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p53-associated miRNAs repress lncRNA *ZFAS1* to retard the proliferation of papillary thyroid carcinoma

***Gang Wang*[1, 2], *Liping Wei*[3], *Hui Yang*[1]**

[1]*Department of General Surgery (Thyroid Surgery), Affiliated Hospital of Southwest Medical University, Southwest Medical University, Luzhou, China*
[2]*Cardiovascular and Metabolic Diseases Key Laboratory of Luzhou, Southwest Medical University, Luzhou, China*
[3]*Pediatric Surgery, the Affiliated Hospital of Southwest Medical University, Southwest Medical University, Luzhou, China*

Abstract

Introduction: Thyroid carcinoma is the most frequent malignancy among endocrine-related tumours. Papillary thyroid carcinoma (PTC) is the main type of thyroid carcinoma, and almost 80% cases of thyroid carcinoma are diagnosed as PTC. The molecular mechanism underlying PTC progression is unclear. This study aims to investigate the potential mechanisms of zinc finger antisense 1 (*ZFAS1*) function in PTC.
Material and methods: The expression of *ZFAS1* and p53 was determined by quantitative polymerase chain analysis (qPCR) in PTC tissues derived from 20 PTC patients. Quantitative chromatin immunoprecipitation assay (qChIP) analysis was performed to validate the target of *ZFAS1*/p53 and miRNAs/p53. The Gene Expression Omnibus (GEO) dataset GSE94908 was analysed to obtain the differentially expressed p53-associated microRNAs (miRNAs). Luciferase assay validated the target of *ZFAS1*/miRNAs, and 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was used to determine the cell proliferation.
Results: The expression of *ZFAS1* was up-regulated in the tissues derived from PTC patients, and the expression of *ZFAS1* was negatively associated with p53 expression in PTC. The expression of *ZFAS1* was significantly higher in the MDA-T120 cells harbouring mutant *p53*. We validated that *ZFAS1* is a direct target of p53. In PTC cells, p53 directly repressed the *ZFAS1* expression. In addition, we determined that miR-135b-5p and miR-193a-3p are directly induced by p53 in PTC cells. Interestingly, p53-targeted miR-135b-5p, miR-193a-3p, and miR-34b repressed the expression of *ZFAS1* via the seed-matching sequences in the 3′-untranslated region (3′-UTR) of *ZFAS1*, and thereby suppressed PTC cell proliferation induced by *ZFAS1*.
Conclusion: The oncogenic lncRNA *ZFAS1* is directly repressed by p53 in PTC. p53-mediated miRNAs including miR-135b-5p, miR-193a-3p, and miR-34b repress *ZFAS1* expression, and thereby inhibit the proliferation of PTC. **(Endokrynol Pol 2024; 75 (1): 12–19)**

Key words: *papillary thyroid carcinoma; ZFAS1; p53; miR-135b-5p; miR-193a-3p*

Introduction

Thyroid carcinoma is the most frequent malignancy among endocrine-related tumours [1]. Thyroid carcinoma occurrence has been gradually increasing in the last 2 decades, and now the occurrence rate ranks ninth in all human cancers [2]. Papillary thyroid carcinoma (PTC) is the main type of thyroid carcinoma, and almost 80% cases of thyroid carcinoma are diagnosed as PTC [1]. Current treatments including surgery and radioactive iodine therapy increase the 5-year survival rate of PTC to over 95%, but further developing progressive diseases are still observed in PTC patients due to metastasis and tumour recurrence [3]. Around 10–15% of PTC patients will develop metastasis after receiving treatments. Therefore, it is an urgent need to reveal the molecular mechanism underlying PTC progression and provide a means to prevent PTC progression.

Long non-coding RNAs (lncRNAs) comprise a class of RNA molecules that exceed 200 nucleotides in length and lack protein coding abilities [4]. A growing body of research has demonstrated that lncRNA expression dysregulation is associated with various diseases, including cancer. Recent biomedical investigations have indicated that lncRNAs are crucial for the progression and development of PTC [5]. Zinc finger antisense 1 (*ZFAS1*), a type of lncRNA, has been identified as an oncogenic lncRNA [6]. In gastric cancer, *ZFAS1* exerts oncogenic effects by interacting with enhancer of zeste homologue 2 (EZH2) [7]. Additionally, upregulation of *ZFAS1* in head and neck squamous cell carcinoma correlates positively with metastasis and cancer initiation [8]. In thyroid carcinoma, downregulation of *ZFAS1* has been shown to hinder PTC progression by modulating microRNAs (miRNAs), which could serve as potential biomarkers for predicting thyroid cancer prognosis [9, 10]. However, due to limited studies, the detailed molecular mecha-

Hui Yang, Department of General Surgery (Thyroid Surgery), Affiliated Hospital of Southwest Medical University, Taiping Rd. No. 25, Luzhou, 646099, PR China, tel: 0086-830-3165431; e-mail: yanghui_lzmu@gmx.net

nism underlying the role of *ZFAS1* in PTC proliferation and progression remains unclear. Therefore, this study aims to investigate the potential mechanisms of *ZFAS1* function in PTC.

Material and methods

Tissues from PTC patients

Tumour and normal PTC samples were derived from inpatients (n = 20, from February 2017 to September 2020) with approval of the Ethis Committee of Southwest Medical University (No. SMU-22-091). RNAs were isolated from tissues following the instruction provided by the manufacturer of the RNeasy Kit (QIAGEN).

Cell culture and transfections

MDA-T32 and MDA-T120 cells were purchased from the American Type Culture Collection (ATCC). Cells were maintained in a base medium of Roswell Park Memorial Institute 1640 medium (RPMI-1640) containing 5% foetal bovine serum (FBS), 0.5% L-glutamine, and 0.5% non-essential amino acid (NEAA) (Gibco 11140-050) at 37°C in 5% carbon dioxide (CO_2). For transfections, 2×10^5 cells/well were seeded into 6-well plates 24 hours before transfection. The final concentrations of small interfering RNA (siRNA) pools (QIAGEN) and vectors were 12.5 nmol/L and 2.0 μg/mL, respectively. Lipofectamine 3000 (Invitrogen, United States) was used for the transfection according to the protocol provided by the manufacturer. Functional experiments were performed after 48-hour transfection. *ZFAS1* and p53 ectopic expression were achieved by transfecting cells with pcDNA3.1 vector containing the mRNA sequence of *ZFAS1* and the coding sequence (CDS) region of *p53*, and thereafter the vectors were named pcD-*ZFAS1* and pcD-p53, respectively. The vectors were constructed by GenePharma (Shanghai, China).

RNA isolation and quantitative polymerase chain (qPCR) analysis

After different treatments, the total RNA was isolated from cells using a High Pure RNA isolation kit (Roche) according to the provided instructions. The isolated RNA was quantified, and a total 100 ng of RNA in each sample was subjected to the cDNA synthesis by a verso cDNA synthesis kit (Thermo Fisher). The quantitative polymerase chain reaction (qPCR) was performed by using SYBR Green mix (Thermo Fisher) on the Applied Biosystems ABI 7900HT Real Time Thermo Cycler (Thermo Fisher) platform. The relative gene expression was calculated by 2^(-ΔΔCT) and normalized to glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*), as previously described. The primers used in qPCR analysis are listed in Table 1.

Western blot analysis

Total protein was extracted with radioimmunoprecipitation assay (RIPA) lysis buffer (Beyotime, China) supplemented with the proteinase inhibitors phenylmethylsulphonyl fluoride (PMSF) (Roche, United States) and PI (Thermo, United States). Proteins were separated by 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) (Bio-Rad, United States). β-actin was used as an internal control.

Table 1. ***Primers used in quantitative polymerase chain reaction (qPCR) analysis***

Primers	Sequences 5′-3′
GAPDH Forward	GGAGCGAGATCCCTCCAAAAT
GAPDH Reverse	GGCTGTTGTCATACTTCTCATGG
ZFAS1 Forward	AAAGAGAGCGTTTCGGGTCC
ZFAS1 Reverse	GCTCTAACGGGCAGGACAAT
p53 Forward	CAGCACATGACGGAGGTTGT
p53 Reverse	TCATCCAAATACTCCACACGC

Table 2. ***Primers used in quantitative chromatin immunoprecipitation (qChIP) analysis***

Primers	Sequences 5′-3′
ZFAS1 qChIP Forward	AGCCGGGCGGGCGGCCCA
ZFAS1 qChIP Reverse	AGGGAAGGGAGGACCAGAGG
MIR135B qChIP Forward	GCAGATTCCAAGCTGACCTCA
MIR135B qChIP Reverse	TATGGCTTTTCATTCCTATGTGATTG
MIR193A qChIP Forward	CCGGGCAGGTCGAGCGAG
MIR193A qChIP Reverse	CGGGGATTTCCCGAATCA

Quantitative chromatin immunoprecipitation assay

The quantitative chromatin immunoprecipitation (qChIP) assay was performed using a commercial kit (Beyotime, Shanghai, China) according to the manufacturer's instructions. An antibody against p53 was used to immunoprecipitate p53-chromatin complexes. Immunoglobulin G antibody (anti-IgG) (Santa Cruz, United States) served as a negative control. The ChIP products were amplified by PCR. The primers used in the qChIP analysis are listed in Table 2.

Luciferase assay

Luciferase vectors were purchased from Genescript (China). pMIR-report luciferase vectors (Promega, USA) containing binding sites for miR-135b-5p and miR-193a-3p on *ZFAS1* 3′-UTR were constructed. The mutations of the binding sites of miR-135b-5p and miR-193a-3p were achieved by using the ClonExpress MultiS One Step Cloning Kit (C113-01, Vazyme, China). When transfecting MDA-T32 cells with luciferase vectors and miRNA mimics purchased from Qiagen (Germany). A β-galactosidase (β-gal) expression vector (Ambion) was transfected together as an internal control. Luciferase activity was tested using a luciferase assay kit (Promega, United States).

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay

MDA-T120 (3×10^3 cells/mL) were seeded onto 96-well plates and incubated for 24 h, then incubated with 20 μl of MTT solution (5 mg/mL in PBS) for another 1 h. After the removal of culture me-

Table 3. ***Up-regulated miRNAs in RKO p53$^{+/+}$ cells compared to RKO p53$^{-/-}$ cells***

Up-regulated miRNAs	logFC	Adj. P. Val.
miR-135b-5p	1.847442689	4.32218E-08
miR-200b	1.409154124	3.26282E-06
miR-193a-3p	1.395380763	9.10192E-07
miR-221	1.317745855	6.93131E-06
miR-34a	1.313187698	3.21103E-05
miR-200a	1.223208973	2.85309E-07
miR-29b-1	1.153742891	8.91551E-05
miR-222	1.126664429	0.000367632
miR-34b	1.015849723	1.324E-05

dium, converted purple formazan dye from MTT was solubilized in dimethyl sulphoxide (DMSO; Sigma-Aldrich; Merck KGaA, Darmstadt, Germany). Finally, optical densities from all the samples were measured at 595 nm by a microplate reader (BioRad, Hercules, CA, United States).

Bioinformatics analysis

The ChIP-seq data was analysed from the Cistrome DB (http://cistrome.org/db/#/). The target between miRNAs and *ZFAS1* was screened by using ENCORI and the TargetScan database. The differentially expressed miRNAs with different *p53* status in RKO cells were obtained and analysed from GSE94908.

Statistics

Data were processed using SPSS 19.0 and presented as mean ± standard deviation. Statistical significance was determined using analysis of variance for multiple comparisons followed by Tukey's test. $p < 0.05$ was considered statistically significant.

Results

ZFAS1 is negatively associated with p53 in PTC

Our previous study indicated that transcription factor directly controls the expression of *ZFAS1* in PTC [11]. In addition, miR-34b, a p53-induced miRNA, is capable of repressing *ZFAS1* via the direct target in the 3'UTR of *ZFAS1* [12]. Therefore, we would like to know whether *ZFAS1* is regulated by p53, the well-studied tumour suppressor and transcription factor. To this end, the expression of *ZFAS1* and *p53* was evaluated in tissues derived from 20 patients of papillary thyroid carcinoma. *ZFAS1* was up-regulated in 12 out of 20 patients, whereas the expression of p53 was down-regulated in 17 out of 20 patients (Fig. 1A and Fig. 1B). Interestingly, the expression of *p53* was repressed in 12 patients with high *ZFAS1* expression. Furthermore, we tested the expression of *ZFAS1* in 2 PTC cell lines with different *p53* status. The expression of *ZFAS1* was significantly lower in MDA-T32 (*p53* wild type, MDA-T32 $p53^{+/+}$) cells compared to MDA-T120 (*p53* mutant, $p53^{R280T}$) cells (Fig. 1C). Next, we silenced p53 in MDA-T32 cells ($p53^{+/+}$) via siRNA pool, and the p53 silencing was evaluated by Western blot (Fig. 1D). The expression of *ZFAS1* was significantly up-regulated after silencing p53 in MDA-T32 ($p53^{+/+}$) compared to MDA-T32 cell ($p53^{+/+}$) transfected with control siRNA (Fig. 1E). Conversely, overexpression of p53 repressed *ZFA1* expression in MDA-T120 cell ($p53^{R280T}$) (Fig. 1F and 1G). Therefore, *ZFAS1* is negatively associated with p53 in PTC.

ZFAS1 is a direct target of p53

Because we confirmed that the expression of *ZFAS1* is negatively associated with p53, we then assessed the possible regulation between *ZFAS1* and p53. Previous studies have shown that p53, as a transcription factor, mediates the expression of a myriad of genes. Here, we first determined whether the p53 binding motif is located in the promoter region of the *ZFAS1* gene. We detected p53 occupancy at the *ZFAS1* promoter (Fig. 2A). The p53 binding motif sequence was obtained from JASPAR 2022, and the sequence information of the binding motif was shown in Figure 2B. q-ChIP analysis validated the target between p53 and *ZFAS1* (Fig. 2C). Taken together, *ZFAS1* is a direct target of p53, and the expression of *ZFAS1* is repressed by p53.

Identification of miRNAs induced by p53 in PTC

p53 has been shown to regulate the expression of non-coding RNAs, including microRNAs, which in turn mediates p53 function [13]. Therefore, we determined the differentially expressed miRNAs relying on the *p53* status in PTC. Because few studies have performed microRNA-Seq in PTC cells with different *p53* status, we selected a dataset GSE94908 containing microRNA-Seq performed in RKO $p53^{+/+}$ and RKO $p53^{-/-}$ colorectal cancer cells. A total of 9 miRNAs (listed in Tab. 3) were up-regulated over 2.0-fold in RKO $p53^{+/+}$ cells compared to RKO $p53^{-/-}$ cells (Fig. 3A). In these miRNAs, miR-34a, miR-34b, miR-200s, and miR-221/222 were validated as p53 direct targets by previous studies. Next, we determined the expression of the 9 up-regulated miRNAs in PTC cells to validate the results obtained from bioinformatics analysis performed in RKO cells. In total, 8 miRNAs including miR-34a, miR-34b, miR-200a, miR-200b, miR-221, miR-222, miR-135b-5p, and miR-193a-3p were up-regulated in MDA-T32 cells ($p53^{+/+}$) compared to MDA-T120 cells ($p53^{R280T}$) (Fig. 3B). Ectopic expression of p53 restored the expression of 8 miRNAs in MDA-T120 ($p53^{R280T}$) cells (Fig. 3C). Because miR-34a/b, miR-200a/b, and miR-222 were previously identified as p53 targets, we determined the target between p53 and the remaining microRNAs: miR-135b-5p and miR-193a-3p. p53 occupancies were observed in the *MIR135B* and *MIR193A* genes (Fig. 3D). q-ChIP analysis confirmed that *MIR135B* and *MIR193A* are direct targets of p53 (Fig. 3E). In summary, p53 mediates a myriad of miRNAs in PTC. As direct targets of p53, miR-135b-5p and miR-193a-3p are induced by p53.

miR-135b-5p and miR-193a-3p repress the metastasis of PTC by inhibiting ZFAS1

Because *ZFAS1* had been identified as a direct target of p53, we aimed to demonstrate the regulation between *ZFAS1* and p53-mediated miRNAs. We first screened and obtained 53 miRNAs that potentially target *ZFAS1*. Interestingly, the *ZFAS1*-targeted miRNAs contained miR-135b-5p, miR193a-3p, and miR-34b, which are p53-associated miRNAs (Fig. 4A). The above results indicated that miR-135b-5p, miR-193a-3p, and miR-34b may mediate the regulation between p53

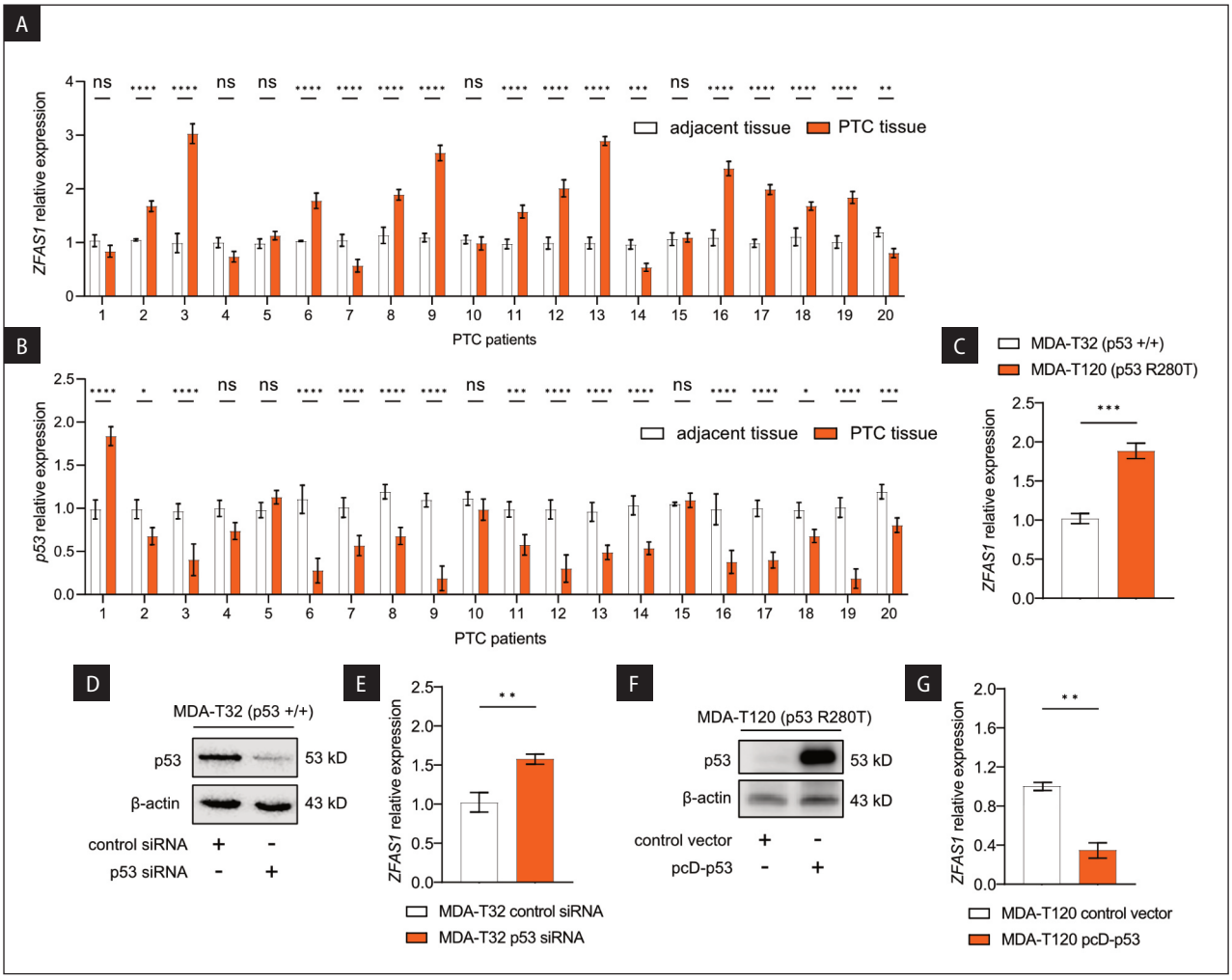

Figure 1. *Zinc finger antisense 1 (ZFAS1) is negatively associated with p53 in papillary thyroid carcinoma (PTC).* **A.** *Quantitative polymerase chain reaction (qPCR) analysis of ZFAS1 in PTC tissues derived from 20 PTC patients and comparison with the adjacent non-cancerous tissues;* **B.** *qPCR analysis of p53 in PTC tissues derived from 20 PTC patients and comparison with the adjacent non-cancerous tissues;* **C.** *qPCR analysis of ZFAS1 in MDA-T32 and MDA-T120 cells. The expression of ZFAS1 in MDA-T120 cells was normalized to the ZFAS1 expression in MDA-T32 cells;* **D.** *Western blot analysis of p53 small interfering RNA (siRNA) efficiency in MDA-T32 cells after transfection of cells with p53 siRNA for 48 hours;* **E.** *qPCR analysis of ZFAS1 in MDA-T32 cells after p53 siRNA transfection for 48 hours;* **F.** *Western blot analysis of ectopic p53 expression by pcD-p53 transfection in MDA-T120 cells;* **G.** *qPCR analysis of ZFAS1 in MDA-T120 cells after ectopic p53 expression for 48 hours. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001*

and *ZFAS1* via miRNAs. The seed-matching sequences of miR-135b-5p, miR193a-3p, and miR-34b were found in the 3'UTR of *ZFAS1* (Fig. 4B). Luciferase assay confirmed that miR-135b-5p, miR193a-3p, and miR-34b directly targeted *ZFAS1* (Fig. 4C). Overexpression of miR-135b-5p, miR193a-3p, or miR-34b repressed the expression of *ZFAS1* in MDA-T120 ($p53^{R280T}$) cells (Fig. 4D). Interestingly, miR-135b-5p, miR-193a-3p, and miR-34b displayed a synergetic effect of repressing *ZFAS1* in MDA-T120 ($p53^{R280T}$) cells (Fig. 4D).

Our previous work showed that *ZFAS1* promotes the metastasis of PTC [11]. Therefore, we would like to know the effect of *ZFAS1*-associated miR-135b-5p, miR193a-3p, and miR-34b in PTC. Ectopic expression of miR-135b-5p, miR193a-3p, and miR-34b suppressed the proliferation of MDA-T120 ($p53^{R280T}$) cells. However, ectopic *ZFAS1* largely abrogated the effect of miR-135b-5p, miR193a-3p, and miR-34b (Fig. 4E).

Therefore, p53-associated miR-135b-5p, miR193a-3p, and miR-34b repress the expression of *ZFAS1* via direct targeting, and thereby suppress the metastasis induced by *ZFAS1*.

Discussion

The current study identified that the long non-coding RNA *ZFAS1* is a direct target of p53. The expression of *ZFAS1* is repressed by p53 or p53-induced miRNAs, and the regulation is involved in the proliferation of PTC cells. Our previous study has shown that *ZFAS1*

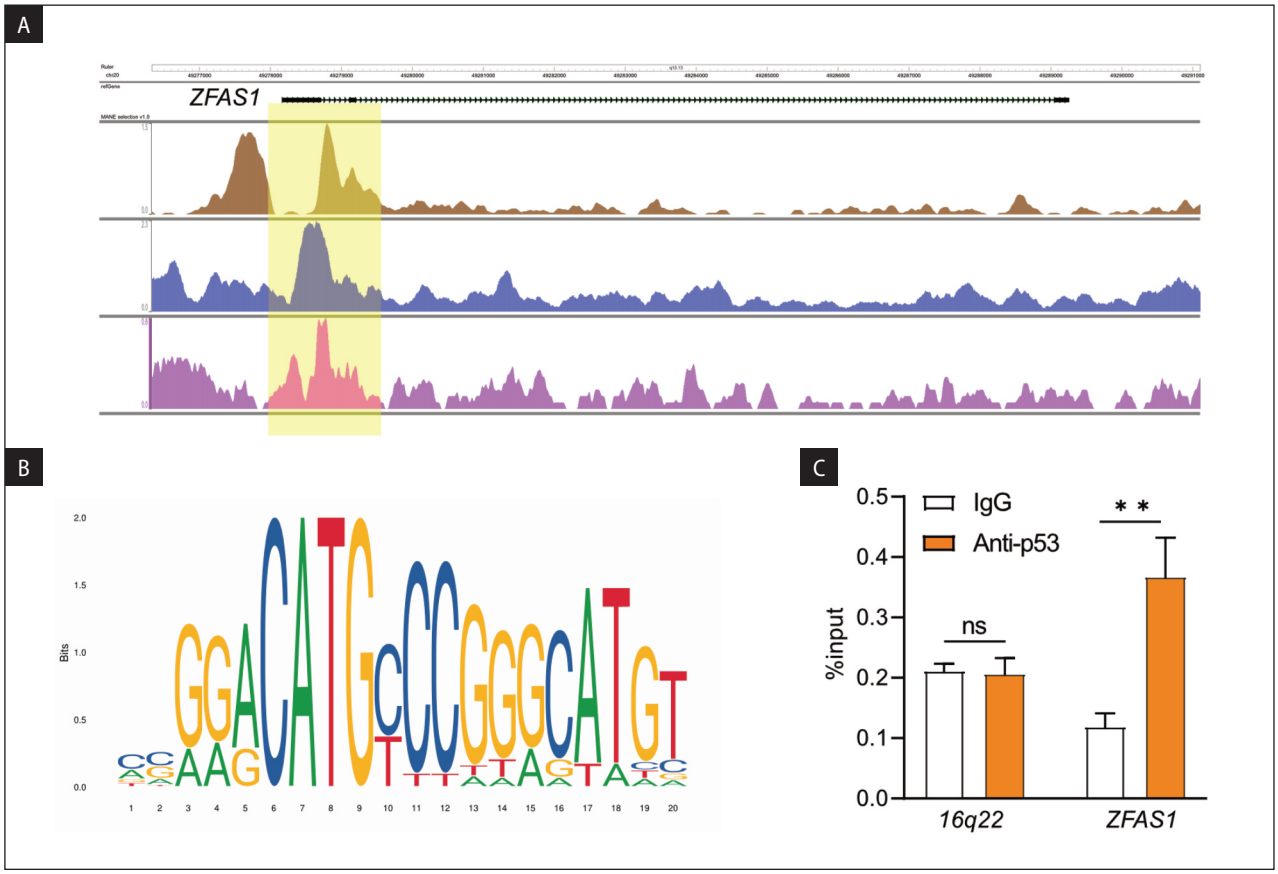

Figure 2. *Zinc finger antisense 1 (ZFAS1) is a direct target of p53.* **A.** *The scheme of p53 quantitative chromatin immunoprecipitation (qChIP) analysis from 3 cell lines were modified from the University of California, Santa Cruz (UCSC) genome browser. The ChIP peaks of p53 on the promoter of ZFAS1 are highlighted and the binding motif was indicated;* **B.** *The probability of binding motif of p53 was obtained from the JASPAR online database;* **C.** *The target between ZFAS1 and p53 was validated by q-ChIP analysis. 16q22 served as a negative control. **$p < 0.01$*

promotes the metastasis of PTC [11]. In addition, the expression of *ZFAS1* is directly mediated by a transcription factor CREB3, which contributes to the progression of metastasis [11]. In line with our previous data, herein we confirmed that p53, as a tumour suppressor and a transcription factor, directly targets *ZFAS1*, which in turn represses *ZFAS1* expression in PTC. Because p53 is mutated or deregulated in most types of cancers [14], *ZFAS1* is supposed to be a mediator of the function of *p53* deficiency in PTC. Indeed, retrained p53 expression and elevated *ZFAS1* were detected in most cases of 20 PTC patients in our study. The mutation and deregulation of *p53* are associated with the initiation of thyroid carcinoma. *p53* mutations account for up to 40% of PTCs [15]. Therefore, our findings suggest that *p53* mutation or deregulation may result in uncontrolled expression of *ZFAS1*, which promotes the progression of PTC.

As a transcription factor, p53 regulates a myriad of coding and non-coding genes [13]. In addition, the regulation of p53 is in turn fine-tuned by non-coding RNAs [16]. Several lncRNAs and miRNAs were identified as direct targets of p53 [13]. *LincRNA-p21* is directly repressed by p53, which is involved in the regulation of apoptosis and cell cycle arrest [17]. Tumour suppressor miR-34 family members are directly induced by p53 in colorectal cancer [18, 19], and thereby multiple downstream target genes of miR-34 are controlled by p53 via the p53-miR-34 regulation. Herein, we clarified that the lncRNA *ZFAS1* is a direct target of p53. In addition, we validated that both miR-135b-5p and miR-193a-3p are directly promoted by p53. Interestingly, *ZFAS1* serves as a target of miR-135b-5p, miR-193a-3p, and miR-34b. Therefore, p53, *ZFAS1*, and p53-mediated miRNAs form a coherent forward feedback loop in PTC. However, in the current study it is still unclear whether the p53-mediated miRNAs are required for the p53-*ZFAS1* regulation. Further experiments with ectopic p53 and miRNA repression are warranted.

We have shown that miR-135b-5p and miR-193a-3p repress the proliferation of PTC. The role of miR-135b-5p is contradictory in different cancers. Overexpression of miR-135b-5p promotes the progression of gastric

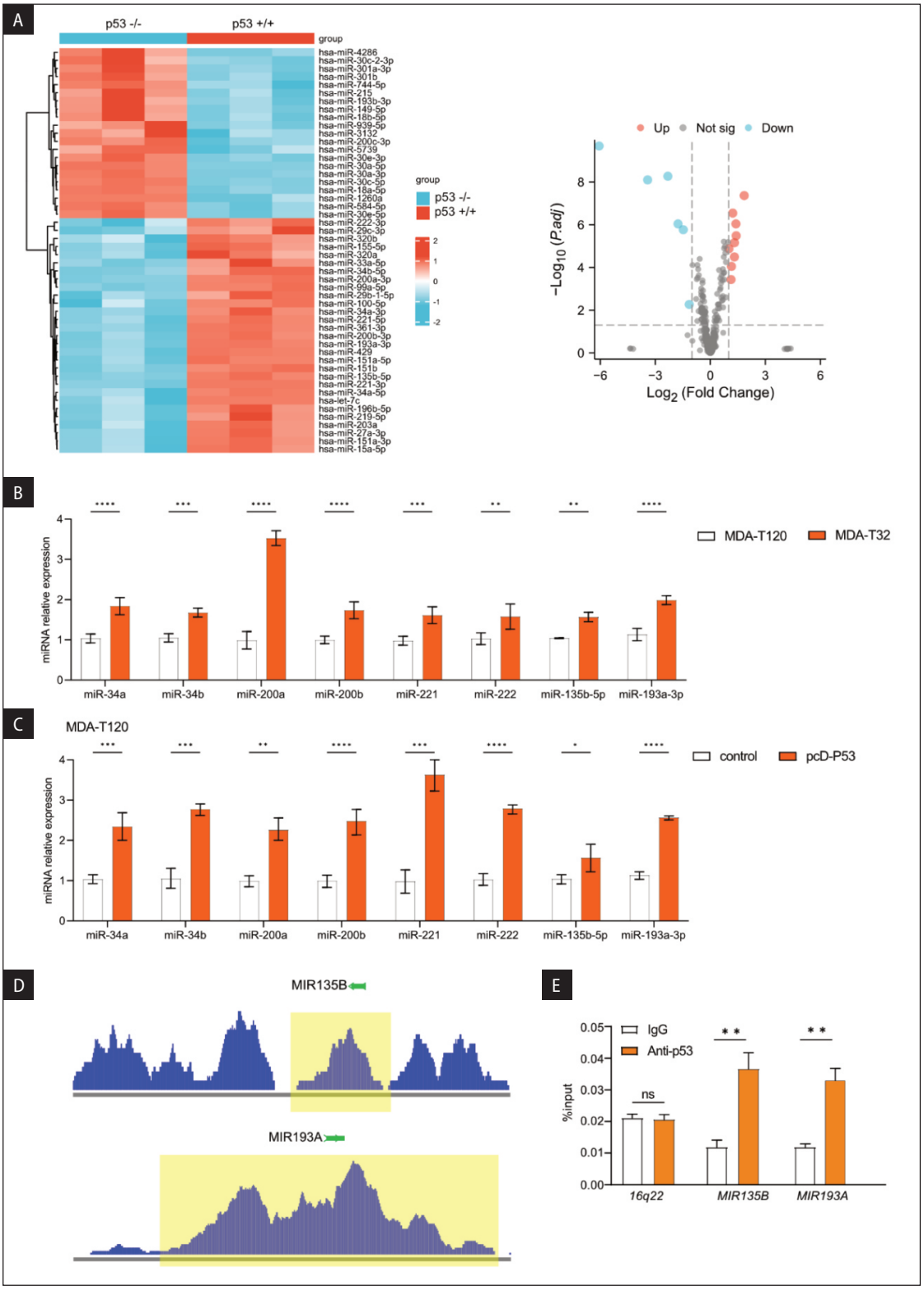

Figure 3. *Identification of miRNAs induced by p53 in papillary thyroid carcinoma (PTC).* **A.** *Left panel: heat map of differentially expressed miRNAs in RKO p53$^{+/+}$ and RKO p53$^{-/-}$ cells. Right panel: the selected differentially expressed microRNAs (miRNAs) with different p53 status. The up-regulated miRNAs are indicated by orange dots, and the down-regulated miRNAs are indicated by blue dots;* **B.** *Quantitative polymerase chain reaction (qPCR) analysis of 9 up-regulated miRNAs in MDA-T120 and MDA-T32 cells;* **C.** *qPCR analysis of 9 up-regulated miRNAs in MDA-T120 cells after ectopic p53 expression by pcD-p53 transfection for 48 hours;* **D.** *The scheme of p53 quantitative chromatin immunoprecipitation (qChIP) analysis was modified from the University of California, Santa Cruz (UCSC) genome browser. The ChIP peaks of p53 on the promoter of MIR135B and MIR193A are highlighted, and the binding motif was indicated;* **E.** *The target between MIR135B, MIR193A and p53 was validated by q-ChIP analysis. 16q22 served as a negative control. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001*

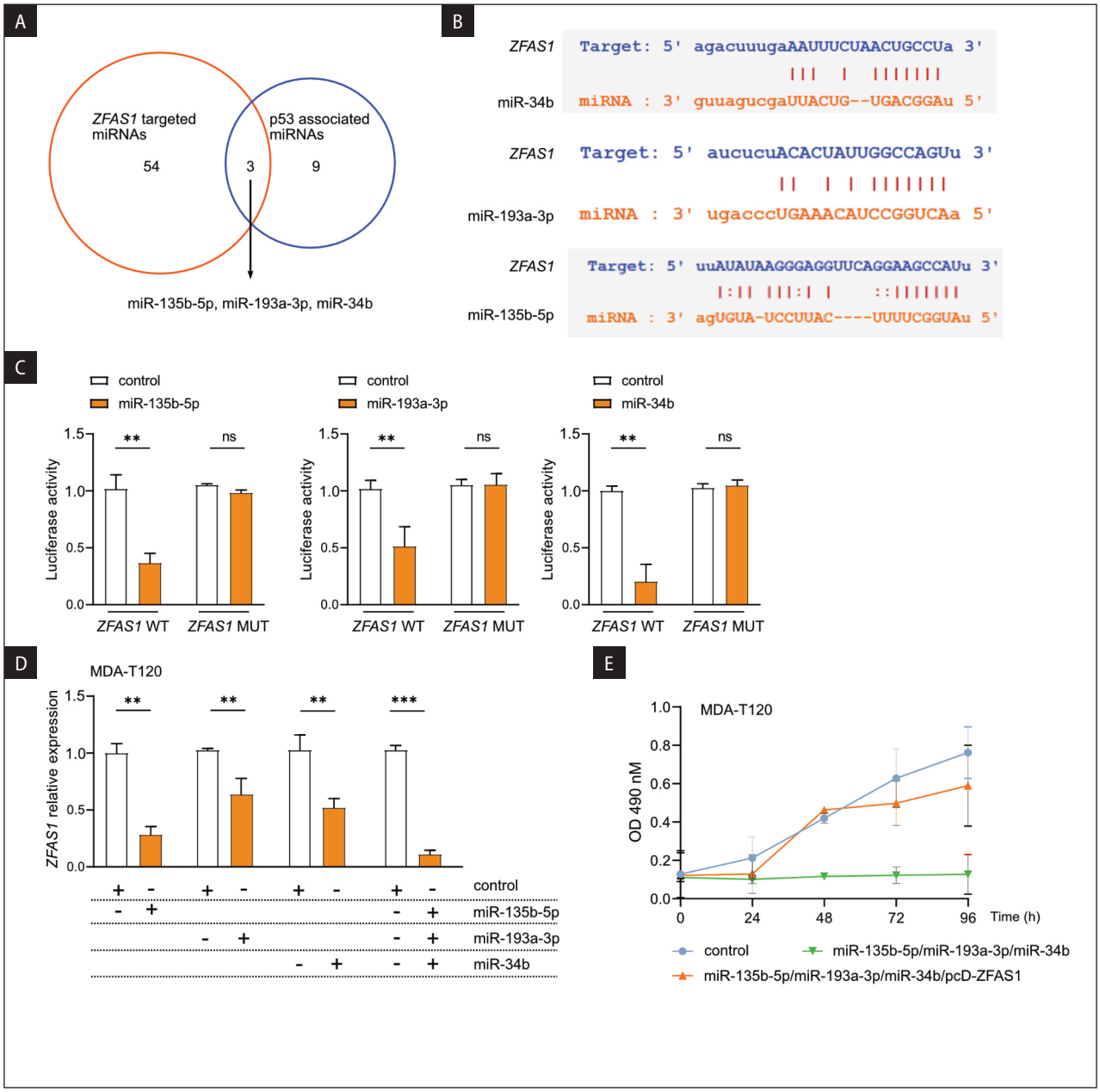

Figure 4. *miR-135b-5p and miR-193a-3p repress the proliferation of in papillary thyroid carcinoma (PTC) by inhibiting zinc finger antisense 1 (ZFAS1).* **A.** *The common miRNAs of ZFAS1-targeted miRNAs and p53-induced miRNAs;* **B.** *The predicted target seed-matching sequences of miR-34b, miR-193a-3p and miR-135b-5p on the 3'UTR of ZFAS1;* **C.** *The ZFAS1-miR-135b-5p, ZFAS1-miR-193a-3p, and ZFAS1-miR-34b targets were validated by luciferase assay;* **D.** *Quantitative polymerase chain reaction (qPCR) analysis of ZFAS1 expression in MDA-T120 cells after transfected with the indicated miRNAs for 48 hours;* **E.** *The proliferation of MDA-T120 cells was determined by MTT assay after transfected with the indicated miRNAs for 48 hours. **$p < 0.01$, ***$p < 0.001$*

cancer [20] and pancreatic cancer [21] by inducing proliferation, migration, and invasion. Nevertheless, in the early stages of breast cancer [22], melanoma [23], and renal cell cancer [24], down-regulation of miR-135-5p induces tumourigenesis. Here we confirm that miR-135b-5p is directly induced by p53 and functions as a tumour suppressor in PTC by targeting *ZFAS1*. Therefore, the role of miR-135b-5p is tissue context dependent. p53-mediated miR-193a-3p is a tumour suppressive miRNA that inhibits proliferation of PTC by targeting *ZFAS1*. Taken together, p53-mediated miRNAs such as miR-135-5p, miR-193a-3p, and miR-34b are negative regulators of *ZFAS1*, and thereby play tumour-suppressive roles in PTC.

Conclusion

Our study validates that oncogenic lncRNA *ZFAS1* is directly repressed by p53 in PTC. In addition, p53-mediated miRNAs including miR-135b-5p, miR-193a-3p,

and miR-34b repress *ZFAS1* expression, and thereby inhibit the proliferation of PTC.

Data availability statement
All datasets for supporting the conclusions of this article are available from the corresponding author upon reasonable request.

Ethics statement
The experiments with tumour samples derived from patients were approved by the Ethics Committee of Southwest Medical University (No. SMU-22-091)

Author contributions
G.W. and H.Y. designed the project. G.W. and L.W. performed experiments. G.W. analysed and presented data. G.W. and H.Y. wrote the paper.

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Conflict of interest
The authors report there are no competing interests to declare.

References
1. Baloch ZW, Asa SL, Barletta JA, et al. Overview of the 2022 WHO Classification of Thyroid Neoplasms. Endocr Pathol. 2022; 33(1): 27–63, doi: 10.1007/s12022-022-09707-3, indexed in Pubmed: 35288841.
2. Chen D, Lang B, McLeod D, et al. Thyroid cancer. Lancet. 2023; 401(10387): 1531–1544, doi: 10.1016/s0140-6736(23)00020-x, indexed in Pubmed: 37023783.
3. Haddad RI, Bischoff L, Ball D, et al. Thyroid Cancer Clinical Practice Guidelines in Oncology. J Natl Compr Canc Netw. 2022; 20(8): 925–951, doi: 10.6004/jnccn.2022.0040, indexed in Pubmed: 35948029.
4. Yan H, Bu P. Non-coding RNA in cancer. Essays Biochem. 2021; 65(4): 625–639, doi: 10.1042/EBC20200032, indexed in Pubmed: 33860799.
5. Lin RX, Yang SL, Jia Y, et al. Epigenetic regulation of papillary thyroid carcinoma by long non-coding RNAs. Semin Cancer Biol. 2022; 83: 253–260, doi: 10.1016/j.semcancer.2021.03.027, indexed in Pubmed: 33785446.
6. Ghafouri-Fard S, Kamali MJ, Abak A, et al. LncRNA *ZFAS1*: Role in tumorigenesis and other diseases. Biomed Pharmacother. 2021; 142: 111999, doi: 10.1016/j.biopha.2021.111999, indexed in Pubmed: 34385106.
7. Nie F, Yu X, Huang M, et al. Long noncoding RNA *ZFAS1* promotes gastric cancer cells proliferation by epigenetically repressing KLF2 and NKD2 expression. Oncotarget. 2017; 8(24): 38227–38238, doi: 10.18632/oncotarget.9611, indexed in Pubmed: 27246976.
8. Kolenda T, Guglas K, Kopczyńska M, et al. Oncogenic Role of *ZFAS1* lncRNA in Head and Neck Squamous Cell Carcinomas. Cells. 2019; 8(4), doi: 10.3390/cells8040366, indexed in Pubmed: 31010087.
9. Carrizzo A, Damato A, Vecchione C. Long non-coding RNA-*ZFAS1*: A novel possible biomarker to monitor and hamper the atherosclerotic process? Int J Cardiol. 2020; 319: 129–130, doi: 10.1016/j.ijcard.2020.05.009, indexed in Pubmed: 32389763.
10. Chen S, Zhang Y, Ding X, et al. Identification of lncRNA/circRNA-miRNA-mRNA ceRNA Network as Biomarkers for Hepatocellular Carcinoma. Front Genet. 2022; 13: 838869, doi: 10.3389/fgene.2022.838869, indexed in Pubmed: 35386284.
11. Wang G, Le Y, Wei L, et al. CREB3 Transactivates lncRNA *ZFAS1* to Promote Papillary Thyroid Carcinoma Metastasis by Modulating miR-373-3/MMP3 Regulatory Axis. Int J Endocrinol. 2021; 2021: 9981683, doi: 10.1155/2021/9981683, indexed in Pubmed: 34249125.
12. Deng H, Wang M, Xu Q, et al. *ZFAS1* Promotes Colorectal Cancer Metastasis Through Modulating miR-34b/SOX4 Targeting. Cell Biochem Biophys. 2021; 79(2): 387–396, doi: 10.1007/s12013-021-00976-z, indexed in Pubmed: 33725330.
13. Hermeking H. p53 enters the microRNA world. Cancer Cell. 2007; 12(5): 414–418, doi: 10.1016/j.ccr.2007.10.028, indexed in Pubmed: 17996645.
14. Zhang C, Liu J, Xu D, et al. Gain-of-function mutant p53 in cancer progression and therapy. J Mol Cell Biol. 2020; 12(9): 674–687, doi: 10.1093/jmcb/mjaa040, indexed in Pubmed: 32722796.
15. Cancer Genome Atlas Research.. Integrated genomic characterization of papillary thyroid carcinoma. Cell. 2014; 159(3): 676–690, doi: 10.1016/j.cell.2014.09.050, indexed in Pubmed: 25417114.
16. Chen R, Liu Y, Zhuang H, et al. Quantitative proteomics reveals that long non-coding RNA MALAT1 interacts with DBC1 to regulate p53 acetylation. Nucleic Acids Res. 2017; 45(17): 9947–9959, doi: 10.1093/nar/gkx600, indexed in Pubmed: 28973437.
17. Zhang Y, Miao Y, Shang M, et al. LincRNA-p21 leads to G1 arrest by p53 pathway in esophageal squamous cell carcinoma. Cancer Manag Res. 2019; 11: 6201–6214, doi: 10.2147/CMAR.S197557, indexed in Pubmed: 31308755.
18. Jiang L, Hermeking H. and Suppress Intestinal Tumorigenesis. Cancer Res. 2017; 77(10): 2746–2758, doi: 10.1158/0008-5472.CAN-16-2183, indexed in Pubmed: 28363996.
19. Rokavec M, Öner MG, Li H, et al. IL-6R/STAT3/miR-34a feedback loop promotes EMT-mediated colorectal cancer invasion and metastasis. J Clin Invest. 2014; 124(4): 1853–1867, doi: 10.1172/JCI73531, indexed in Pubmed: 24642471.
20. Lu M, Huang Y, Sun W, et al. miR-135b-5p promotes gastric cancer progression by targeting CMTM3. Int J Oncol. 2018; 52(2): 589–598, doi: 10.3892/ijo.2017.4222, indexed in Pubmed: 29345297.
21. Zhang Z, Che X, Yang Ni, et al. miR-135b-5p Promotes migration, invasion and EMT of pancreatic cancer cells by targeting NR3C2. Biomed Pharmacother. 2017; 96: 1341–1348, doi: 10.1016/j.biopha.2017.11.074, indexed in Pubmed: 29196101.
22. Pu T, Shen M, Li S, et al. Repression of miR-135b-5p promotes metastasis of early-stage breast cancer by regulating downstream target SDCBP Lab Invest. 2019; 99(9): 1296–1308, doi: 10.1038/s41374-019-0258-1, indexed in Pubmed: 31024042.
23. Zhang XH, Xin ZM. MiR-135b-5p inhibits the progression of malignant melanoma cells by targeting RBX1. Eur Rev Med Pharmacol Sci. 2020; 24(3): 1309–1315, doi: 10.26355/eurrev_202002_20188., indexed in Pubmed: 32096167.
24. Wang S, Yang X, Xie W, et al. LncRNA GAPLINC Promotes Renal Cell Cancer Tumorigenesis by Targeting the miR-135b-5p/CSF1 Axis. Front Oncol. 2021; 11: 718532, doi: 10.3389/fonc.2021.718532, indexed in Pubmed: 34722262.