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MIR22HG inhibits cell growth, migration and invasion through regulating the miR-24-3p/p27kip1 axis in thyroid papillary carcinomas

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Abstract. – OBJECTIVE: To explore the underlying mechanism of ncRNA (MIR22HG) in thyroid papillary carcinomas.

PATIENTS AND METHODS: 40 pairs of thyroid papillary carcinomas tissues and adjacent normal tissues were collected from patients of the First Affiliated Hospital of Guangxi Medical University, who underwent oral surgery. qRT-PCR was applied to detect the expression of MIR22HG, miR-24-3p and p27kip1 in tissues and cells. Western blot was used to measure the protein level of p27kip1 in tissues and cells. Kaplan-Meier plot was used to analyze the overall survival rates in thyroid papillary carcinomas. Pearson's correlation analysis was used to analyze the correlation relationship among MIR22HG, miR-24-3p and p27kip1 expression. Flow cytometric assay was applied to measure cell apoptosis. Transwell assay was used to assess cell migration and invasion abilities. Luciferase reporter assay was applied to verify the molecular relationships among MIR22HG, miR-24-3p and p27kip1 in thyroid papillary carcinomas.

RESULTS: LncRNA MIR22HG and p27kip expressed low while miR-24-3p expressed high in thyroid papillary carcinomas and cells. Overexpression of MIR22HG inhibited cell proliferation, migration and invasion, whereas promoted cell apoptosis in thyroid papillary carcinomas cells. However, these effects were reversed by upregulation of miR-24-3p. Further exploration showed that the promoted effects of miR-24-3p mimics on thyroid papillary carcinomas cells were suppressed by enhancing p27kip1 expression. Meanwhile, MIR22HG induced p27kip1 expression by binding miR-24-3p in thyroid papillary carcinomas.

CONCLUSIONS: MIR22HG inhibited cell growth through modulating p27kip1 by decreasing miR-24-3p expression in thyroid papillary carcinomas, providing a new modulate mechanism and therapeutic targets in thyroid papillary carcinomas.

Key Words:
MIR22HG, miR-24-3p, p27kip1, Regulatory network, Thyroid papillary Carcinomas, Cell growth.

Abbreviations
ncRNAs=Non-Coding RNAs, miRNA=micro RNAs, TPC=Thyroid Papillary Carcinomas, DMEM=Dulbecco's Modified Eagle's Medium, FBS=Fetal Bovine Serum, GAPDH=Glyceraldehyde Phosphate Dehydrogenase, HRP=Horseradish Peroxidase, SD=Standard Deviation.

Introduction

Thyroid cancer is one of the most common malignant cancers, with a rapidly increasing rate worldwide[1]. Thyroid papillary carcinomas are well-differentiated tumors formed by thyroid tumor accumulation and differentiation[2]. Cell proliferation, apoptosis, migration and invasion are important phenotypes for cancer research and play an important role in the understanding of the regulatory mechanisms of tumor cells[3]. At present, many molecular mechanisms for tumors have been studied[4-8], but the regulatory mechanisms between mRNA and ncRNA have not been fully elucidated. Recently, investigations suggested that non-coding RNAs (ncRNAs) widely took part in the regulatory mechanisms of cancer occurrence, development, invasion and migration, including gastric cancer, lung cancer and thyroid cancer[9-12]. Long non-coding RNAs are members of ncRNAs with more than 200 nucleotides and micro RNAs (miRNA) belonged to ncRNAs with 18-24 nucleotides[13,14]. Increasing evidence indicated that lncRNAs act as ceRNA to bind miRNAs to regulate mRNA in cancers cell progression[15-18].

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In thyroid cancer, some regulatory mechanisms of lncRNAs have been verified to participate in cell proliferation and apoptosis. For example, suppression of LncRNA-PANDAR reduced cell proliferation, cell cycle and induced cell apoptosis[19]. Zhou et al[20] showed that lncRNA SPRY4-IT was associated with poor prognosis and promoted cell progression. In addition, lncRNA CRNDE also promoted cell proliferation, invasion and migration in papillary thyroid cancer[21]. In this research, we found that lncRNA MIR22HG has abnormally expressed in thyroid papillary carcinomas. The regulatory network of MIR22HG was predicted using bioinformatics analyses. MIR22HG has been determined to participate in cell progression in hepatocellular carcinoma, endometrial cancer and lung cancer[22-24]. Moreover, MIR22HG was a biomarker and expressed low in thyroid cancer[25], consistently with our results. However, the regulatory mechanism related to MIR22HG has not been fully explored. Therefore, we investigated whether the potential regulatory mechanisms of MIR22HG regulate proliferation, apoptosis, migration and invasion of thyroid cancer cells, demonstrating that MIR22HG regulates p27kip1 by binding to miR-24-3p to affect cell progression in thyroid papillary carcinomas.

Patients and Methods

Patients

40 pairs of thyroid papillary carcinomas tissues and adjacent normal tissues were collected from patients of the First Affiliated Hospital of Guangxi Medical University (Nanning, Guangxi, China), who underwent oral surgery. No patients had received radiotherapy or chemotherapy to surgery. Informed consent was obtained from all patients and/or guardians. All samples were stored at -80°C. This study was approved by Research Ethics of the First Affiliated Hospital of Guangxi Medical University.

Cell Culture and Transfection

The thyroid papillary carcinomas cell lines (TPC-1 and KAT-5 BCPAP, K1, BHP5-16) and normal cell line HT-ori3 were purchased from Cell Bank of Chinese Academy of Sciences and had no mycoplasma contamination. All cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM; Thermo Fisher Scientific, Inc., Waltham, MA, USA) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin (100× solution, Invitrogen, Carlsbad, CA, USA) in a humidified atmosphere containing 5% CO_2. The medium was replaced every other day. pcDNA vector (vector), pcDNA-MIR22HG (MIR22HG), pcDNA-p27kip1 (p27kip1), si-NC, si-MIR22HG#1, si-MIR22HG#2, miRNA scramble control (miR-NC and anti-miR-NC), miR-24-3p mimics and miR-24-3p inhibitors were obtained from Genepharma (Shanghai, China). These oligonucleotides or plasmids were transfected into TCP-1 and BCPAP cell lines using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA).

qRT-PCR

Total RNAs were isolated from thyroid papillary carcinomas tissues and normal tissues using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). TaqMan® MicroRNA Real-time PCR Assay reagents and primers (Applied Biosystems; Foster City, CA, USA) were used to detect miR-24-3p expression. For the expression analysis of MIR22HG and p27kip1, High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA) and random primers were used to generate cDNA. SYBR® Premix Ex Taq™ reagent (TaKaRa, Otsu, Shiga, Japan) was used to measure the relative expression of MIR22HG and p27kip1. Glyceraldehyde phosphate dehydrogenase (GAPDH) and U6 were employed to normalize for MIR22HG / p27kip1 and miR-24-3p, respectively. $2^{-\Delta\Delta}$Ct method was used to calculate the relative expression of MIR22HG /p27kip1 and miR-24-3p. The primer sequences were as follows:

lncRNA MIR22HG forward, 5′-GAUUGAUGGAGGGUGUUGGA-3′ and reverse, 5′-UUCUUCACUUCCAUCCCAUC-3′

miR-24-3p: 5′-TGCGGTGGCTCAGTTCAGCAGGAAC-3′;

U6 forward, 5′-CTCGCTTCGGCAGCACA-3′ and reverse, 5′-AACGCTTCACGAATTTGCGT-3′

P27kip1 Forward, 5′-GGCCCGGTCAATCATGAA-3′ and reverse, 5′-TTGCGCTGACTCG CTTCTTC

GAPDH forward, 5′-GGAGCGAGATCCCTCCAAAAT-3′; GAPDH reverse, 5′-GGCTGTTGTCATACTTCTCATGG-3′.

Western Blot

Total proteins were extracted using RIPA Buffer (Beyotime Biotechnology, Shanghai, China). Equivalent protein was separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto polyvinylidene difluoride (PVDF) membranes (Immobilon-P

membrane, Millipore, Billerica, MA, USA). After that, the membranes were incubated with primary antibodies (primary antibody against p27kip1 and GAPDH; Abcam, Cambridge, MA, USA) and second antibodies (horseradish peroxidase (HRP)-conjugated goat anti-rabbit secondary antibody; Santa Cruz Biotechnology, Santa Cruz, CA, USA). Next, the blots were detected and analyzed using Pierce™ ECL Western Blotting Substrate (Thermo Fisher Scientific, Waltham, MA, USA) and Quantity One software (Bio-Rad Laboratories, Hercules, CA, USA).

Luciferase Reporter Assay

Luciferase reporter vectors (MIR22HG-WT, MIR22HG-MUT, p27kip1-WT, p27kip1-MUT) were constructed based on pRL-TK (Promega, Madison, WI, USA) and were co-transfected with miR-24-3p into TCP-1 and BCPAP cell lines using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA). After harvested at 48 h post-transfection, the relative luciferase activity was measured with the Dual-Luciferase Reporter Assay System (Promega, Madison, WI, USA).

Cell Counting Kit-8 and Colony Formation Assays

Cell Counting Kit-8 and colony formation assays (CCK-8; Dojindo, Laboratories, Kumamoto, Japan) were applied to measure cell proliferation ability according to the manufacturer's protocol. 2×10^3 cells were seeded into 96-well plates and incubated at 37°C for 24 h, 48 h and 72 h. Next, 10 µl CCK-8 solutions were added into each well. After incubation at 37°C for 1 h, the optical density was detected using Microplate Reader at a wavelength of 490 nm.

Transwell Migration and Invasion Assay

Cell migration assay was performed using Transwell chambers (8 µm pore size; Costar, Corning, NY, USA). The transfected TCP-1 and BCPAP cells were cultured in serum-free media (2×10^3 cells per transwell) and seeded in the upper chambers. DMEM media containing 10% FBS were added into the lower chamber as a chemoattractant. After cells were cultured at 37°C for 24 h, cells on the upper chamber were removed using cotton swabs and cells on the lower chamber were stained with crystal violet at 37°C for 15 min. Then, the number of cells was counted using a light microscope (Olympus, Tokyo, Japan). Cell invasion assay was performed in a similar method with Matrigel coatings.

Flow Cytometric Assay

Flow cytometric assay was applied to detect cell apoptosis using Annexin V-FITC Apoptosis Detection Kit (BD Biosciences, Franklin Lakes, NJ, USA) according to the manufacturer's instructions. The transfected TCP-1 and BCPAP cells collected were re-suspended in binding solution (200 µl). After harvesting the transfected cells, cells were stained with Annexin V-FITC (10 µl) and propidium iodide (PI) (10 µl) at 37°C for 15 min. Cell apoptosis rate was analyzed by flow cytometry (BD Biosciences, Franklin Lakes, NJ, USA).

Statistical Analysis

All data in this study were performed and displayed using SPSS 18.0 software (SPSS Inc., Chicago, IL, USA) and GraphPad Prism (GraphPad Software, La Jolla, CA, USA). Each experiment has at least 3 times independent experiments and data are presented as the mean ± standard deviation (SD). Student's *t*-test was used to analyze all comparisons to assess the significance of the results. Pearson's correlation analysis was used to analyze the correlation relationship among MIR22HG, miR-24-3p and p27kip1expression. $p < 0.05$ was considered as statistically significant.

Results

LncRNA MIR22HG Expressed Low in Thyroid Papillary Carcinomas

In clinical treatment, we found that the expression of lncRNA MIR22HG was correlated with clinical characteristics in thyroid papillary carcinomas. Thus, we selected 40 patients with thyroid papillary carcinomas to carry out further investigations. The results showed that down-regulation of lncRNA MIR22HG was related with the tumor size (p=0.015, *p< 0.05), TNM stage (p=0.022, *p< 0.05) (Table I). Moreover, lncRNA MIR22HG was downregulated in tumor tissues compared with non-tumor tissues (Figure 1A). In addition, the results of qRT-PCR showed that lncRNA MIR22HG significantly expressed low in TPC-1, KAT-5, BCPAP, K1 and BHP5-16 cells compared with normal cell line (HT-ori3) (Figure 1B). Meanwhile, the total survival rate of low MIR22HG was lower than that of high MIR22HG in thyroid papillary carcinomas (Figure 1C). Therefore, MIR22HG was remarkably correlated with thyroid papillary carcinomas.

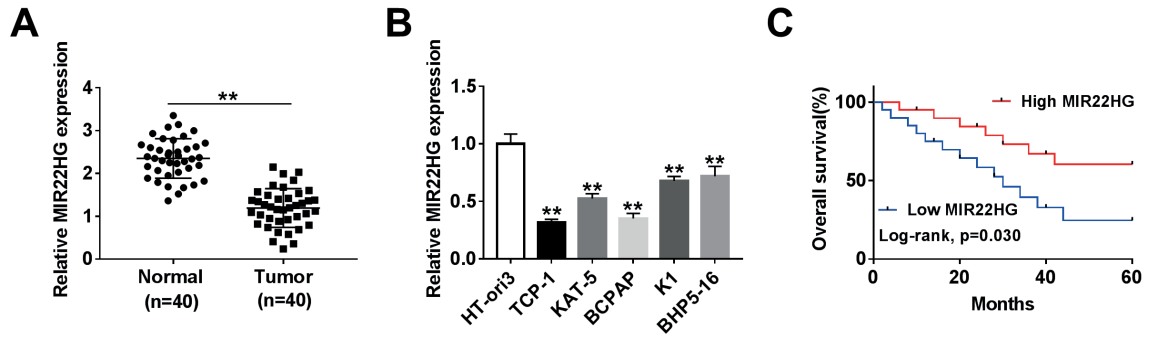

Figure 1. LncRNA MIR22HG expressed low in thyroid papillary carcinomas. ***(A)*** The mRNA expression of MIR22HG was detected in thyroid papillary carcinomas tissues and non-tumor tissues using qRT-PCR assay. ***(B)*** The mRNA expression of MIR22HG was detected in thyroid papillary carcinomas cell lines (TPC-1 and KAT-5 BCPAP, K1, BHP5-16) and normal cell line (HT-ori3) using qRT-PCR. ***(C)*** The relationship between MIR22HG and overall survival rates was analyzed using Kaplan-Meier plot. *$p < 0.05$.

Table I. The clinical characteristics and MIR22HG expression in patients with thyroid cancer.

Parameters	Total	LINC00460 expression		*p*-value
		Low (n=20)	High (n=20)	
Age				
< 60	18	10	8	0.540
≥ 60	22	10	12	
Gender				
Male	14	8	6	0.621
Female	26	12	14	
Tumor size (cm)				
< 1	18	6	12	0.015*
≥ 1	22	14	8	
TNM stage				
I-II	23	9	14	0.022*
III-IV	17	11	6	
Lymph node metastasis				
No	24	10	14	0.036*
Yes	16	10	16	

Up-Regulation of MIR22HG Affected Cell Progression in Thyroid Papillary Carcinomas

We selected TPC-1 and BCPAP cell lines to verify the function of MIR22HG. The vector of MIR22HG and vector were transfected into TPC-1 and BCPAP cell lines, respectively, and the expression of MIR22HG was significantly higher in MIR22HG groups than that in Vector groups (Figure 2A). CCK-8 revealed that cell proliferation of MIR22HG groups was obviously decreased compared with that of Vector groups in TCP-1 and BCPAP cell lines (Figure 2B and 2C). In addition, we used flow cytometric to detect cell apoptosis and the results revealed that cell apoptosis rate was significantly increased in MIR22HG groups (Figure 2D). Furthermore, the results of transwell showed that upregulated MIR22HG expression remarkably inhibited cell migration and invasion (Figure 2E and 2F). Therefore, overexpression of MIR22HG could suppress cell growth and promote cell apoptosis.

miR-24-3p was a Direct Target of MIR22HG

To further explore the underlying regulatory mechanism of MIR22HG, the bioinformatics analysis was used to predict its target miRNA; the results showed that mir-24-3p was a potential target of MIR22HG (Figure 3A). Then, by using

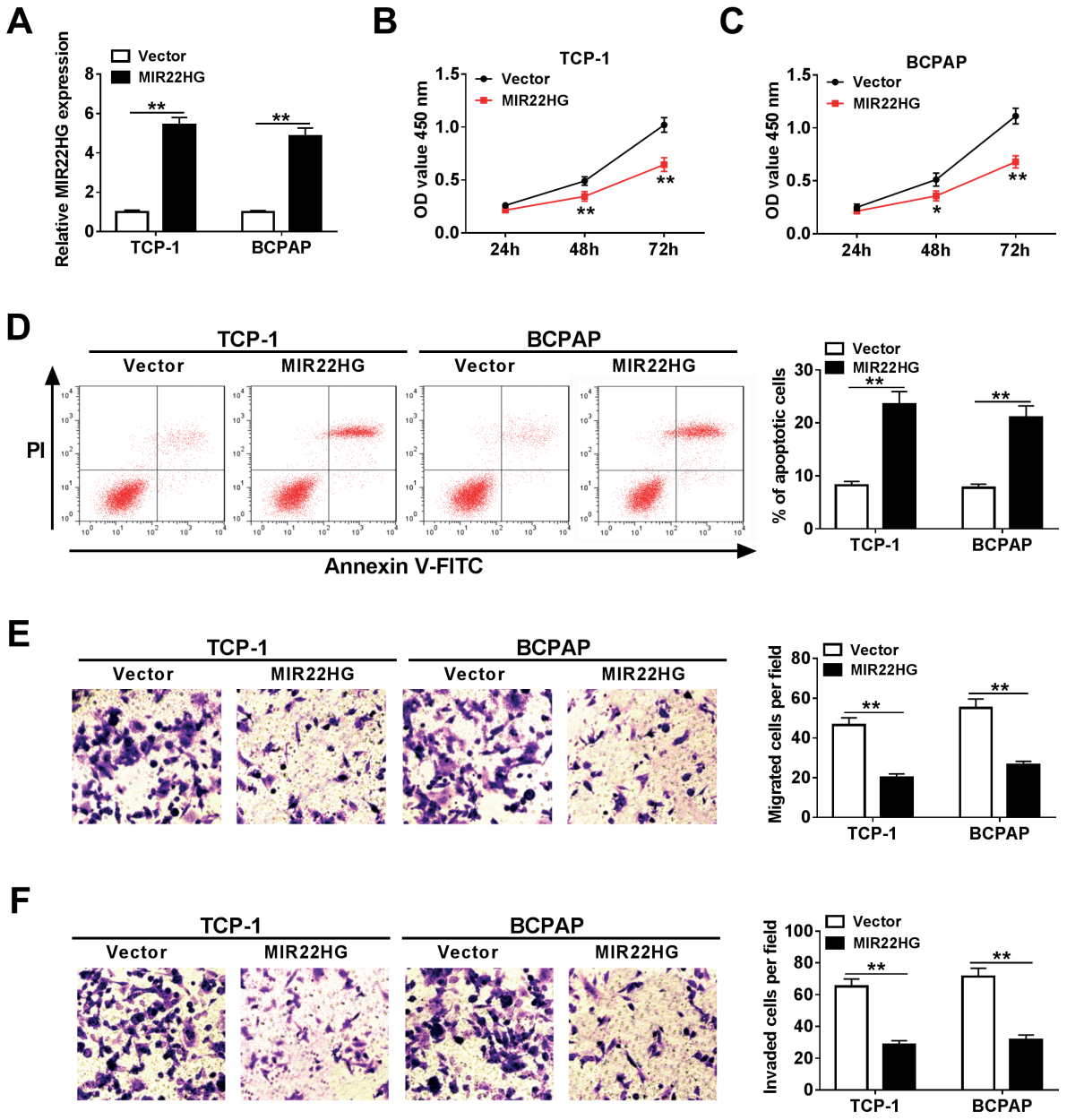

Figure 2. Up-regulation of MIR22HG affected cell progression in thyroid papillary carcinomas. ***(A)*** The mRNA expression of MIR22HG was detected in TCP-1 and BCPAP cells transfected with vector and MIR22HG using qRT-PCR. (B and C) cell proliferation was measured in TCP-1 ***(B)*** and BCPAP ***(C)*** cells transfected with vector and MIR22HG with CCK-8 assay. ***(D)*** Cell apoptosis was calculated in TCP-1 and BCPAP cells transfected with vector and MIR22HG using flow cytometric assay. (E and F) cell migration ***(E)*** and invasion ***(F)*** was detected in TCP-1 and BCPAP cells transfected vector and MIR22HG using transwell assay. * $p < 0.05$.

luciferase reporter assays, we found that the luciferase activity of MIR22HG-WT was significantly decreased by binding to the sites of miR-24-3p, but not MIR22HG-MUT (Figure 3B and 3C). Otherwise, overexpression of MIR22HG inhibited miR-24-3p expression in TCP-1 and BCPAP cell lines (Figure 3D). Meanwhile, we also transfected si-NC, si-MIR22HG#1and si-MIR22HG#2 into TCP-1 and BCPAP cell lines and qRT-PCR analysis showed that MIR22HG expressed lower and miR-24-3p expressed higher in si-MIR22HG#1 and si-MIR22HG#2 than that in si-NC (Figure 3E and 3F). We also detected that miR-24-3p expression was significantly en-

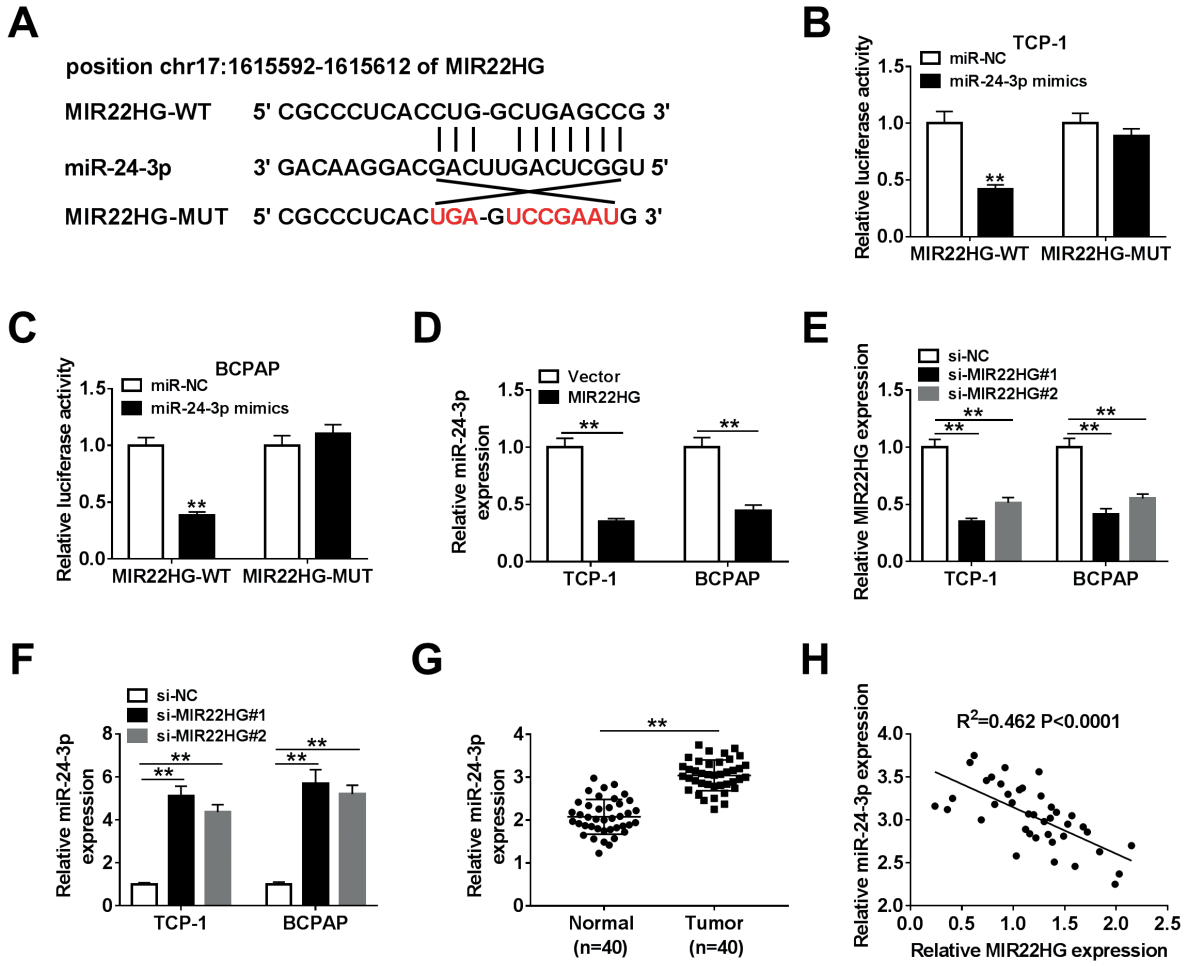

Figure 3. miR-24-3p was a direct target of MIR22HG. ***(A)*** The prediction of miR-24-3p binding sites on the MIR22HG transcript using software of starBase. (B and C) Luciferase activities were measured in TCP-1 ***(B)*** and BCPAP ***(C)*** cells cotransfected with MIR22HG-WT, MIR22HG-MUT and miR-NC, miR-24-3p mimics respectively using luciferase reporter assay. ***(D)*** The mRNA expression of miR-24-3p was measured in TCP-1 and BCPAP cells transfected with vector and MIR22HG using qRT-PCR. ***(E*** and ***F)*** The mRNA expression of MIR22HG ***(E)*** and miR-24-3p ***(F)*** were detected in TCP-1 and BCPAP cells transfected with si-NC, si-MIR22HG#1 and si-MIR22HG#2 using qRT-PCR. ***(G)*** The mRNA expression of miR-24-3p was detected in thyroid papillary carcinomas tissues and non-tumor tissues using qRT-PCR assay. ***(H)*** Pearson's correlation analysis was used to detect the relationship between MIR22HG and miR-24-3p. * $p < 0.05$.

hanced in tumor tissues compared with normal tissues (Figure 3G). More than that, correlation analysis indicated that MIR22HG expression was negatively correlated with miR-24-3p expression in tumor tissues of thyroid papillary carcinomas (Figure 3H). In summary, these results displayed that miR-24-3p was a target of MIR22HG in thyroid papillary carcinomas.

miR-24-3p Rescue the Effects of MIR22HG on Cell Progression in Thyroid Papillary Carcinomas

As shown in Figure 4A, the vector, MIR22HG, MIR22HG + miR-NC mimics and MIR22HG + miR-24-3p mimics were transfected into TCP-1 and BCPAP cell lines, respectively, and we found that upregulating MIR22HG inhibited the expression of miR-24-3p which was rescued by miR-24-3p mimics. Furthermore, cell proliferation, apoptosis, migration and invasion were detected, and the analysis of results showed that overexpression of MIR22HG inhibited cell proliferation, migration and invasion, and induced cell apoptosis. However, upregulating miR-24-3p could reverse the effects of high MIR22HG on TCP-1 and BCPAP cell lines in thyroid papillary carcinomas (Figure 4B-4F).

p27kip1 was a Target Gene of miR-24-3p

To further elucidate the regulatory network of miR-24-3p, we found that p27kip1 has comple-

ment sites to bind to miR-24-3p in 3'UTR through using bioinformatics software targetScan, which could be a potential target gene of miR-24-3p (Figure 5A). To verify these predicted results, we also constructed a luciferase reporter vector containing p27kip1-WT and p27kip1-MUT. The result showed that miR-24-3p mimics observably inhibited the luciferase activity of p27kip1-WT, but not p27kip1-MUT in TCP-1 and BCPAP cell lines (Figure 5B and 5C). Then, qRT-PCR and Western blot analysis showed that overexpression of miR-24-3p decreased the p27kip1 mRNA and protein expression (Figure 5D and 5F) and downregulation of miR-24-3p promoted the p27kip1 mRNA and protein expression in TCP-1 and BCPAP cell lines (Figure 5E and 5G). Notably, p27kip1 expressed lower in tumor tissues than that in normal tissues (Figure 5H). Pearson's correlation analysis indicated that miR-24-3p expression was negatively correlated with p27kip1 expression in thyroid papillary carcinomas (Figure 5I). These results suggested that p27kip1 was a target gene of miR-24-3p.

MiR-24-3p Regulated Cell Proliferation, Apoptosis, Migration and Invasion Through Targeting p27kip1 in Thyroid Papillary Carcinomas

Here, we further investigated the function of miR-24-3p and p27kip1 in thyroid papillary carcinomas. miR-NC, miR-24-3p mimics, miR-24-3p mimics + Vector and miR-24-3p+p27kip1 were transfected into TCP-1 and BCPAP cell lines, respectively. As shown in Figure 6A-6C, the p27kip1 mRNA and protein expression were significantly decreased by overexpression of miR-24-2p, but transfection of p27kip1 could reverse its mRNA and protein expression in TCP-1 and BCPAP cell lines by qRT-PCR and Western blot assays. Also, miR-24-3p mimics transfection impaired cell proliferation, migration and invasion (Figure 6D, 6E, 6G, 6H), which were recovered by upregulating p27kip1 expression. Otherwise, cell apoptosis was promoted by miR-24-3p mimics, which were significantly reversed by p27kip1 overexpression (Figure 6F). Thus, the suppressive effects of

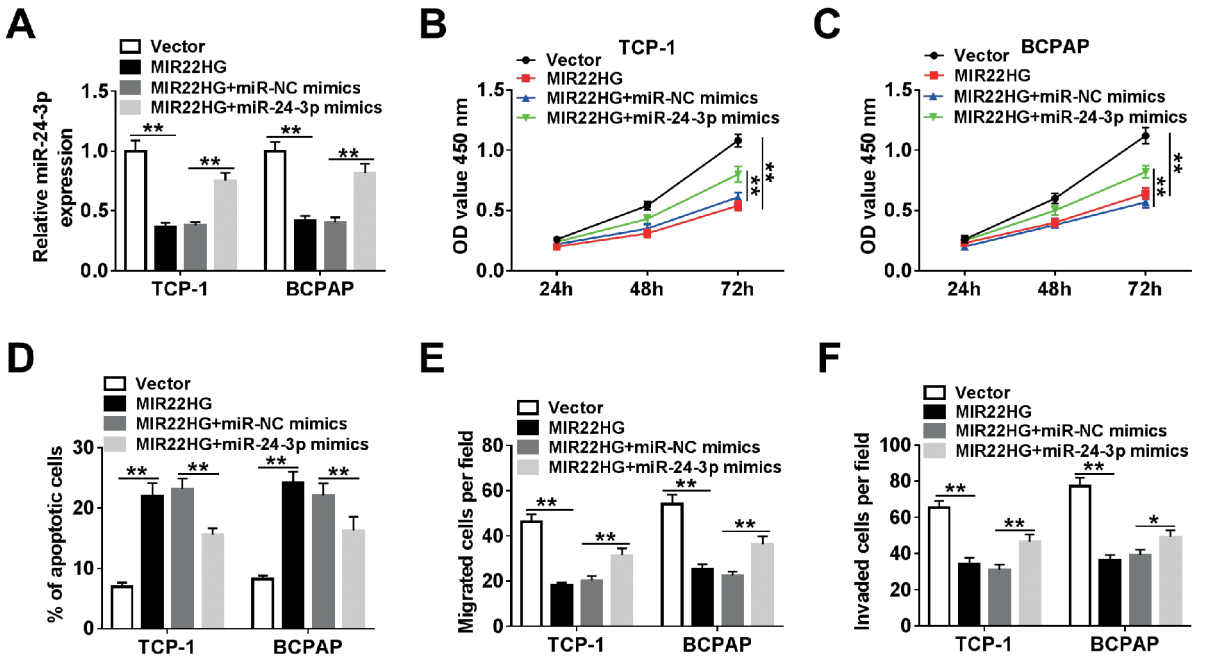

Figure 4. miR-24-3p rescue the effects of MIR22HG on cell progression in thyroid papillary carcinomas. ***(A)*** The mRNA expression of miR-24-3p was detected in TCP-1 and BCPAP cells transfected with vector, MIR22HG, MIR22HG + miR-NC mimics and MIR22HG + miR-24-3p mimics using qRT-PCR assay. (B and C) Cell proliferation was determined in TCP-1 ***(B)*** and BCPAP ***(C)*** cells transfected with vector, MIR22HG, MIR22HG + miR-NC mimics and MIR22HG + miR-24-3p mimics using CCK-8 assay. ***(D)*** Cell apoptosis rate was calculated in TCP-1 and BCPAP cells transfected with vector, MIR22HG, MIR22HG + miR-NC mimics and MIR22HG + miR-24-3p mimics using flow cytometric assay. (E and F) cell migration ***(E)*** and invasion ***(F)*** was determined in TCP-1 and BCPAP cells transfected with vector, MIR22HG, MIR22HG + miR-NC mimics and MIR22HG + miR-24-3p mimics using transwell assay. * $p < 0.05$.

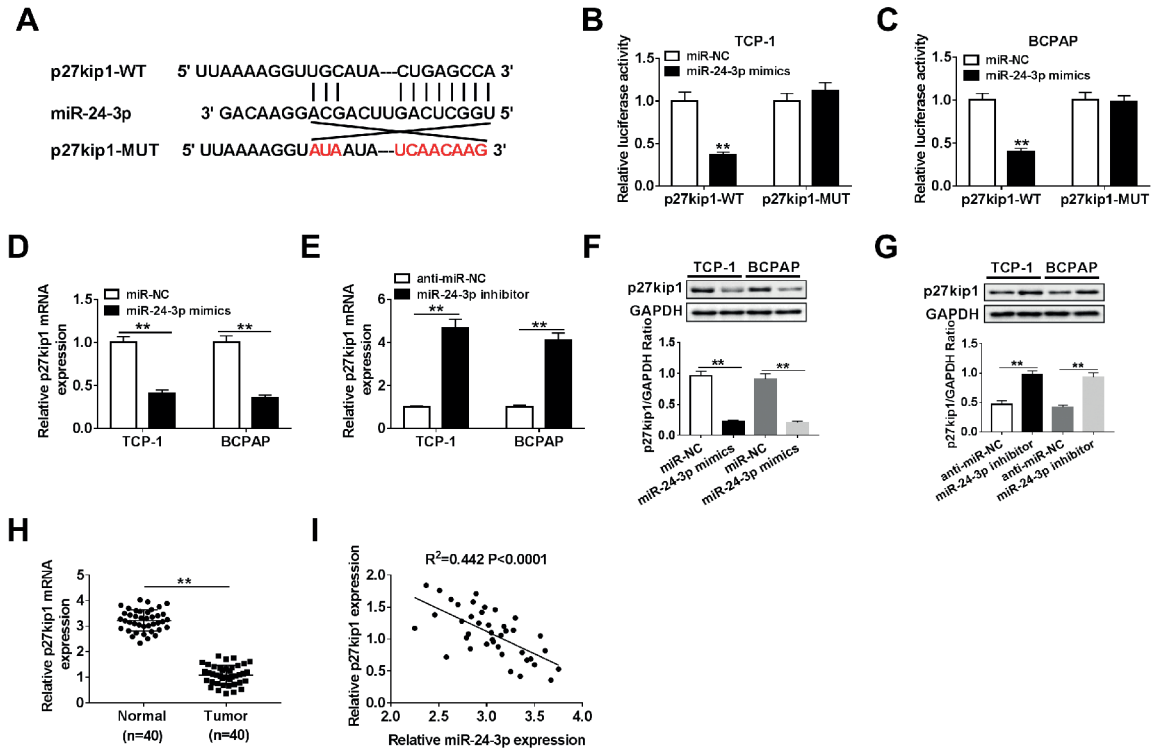

Figure 5. p27kip1 was a target gene of miR-24-3p. ***(A)*** The prediction of miR-24-3p binding sites on the p27kip1 transcript using software of Targetscan. (B and C) Luciferase activities were measured in TCP-1 ***(B)*** and BCPAP ***(C)*** cells cotransfected with p27kip1-WT, p27kip1-MUT and miR-NC, miR-24-3p mimics respectively using luciferase reporter assay. (D and F) The mRNA ***(D)*** and protein ***(F)*** expression of p27kip1 were measured in TCP-1 and BCPAP cells transfected with miR-NC and miR-24-3p mimics using qRT-PCR and Western blot assays respectively. (E and G) The mRNA ***(E)*** and protein ***(G)*** expression of p27kip1 were measured in TCP-1 and BCPAP cells transfected with anti-miR-NC and miR-24-3p inhibitor using qRT-PCR and Western blot assays respectively. ***(H)*** The mRNA expression of p27kip1was detected in thyroid papillary carcinomas tissues and non-tumor tissues using qRT-PCR assay. ***(I)*** Pearson's correlation analysis was used to detect the relationship between p27kip1 and miR-24-3p. *$p < 0.05$.

miR-24-3p mimics on cell progression were alleviated by p27kip1 overexpression.

MIR22HG Regulated Cell Growth Through Modulating miR-24-3p/p27kip1 Axis in Thyroid Papillary Carcinomas

Western blot and qRT-PCR analysis determined that p27kip1 mRNA and protein expression were promoted by MIR22HG overexpression, which was inhibited by miR-24-3p mimics in TCP-1 and BCPAP cell lines (Figure 7A-7C). Meanwhile, the expression of MIR22HG was positively correlated with p27kip1 expression in tumor tissues (Figure 7D). In summary, MIR22HG affects cell progression by regulating miR-24-3p/p28kip1 axis in thyroid papillary carcinomas.

Discussion

With the development of research techniques, many lncRNA regulatory mechanisms in thyroid cancer have been reported, most of which are combined with miRNAs to regulate downstream target mRNAs affecting the development and growth of cancer cells[21,26-28]. lncRNA TUG1 affected cell progression and EMT formation by targeting miR-145[29]. Zhang et al[30] showed that lncRNA Gas5 acts as a ceRNA to modulate PTEN by binding miR-222-3p in thyroid papillary carcinomas. Therefore, the study of regulatory networks in thyroid cancer could be helpful to further clarify the occurrence of cancer and provide new therapeutic targets and new ideas for clinical treatment. To date, increasing evidence has de-

termined that MIR22HG plays a crucial role in cancer development, such as cell invasion, proliferation, migration and apoptosis[22,24]. In addition, MIR22HG is also an essential regulator of thyroid cancer and a hallmark of its survival prognosis[25]. Therefore, we focus on the regulation of MIR22HG in thyroid cancer. In our results, MIR22HG expression was significantly reduced in thyroid cancer tissues, and low expression of MIR22HG was obviously associated with thyroid tumor size, TNM stage, and overall patient survival. At the same time, we further studied that high expression of MIR22HG remarkably inhibited cell proliferation, migration and invasion, and significantly induced cell apoptosis rate in thyroid papillary carcinomas. These results suggested that MIR22HG does play an important regulatory role in the development and prognosis of thyroid papillary carcinomas. It is well known that lncRNA does not function alone in cell progression,

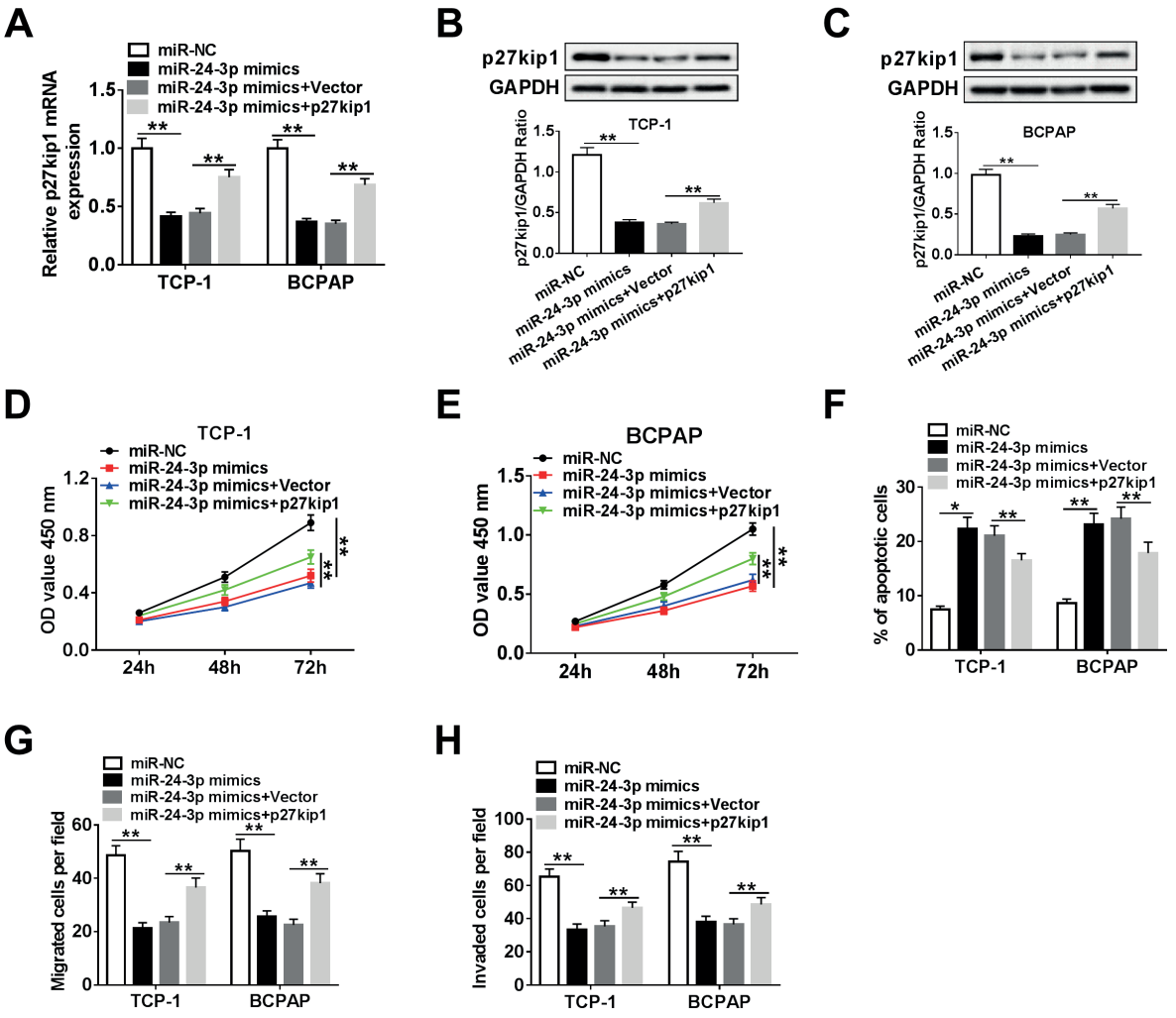

Figure 6. miR-24-3p regulated cell proliferation, apoptosis, migration and invasion through targeting p27kip1 in thyroid papillary carcinomas. ***(A)*** The mRNA expression of p27kip1 was detected in TCP-1 and BCPAP cells transfected with miR-NC, miR-24-3p mimics, miR-24-3p mimics + Vector and miR-24-3p+p27kip1 using qRT-PCR assay. (B and C) The protein expression of p27kip1 was detected in TCP-1 and BCPAP cells transfected with miR-NC, miR-24-3p mimics, miR-24-3p mimics + Vector and miR-24-3p+p27kip1 using Western blot. (D and E) Cell proliferation was determined in TCP-1 ***(D)*** and BCPAP ***(E)*** cells transfected with miR-NC, miR-24-3p mimics, miR-24-3p mimics + Vector and miR-24-3p+p27kip1 using CKK-8 assay. ***(F)*** Cell apoptosis rate was calculated in TCP-1 and BCPAP cells transfected with miR-NC, miR-24-3p mimics, miR-24-3p mimics + Vector and miR-24-3p+p27kip1 using flow cytometric assay. (G and H) Cell migration ***(G)*** and invasion ***(H)*** was determined in TCP-1 and BCPAP cells transfected with miR-NC, miR-24-3p mimics, miR-24-3p mimics + Vector and miR-24-3p+p27kip1 using transwell assay. *$p < 0.05$.

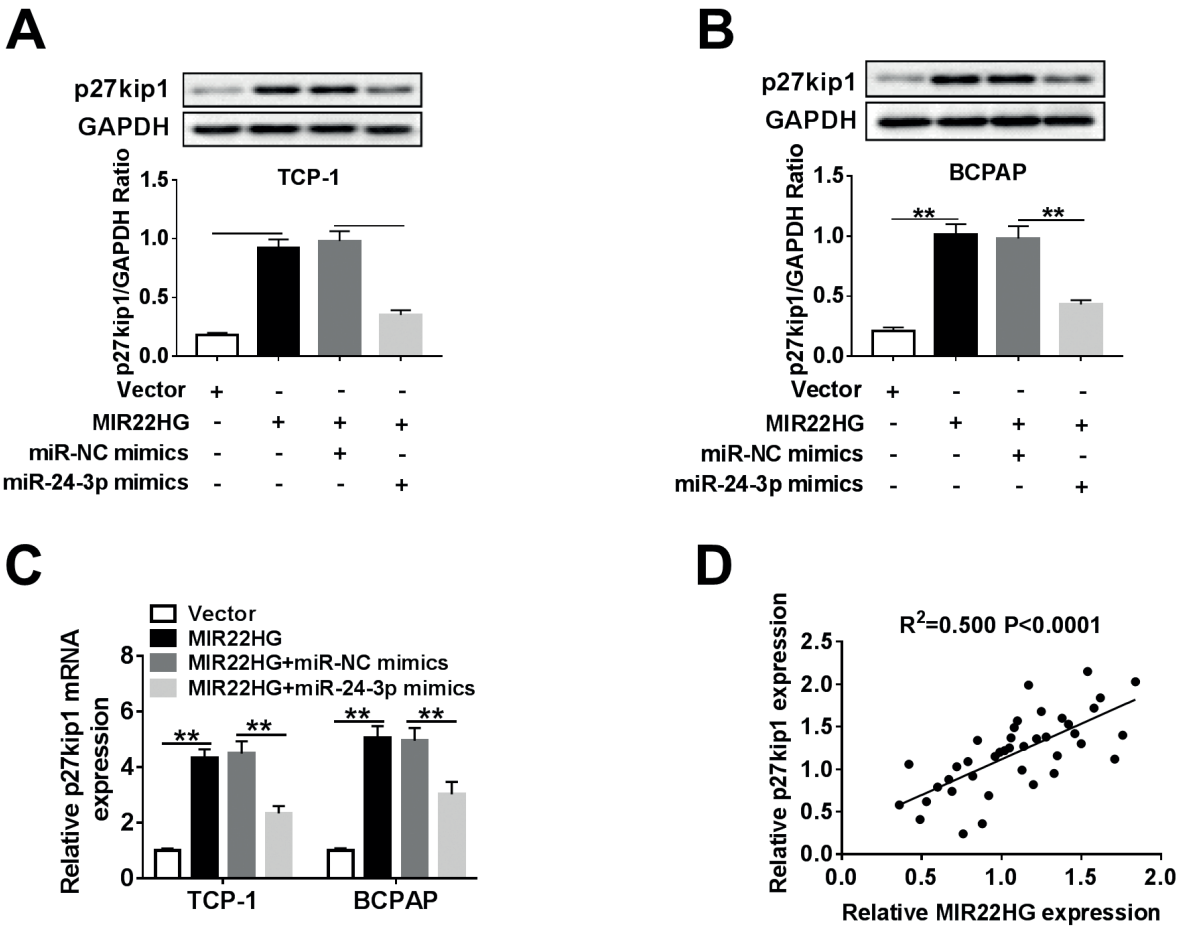

Figure 7. MIR22HG regulated cell growth through modulate miR-24-3p/p27kip1 axis in thyroid papillary carcinomas. (A and B) The protein expression of p27kip1 was determined in TCP-1 ***(A)*** and BCPAP ***(B)*** cells transfected with vector, MIR22HG, MIR22HG + miR-NC mimics and MIR22HG + miR-24-3p mimics using Western blot. ***(C)*** The mRNA expression of p27kip1 was determined in TCP-1 and BCPAP cells transfected with vector, MIR22HG, MIR22HG + miR-NC mimics and MIR22HG + miR-24-3p mimics using qRT-PCR assay. ***(D)*** Pearson's correlation analysis was used to detect the relationship between MIR22HG and p27kip1. *$p < 0.05$.

and it is usually associated with miRNAs and mRNAs to form regulatory networks that affect cell growth and development in cancers[31,32]. Thus, we used bioinformatics analysis and luciferase reporter assay to determine that miR-24-3p was a target miRNA of MIR22HG. Evidence showed that miR-24-3p was also closely associated with cancer development and drug resistance, such as colorectal cancer[33], small cell lung cancer[34] and hepatocellular carcinoma[35]. In this study, we found that miR-24-3p expressed high in thyroid papillary carcinomas and Pearson's correlation assay determined that MIR22HG expression was negatively correlated with miR-24-3p expression. Further functional experiments indicated that inhibition of miR-24-3p expression could significantly reduce the inhibitory effect of high expression of MIR22HG on cell growth in thyroid papillary carcinomas. To further clarify the regulatory network of MIR22HG, we predicted and demonstrated that p27kip1 is a downstream target gene regulated by miR24-3p. Both qRT-PCR and Western blot showed that p27kip1 was down-regulated in thyroid carcinoma, negatively correlated with miR-24-3p expression, and positively correlated with MIR22HG expression. Some studies determined that p27kip1 acts as a key tumor suppressor, and could take part in tumor occurrence, development, prognostic and drug resistance [36,37], including breast cancer and

thyroid cancer [38-40]. However, the regulatory network among MIR22HG, miR-24-3p and p27kip1 was first verified in this paper. In our study, the analysis of CCK-8, transwell and flow cytometry showed that the inhibition of cell progression by miR-24-3p mimics can be reversed by high expression of p27kip1, demonstrating that miR-24-3p regulates cell proliferation, migration, invasion and apoptosis by regulating the expression of p27kip1 in thyroid papillary carcinomas. Finally, the results of the reversal experiments indicated that MIR22HG regulates the expression of p27kip1 by miR-24-3p. Therefore, based on all the results, we have reached an important conclusion that MIR22HG affects the proliferation and apoptosis of cells by regulating the miR-24-3p/p27kip1 axis in thyroid papillary carcinomas. The identification of this regulatory network marks the birth of a new therapeutic target and treatment idea.

Conclusions

We indicated that MIR22HG inhibited cell growth through modulating p27kip1 by decreasing miR-24-3p expression in thyroid papillary carcinomas, providing a novel modulate mechanism and therapeutic targets in thyroid papillary carcinomas.

Conflict of interest

The authors declare no conflicts of interest.

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