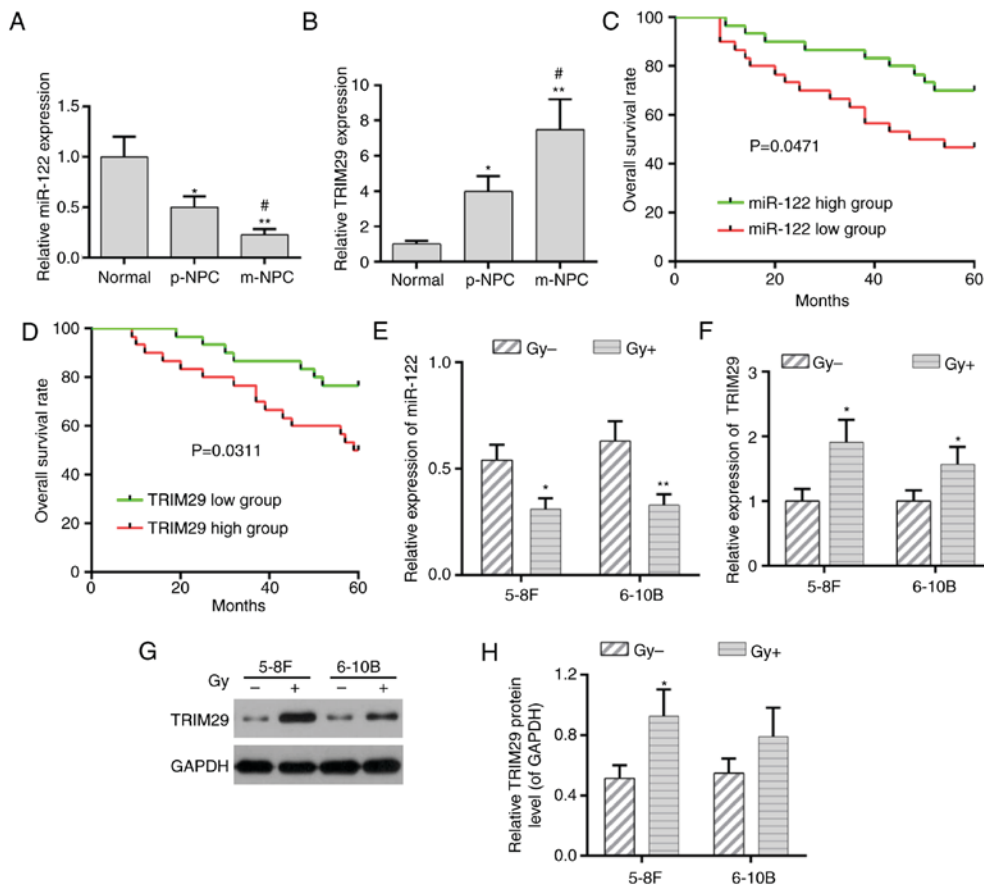


Figure 1. Expression of miR-122 and TRIM29 in patients with NPC and cell lines. Quantitative analysis of (A) miR-122 and (B) TRIM29 in NPC samples with or without metastasis, respectively, was performed. p-NPC, NPC samples without metastasis; m-NPC, NPC samples with metastasis. Data are presented as the mean  $\pm$  standard deviation,  $n=3$ . \* $P<0.05$  and \*\* $P<0.01$  vs. normal; # $P<0.05$  vs. p-NPC. (C) The overall survival rate of patients with NPC in miR-122 high and low expression level groups. (D) The overall survival rate of NPC patients in TRIM29 high and low expression level groups. Quantitative analyses of (E) miR-122 and (F) TRIM29 in NPC cell lines were performed following radiation treatment. (G) Western blot analysis and (H) determination of the expression of TRIM29 in NPC cell lines following radiation treatment. Data are presented as mean  $\pm$  standard deviation,  $n=4$ . \* $P<0.05$  and \*\* $P<0.01$  vs. groups without radiation treatment. NPC, nasopharyngeal carcinoma; TRIM29, tripartite motif-containing protein 29; miR, microRNA.

**Statistical analysis.** Data are presented as mean  $\pm$  standard deviation. All experiments were performed independently at least three times. A  $\chi^2$  test was used to analyze categorical variables. A two-tailed Student's t-test or one-way analysis of variance followed by Dunnett's post-hoc multiple comparison test was employed to compare the differences between groups. Kaplan-Meier analysis was used to draw the survival curves. Spearman's rank correlation analysis was used to analyze bivariate correlations. SPSS v.14.0 (SPSS, Inc., Chicago, IL, USA) and GraphPad Prism 6.0 (GraphPad Software, Inc., La Jolla, CA, USA) software was used for all the statistical analysis and graph-drawing.  $P<0.05$  was considered to indicate a statistically significant difference.

## Results

**Expression of miR-122 and TRIM29 in patients with NPC and cell lines.** In order to determine the expression of miR-122 and TRIM29 in patients with NPC, RT-qPCR was performed to analyze their expression levels in cases with and without metastasis. It was demonstrated that miR-122 expression was decreased in NPC cases without metastasis (p-NPC), and more so in cases with metastasis (m-NPC). By contrast, the expression of TRIM29 was increased in

p-NPC and further enhanced in m-NPC (Fig. 1A and B). Furthermore, the decreased miR-122 expression and the increased TRIM29 expression were significantly correlated with advanced TNM stage and distant metastasis (Tables I and II). According to a previous study (23), the NPC patients were divided into two groups based on the median value of miR-122 or TRIM29 expression: miR-122/TRIM29 high expression group ( $n=15$ ), and miR-122/TRIM29 low expression group ( $n=5$ ). The overall survival (OS) rate in the patients with low miR-122 expression was decreased compared with those in the high miR-122 group (Fig. 1C;  $P<0.05$ ). However, patients with low TRIM29 expression had a higher OS rate compared with those with high TRIM29 expression (Fig. 1D;  $P<0.05$ ). In addition, the expression of miR-122 and TRIM29 in patients with NPC was negatively correlated (Table III).

Subsequently, the expression of miR-122 and TRIM29 was detected in NPC cell lines, including 5-8F and 6-10B, following radiation treatment. The RT-qPCR analysis demonstrated that, in all NPC cell lines following radiation treatment, miR-122 and TRIM29 were downregulated and upregulated, respectively (Fig. 1E and F). The expression of TRIM29 was further confirmed by western blot analysis (Fig. 1G and H).



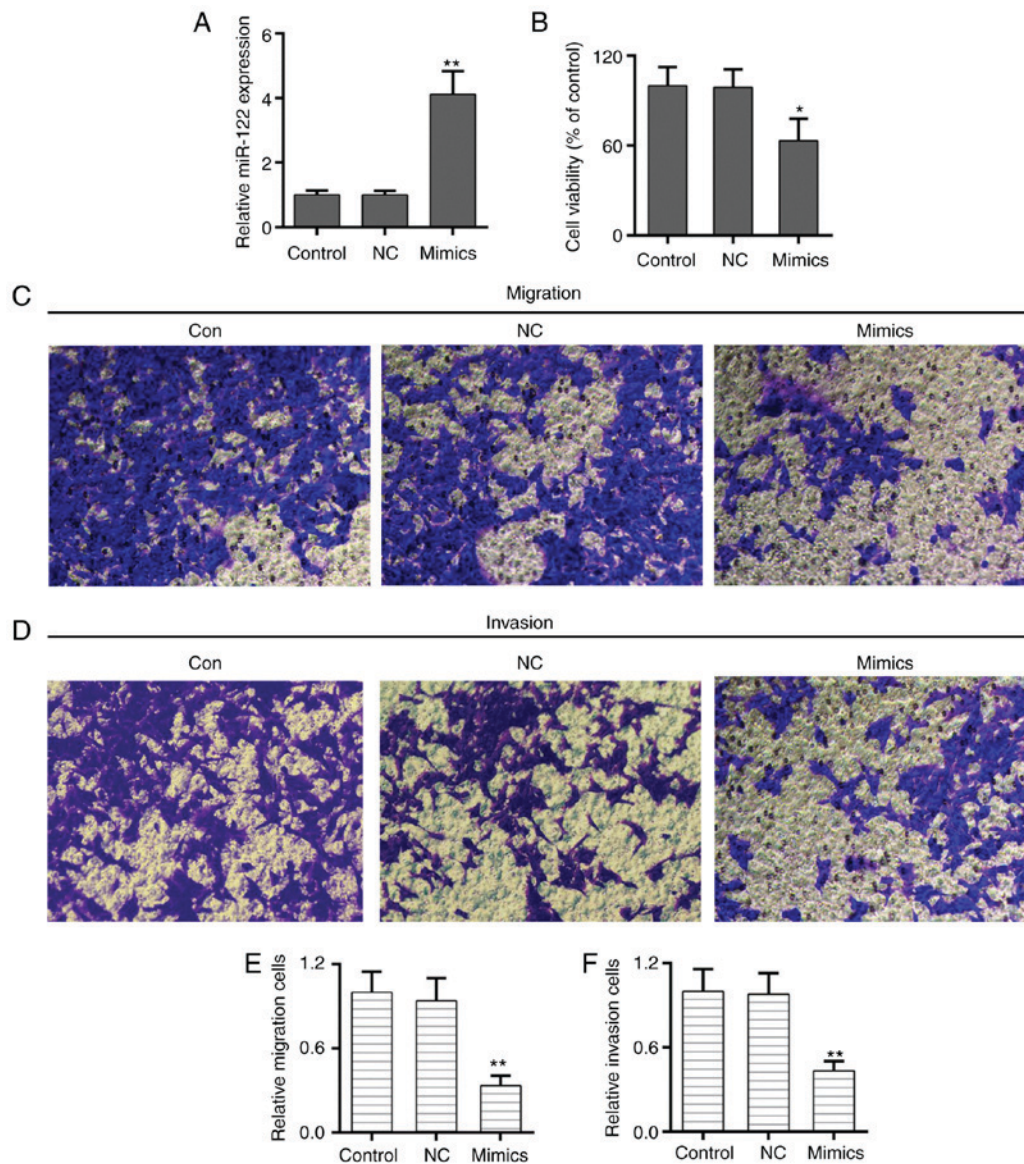


Figure 2. miR-122 suppresses cell proliferation, migration and invasion in NPC cells. (A) The significantly elevated expression of miR-122 in NPC cells transfected with miR-122 mimics. (B) Relative cell survival, demonstrated using Cell Counting kit-8 methods. Data are presented as the mean  $\pm$  standard deviation,  $n=3$ . \* $P<0.05$  and \*\* $P<0.01$  vs. control. Representative images of the (C) migratory and (D) invasive abilities of NPC cells transfected with miR-122 are presented (magnification,  $\times 100$ ). (E) miR-122 inhibited the migratory ability of NPC cells. (F) miR-122 inhibited the invasive ability of NPC cells. Data are presented as the mean  $\pm$  standard deviation,  $n=4$ . \*\* $P<0.01$  vs. control. NPC, nasopharyngeal carcinoma; miR, microRNA; NC, negative control; Con, control.

*miR-122 inhibits the proliferation, migration and invasion of NPC cells.* The potential role of miR-122 and TRIM29 in the progression and metastasis of NPC was determined. The results of the present study demonstrated that the expression of miR-122 was increased significantly in NPC cells that were transfected with miR-122 mimics (Fig. 2A). Furthermore, the cell survival rate was decreased in the miR-122 mimics group compared with the control and negative groups (Fig. 2B). The Transwell assays illustrated that the migratory and invasive abilities of NPC cells were decreased by overexpression of miR-122 (Fig. 2C-F).

*miR-122 regulates the expression of metastasis-associated genes.* The expression of metastasis-associated genes in the miR-122 mimics group was assessed. MTA1 is associated with tumor metastasis (24). As presented in Fig. 3, the expression

of E-cadherin and MTA1 was increased and decreased in the miR-122 mimics group, respectively. The extracellular matrix may be degraded by MMP-2 during the progression of cell invasion (25). It was noted that the expression of MMP-2 was decreased in the miR-122 mimics group compared with the control and negative control groups. Conversely, TIMP2, a suppressor of MMP-2 (26), was upregulated by the overexpression of miR-122 (Fig. 3A-C).

*TRIM29 may be a direct target of miR-122.* The expression of TRIM29 was inhibited at the mRNA and protein levels in the miR-122 mimics group (Fig. 4A-C). In addition, bioinformatics analysis from available databases (TargetScan and microRNA.org) indicated that TRIM29 was a possible target of miR-122 (Fig. 4D). Meanwhile, the results of the luciferase reporter assay demonstrated that the luciferase activity of the

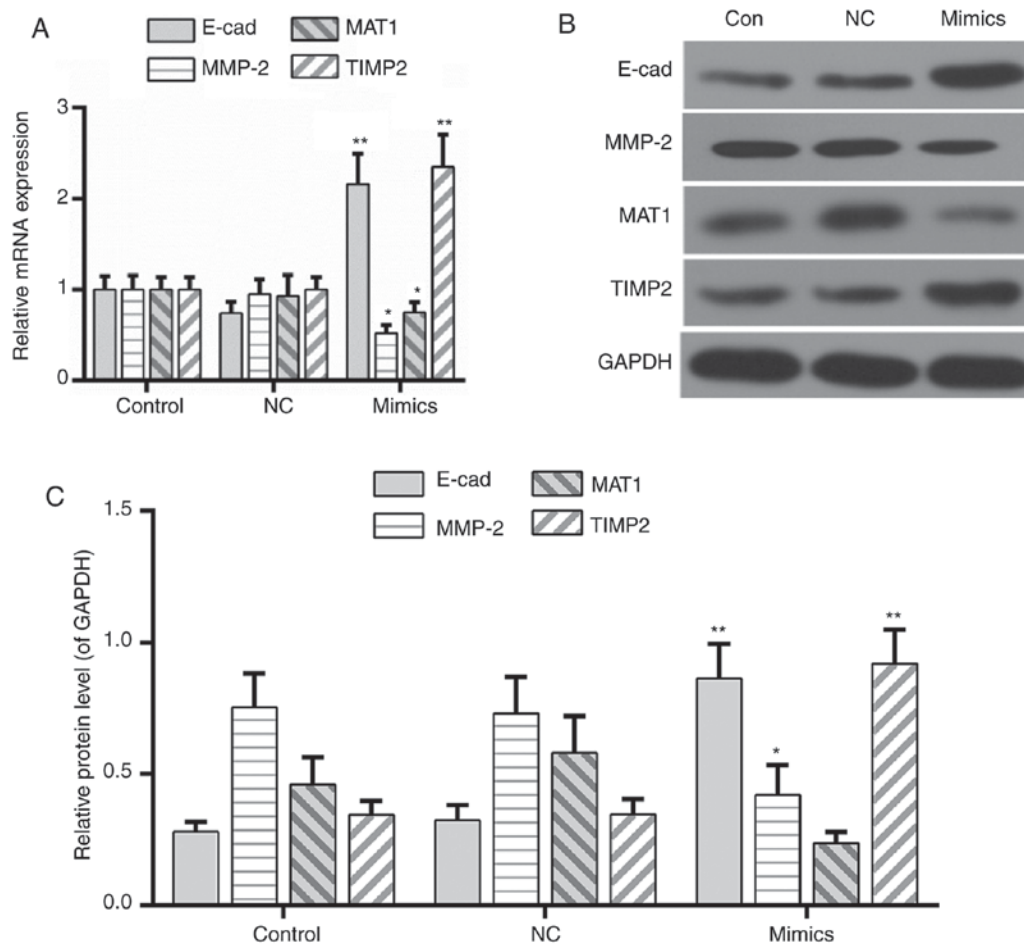


Figure 3. MicroRNA-122 regulates the expression of metastasis-associated genes. (A) Quantitative analysis of E-cad, MTA1, MMP-2 and TIMP2. (B) Western blot assay and (C) determination of the expression of E-cad, MTA1, MMP-2, and TIMP2. Data are presented as the mean  $\pm$  standard deviation, n=4. \*P<0.05 and \*\*P<0.01 vs. control. E-cad, E-cadherin; MMP2, 72 kDa type IV collagenase; MTA1, CDK-activating kinase assembly factor MTA1; TIMP2, metalloproteinase inhibitor 2; NC, negative control.

wild-type TRIM29 3'-UTR was inhibited by the excessive expression of miR-122, whereas the luciferase activity of the mutant TRIM29 3'-UTR was not affected (Fig. 4E). To confirm this result, the luciferase activity reporter assay was performed in another NPC cell line, 5-8F. The results were consistent between the two NPC cell lines (Fig. 4F). These data indicated that TRIM29 may be a direct target of miR-122.

*miR-122 decreases the expression of p-PI3K and p-AKT.* To further clarify the underlying molecular mechanisms, the activity of PI3K/AKT was evaluated in NPC cells transfected with miR-122. The results of the western blotting demonstrated that the expression levels of p-PI3K and p-AKT were decreased by the overexpression of miR-122 (Fig. 5).

## Discussion

Recently, varieties of miRNAs have been demonstrated to be the key regulators in tumor metastatic processes (27). Previous studies have reported that miR-122 serves as a tumor suppressor and is suppressed in a number of types of cancer, including gallbladder cancer, gastric cancer, non-small-cell lung cancer and hepatocellular carcinoma (12,28,29). High expression of TRIM29 has been identified in a number of

aggressive diseases, and is an important oncogenic factor in cancer (15). It has been reported that poor prognosis may be associated with dysregulated miR-122 and TRIM29 (30,31). Consistently, the results of the present study demonstrated that miR-122 and TRIM29 expression was downregulated and upregulated in NPC tissues, particularly in cases with metastasis, respectively. Furthermore, low miR-122 expression and high TRIM29 expression were significantly associated with advanced TNM stage and distant metastasis in patients with NPC. A poor patient overall survival rate was observed in the low miR-122 group and the high TRIM29 group. Additionally, a significant negative correlation between the expression of miR-122 and TRIM29 was observed in patients with NPC. It was indicated that the dysregulation of miR-122 and TRIM29 may serve as a prognostic biomarker and optional target for NPC. Distant metastasis is the primary cause of treatment failure in patients with NPC following radiation therapy (32). The prevention of metastasis in radiation therapy may be a promising strategy for the treatment of NPC. Hence, the expression of miR-122 and TRIM29 was determined in NPC cell lines following radiation treatment. As hypothesized, decreased miR-122 expression and increased TRIM29 expression was identified in NPC cell lines with radiation treatment.

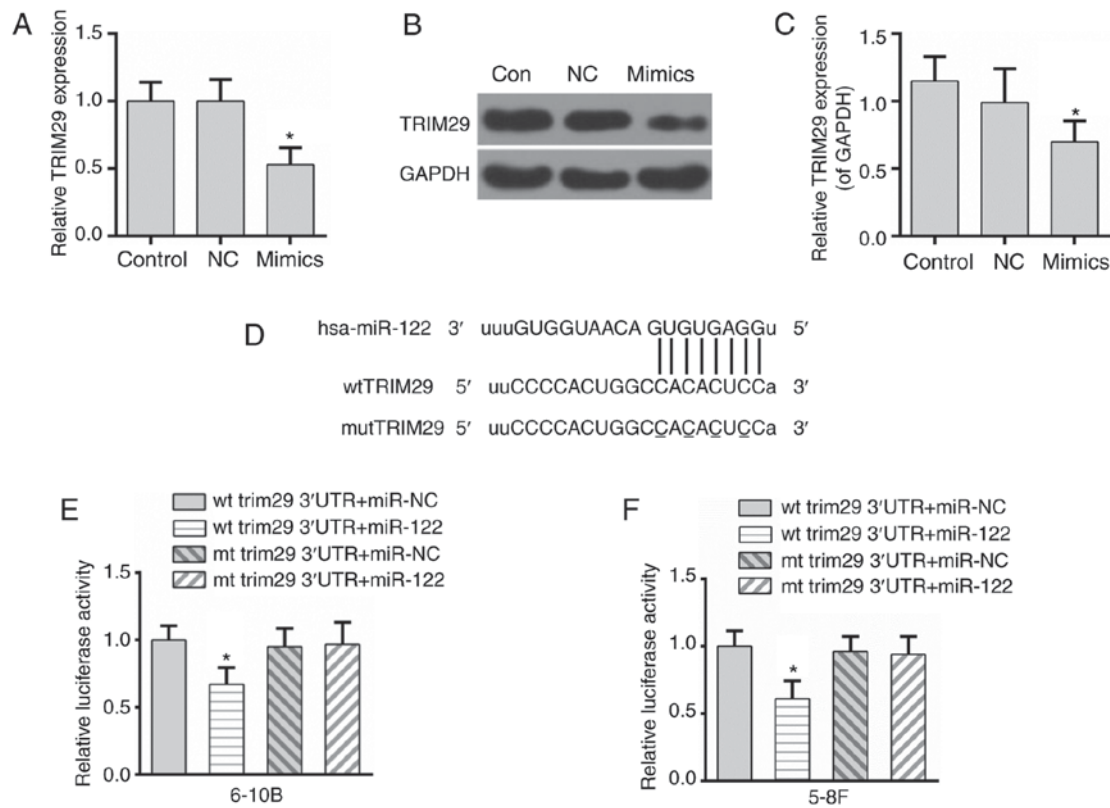


Figure 4. TRIM29 may be a direct target of miR-122. (A) miR-122 decreased the expression of TRIM29 at the mRNA level. miR-122 decreased the expression of TRIM29 at the protein level, as demonstrated by (B) western blotting and (C) densitometry. (D) The potential complementary sequences of the wt TRIM29 3'UTR and mut TRIM29 3'UTR, and the miR-122 binding sequence. (E) miR-122 depressed the luciferase activity of the wt 3'-UTR of TRIM29 in 6-10B cells. (F) miR-122 depressed the luciferase activity of the wt 3'-UTR of TRIM29 in 5-8F cells. Data are presented as the mean  $\pm$  standard deviation, n=3. \*P<0.05 vs. control. TRIM29, tripartite motif-containing protein 29; NC, negative control; wt, wild-type; mut, mutant; UTR, untranslated region; miR, microRNA; Con, control.

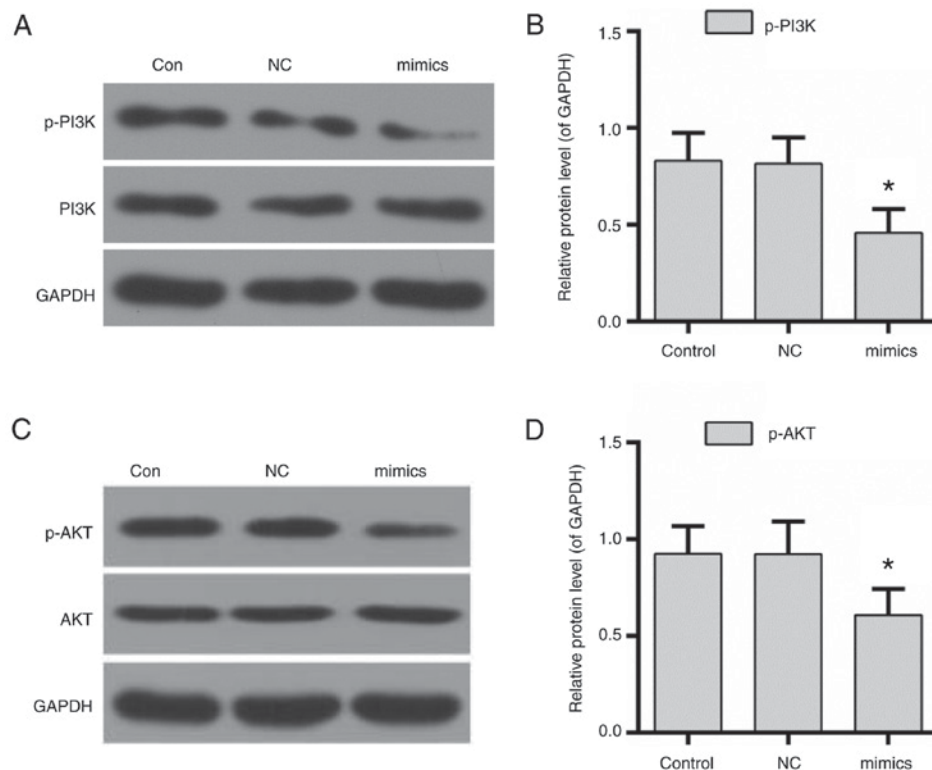


Figure 5. miR-122 decreases the expression levels of p-PI3K and p-AKT. (A) Western blot analysis and (B) determination of the expression of p-PI3K. (C) Western blot analysis and (D) determination of the expression of p-AKT. Data are presented as the mean  $\pm$  standard deviation, n=3. \*P<0.05 vs. control. miR, microRNA; p, phosphorylated; PI3K, phosphatidylinositol 3-kinase; AKT, RAC- $\alpha$  serine/threonine-protein kinase; NC, negative control; Con, control.



Unlimited proliferation and metastasis are the prominent properties of malignant tumors (33). The failure of radiation therapy is primarily caused by distant metastasis. The present study further clarified the potential effect of miR-122 on the metastasis of NPC *in vitro*. It was observed that the overexpression of miR-122 was able to inhibit tumor progression by suppressing the viability, migration and invasion of NPC cells, which indicated the antitumor effects of miR-122. These data indicated that miR-122 acted as a tumor suppressor in NPC cells, and were supported by previous observations in multiple types of cancer (29,34,35). However, it was likely that the decrease in cell viability may also indirectly suppress the invasion and migration of NPC cells. Therefore, the effect of miR-122 on the activity and expression of metastasis-associated genes was assessed. It was revealed that the expression of metastasis-associated genes, including E-cadherin, MTA1, MMP-2 and TIMP2, was regulated by miR-122. However, due to the complexity of tumorigenesis and the intracellular signal transduction network, certain studies have claimed that the overexpression of miR-122 may be associated with poor patient prognosis in colorectal cancer (36) and renal cancer (37). In addition, the expression of TRIM29 was downregulated in NPC cells that were transfected with miR-122. Based on the above investigations, it was speculated that miR-122 may exert its role by blocking the expression of TRIM29. To verify this possibility, bioinformatics databases were searched to predict the downstream targets of miR-122. The analysis results demonstrated that possible miR-122 binding sites were located in the 3'-UTR of TRIM29. Further evidence of this was provided by the results of the luciferase reporter assay, in the 6-10B and 5-8F cell lines, in the present study. It was therefore suggested that TRIM29 may be a direct target of miR-122 and serve as an oncogene in NPC. However, the tumor suppressor role of TRIM29 has additionally been reported in certain studies (38,39). Thus, the function of TRIM29 may be context-dependent in the progression of different tumors. To illustrate the in-depth molecular mechanisms, the activity of PI3K/AKT signaling, which is dysregulated in a number of types of cancer, was detected (40). The results revealed that the phosphorylation of PI3K and AKT was decreased by miR-122. A study in thyroid cancer reported that the activation of the PI3K/AKT signaling pathway was inhibited by decreased TRIM29 expression (21). However, it remains unclear whether or not PI3K/AKT was modulated by TRIM29 in the present study and thus requires further investigation.

The results of the present study suggested a potential role of miR-122 and TRIM29 in metastatic progression following radiation therapy. However, the present study did not provide further evidence for the effect of miR-122 in the radiation treatment of NPC. However, it was necessary to undertake *in vivo* studies to assess the antitumor effect of miR-122 in patients with NPC following radiation therapy. These investigations implied that the antitumor effect of miR-122 in NPC was promising, although this requires validation in future studies.

In conclusion, decreased expression of miR-122 and increased expression of TRIM29 was significantly associated with poor prognosis in patients with NPC. miR-122 may exert antitumor effects through suppression of tumor proliferation, invasion and migration, and modulation of the expression and activity of metastasis-associated genes, including E-cadherin,

MTA1, MMP-2 and TIMP2. Notably, miR-122 may suppress NPC progression by targeting TRIM29 and blocking the activity of PI3K/AKT signaling. Therefore, miR-122 and TRIM29 may be developed as biomarkers and possible molecular targets for the metastasis of NPC.

## Acknowledgements

Not applicable.

## Funding

No funding was received.

## Availability of data and materials

All data generated or analyzed during the present study are included in this published article.

## Authors' contributions

LG designed the present study. QL and YY wrote the manuscript. YY and QL performed the experiments. QL performed data analysis. YY, QL and LG revised the manuscript for important intellectual content.

## Ethics approval and consent to participate

Written informed consent was obtained from all patients prior to enrolling in the present study. The protocols associated with the use of patient tissues were approved by the ethics committee of Jing Men No. 1 People's Hospital (Jingmen, China).

## Consent for publication

Written informed consent was obtained from all patients prior to enrolling in the present study.

## Competing interests

The authors declare that they have no competing interests.

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