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MicroRNA-1225-5p inhibits the development and progression of thyroid cancer via targeting sirtuin 3

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The objective of this study was to grab the expression levels and biological function of microRNA-1225-5p in human thyroid cancer. The miR-1225-5p expression in thyroid cancer tissue and cell lines was detected, followed by detecting the effects of overexpression of miR-1225-5p on the proliferation, apoptosis, migration and invasion of thyroid cancer cells. Moreover, the target relationship between miR-1225-5p and sirtuin 3 (SIRT3) was investigated. Besides, the effect of miR-1225-5p on the activation of Wnt/β-catenin pathway was elucidated. The miR-1225-5p expression was downregulated in thyroid cancer tissues and cell lines. Overexpression of miR-1225-5p significantly inhibited TPC-1 cell proliferation, increased cell apoptosis, and suppressed migration and invasion. In addition, SIRT3 was verified as a functional target of miR-1225-5p. SIRT3 expression was overtly increased in thyroid cancer tissues and cell lines. Overexpression miR-1225-5p and SIRT3 at the same time could significantly reverse the effects of miR-1225-5p overexpression alone on the malignant phenotypes of thyroid cancer cells. Furthermore, overexpression of miR-1225-5p significantly inhibited the activation of the Wnt/β-catenin pathway, which was remarkably reversed after overexpression of SIRT3. Our data reveal that downregulation of miR-1225-5p may promote tumor cell proliferation and metastasis in thyroid cancer *via* a direct targeting of SIRT3. Activation of the Wnt/β-catenin pathway may be a key mechanism to mediate the role of miR-1225-5p/SIRT3 axis in thyroid cancer. miR-1225-5p may serve as a potential anti-cancer target for thyroid cancer treatment.

1. Introduction

Thyroid cancer is the most prevalent endocrine neoplasm, accounting for approximately 1.7 % of all human malignancies (Lodewijk et al. 2012). Its incidence rate has been steadily increasing over the past several years (Davies and Welch 2006). The average overall 5-year survival rate of thyroid cancer patients is up to 95 % (Haugen et al. 2016; Hay et al. 2002), but about 10-20 % of the patients lose their life due to disease recurrence or progression (Hollenbeak et al. 2013). To deepen the understanding of the molecular characteristics of this disease is imperative (Esin et al. 2016), and will help to design an effective therapeutic strategy for improving clinical outcomes.

MicroRNAs (miRNAs) are endogenous small non-coding RNAs that have emerged as key players in the development of many types of cancers *via* negatively regulating their target genes (Abba et al. 2017; Adams et al. 2014; Bartel 2009; Trang et al. 2017). Studies have shown the important role of miRNAs in tumor initiation and development of thyroid cancer. For instance, miR-613 can function as a tumor suppressor in papillary thyroid carcinoma by regulating sphingosine kinase 2 expression (Qiu et al. 2016);

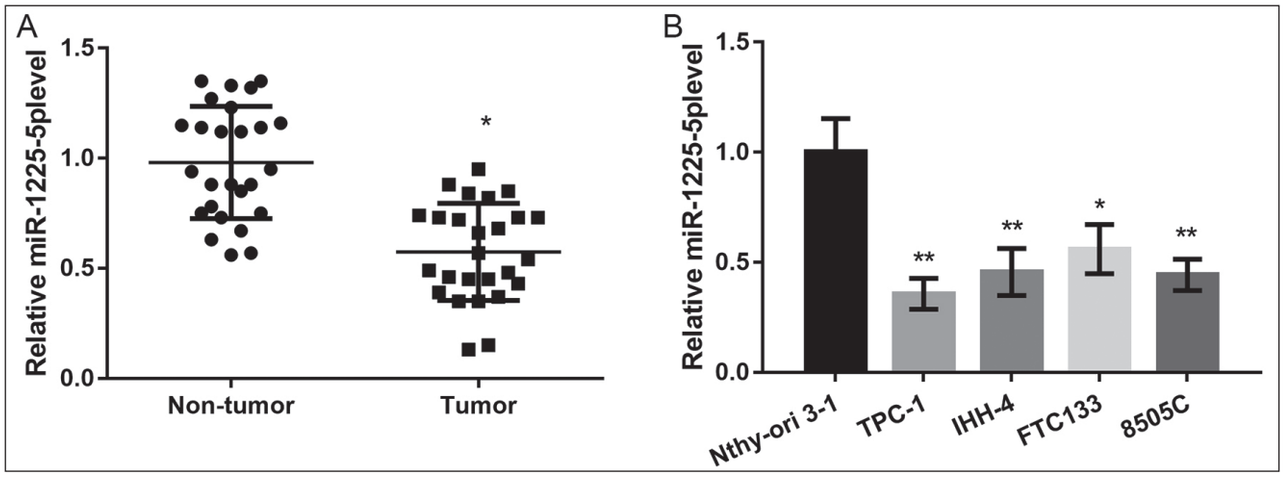

Fig. 1: miR-1225-5p expression was downregulated in thyroid cancer tissues (A) and cell lines (B). * $P < 0.05$ and ** $P < 0.01$ compared to controls.

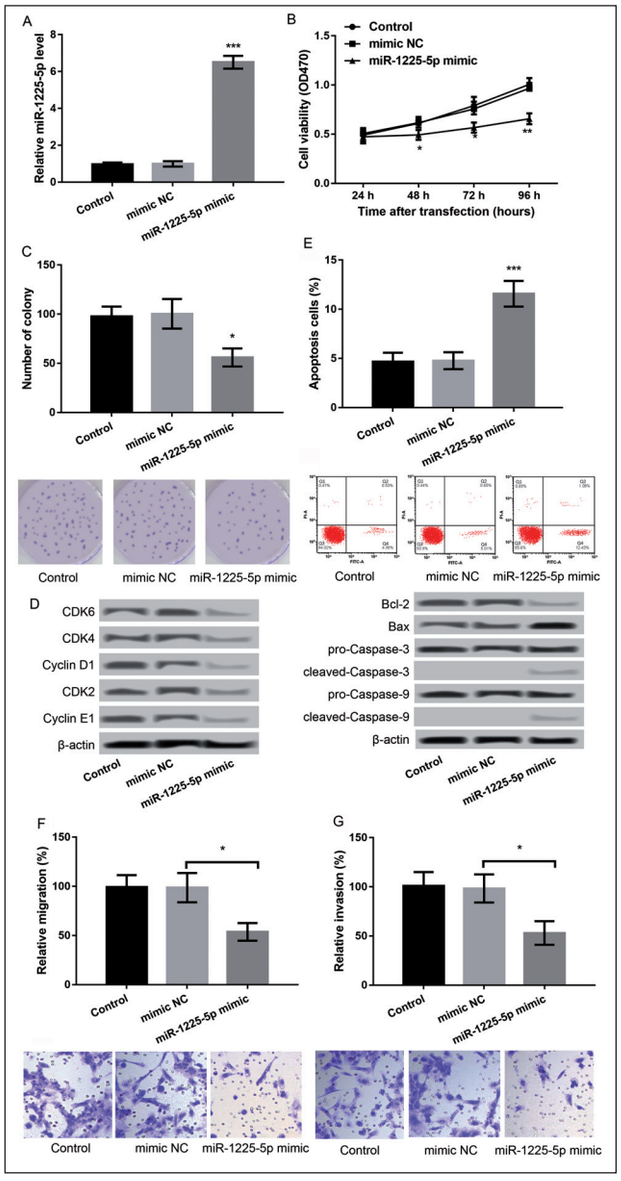

Fig. 2: Overexpression of miR-1225-5p inhibited TPC-1 cell proliferation, increased cell apoptosis, and suppressed migration and invasion. (A) The miR-1225-5p was overexpressed in TPC-1 cell by transfection with miR-1225-5p. (B) TPC-1 cell viability after overexpression of miR-1225-5p. (C) Colony formative ability of TPC-1 cells after overexpression of miR-1225-5p. (D) The expression of cell cycle-related proteins including CDK4, CDK6, CDK2, Cyclin D1, and Cyclin E1 after overexpression of miR-1225-5p. (E) TPC-1 cell apoptosis and the expression of Bax/Bcl-2, cleaved/pro-caspase-3 and cleaved/pro-caspase-9 after overexpression of miR-1225-5p. (F) TPC-1 cell migration after overexpression of miR-1225-5p. (G) TPC-1 cell invasion after overexpression of miR-1225-5p. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$ compared to controls.

miR-141 can inhibit the growth and metastasis of thyroid cancer cells by targeting insulin receptor substrate 2 (Dong et al. 2016); knockdown of PAK1 is shown to inhibit the tumor cell proliferation, migration and invasion in thyroid cancer (Yue et al. 2016).

Notably, miRNAs have received widespread attention due to their potential use in diagnostics and treatment of thyroid carcinomas (Wójcicka et al. 2016). Therefore, identification of key miRNAs involved in thyroid carcinomas could be beneficial for the diagnosis, treatment and prognosis of this disease.

In a previous study, miR-1225-5p is found to be downregulated in aggressive papillary thyroid carcinoma tissues compared with non-aggressive tissues (Yang et al. 2013), implying that miR-1225-5p may participate in the progression of thyroid cancer. However, knowledge about the role of miR-1225-5p in thyroid cancer is limited. In this study, we aimed to explore the expression and molecular function of miR-1225-5p in thyroid cancer. The miR-1225-5p expression in thyroid cancer tissue and cell lines was detected, followed by detecting the effects of overexpression of miR-1225-5p on the proliferation, apoptosis, migration and invasion of thyroid cancer cells. Moreover, the target relationship between miR-1225-5p and sirtuin 3 (SIRT3) was investigated. Besides, the effect of miR-1225-5p on activation of the Wnt/β-catenin pathway was elucidated. This promising finding may lead to future strategies for the therapy of thyroid cancer.

2. Investigations and results

2.1. miR-1225-5p expression is downregulated in thyroid cancer tissues and cell lines

As shown in Fig. 1A, miR-1225-5p expression was significantly downregulated in thyroid cancer tissues compared to that in matched normal tissues ($P < 0.05$). This is consistent with results showing that miR-1225-5p was remarkably downregulated in thyroid cancer cell lines TPC-1, IHH-4, FTC133, and 8505C related to that in normal human thyroid follicular epithelial Nthy-ori 3-1 cell line ($P < 0.05$, Fig. 1B). TPC-1 cells were selected for subsequent experiments due to the lowest expression among the above four thyroid cancer cell lines.

2.2. Overexpression of miR-1225-5p inhibits TPC-1 cell proliferation, increases cell apoptosis, and suppresses migration and invasion

To investigate the role of miR-1225-5p in thyroid cancer, miR-1225-5p was overexpressed in TPC-1 cells by transfection with miR-1225-5p and the high transfection efficiency was confirmed by qPCR ($P < 0.001$, Fig. 2A). Subsequently, the results showed that overexpression of miR-1225-5p significantly inhibited TPC-1 cell viability ($P < 0.05$, Fig. 2B), colony formative ability ($P < 0.05$, Fig. 2C) and decreased the expression of cell cycle-related

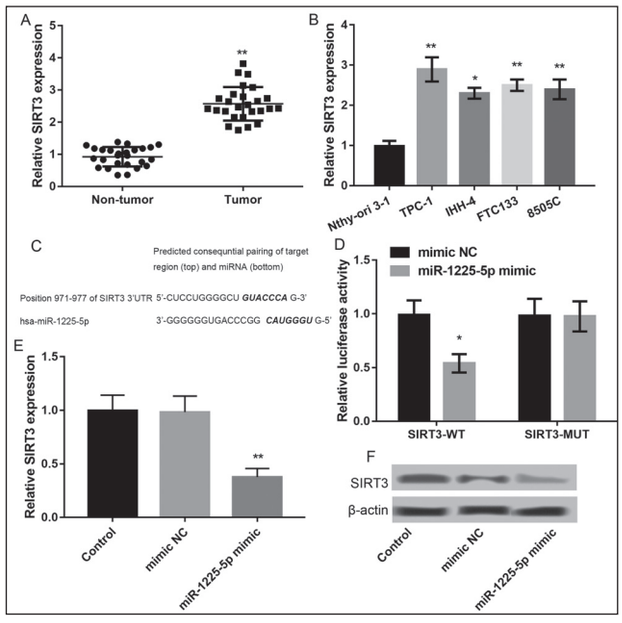

Fig. 3: SIRT3 was verified as a functional target of miR-1225-5p. (A) The SIRT3 expression was increased in thyroid cancer tissues compared to non-tumor tissues. (B) The SIRT3 expression was increased in thyroid cancer cell lines compared to that in normal Nthy-ori 3-1 cells. (C) The complementary pairing sequences between miR-1225-5p and SIRT3. (D) Dual-luciferase reporter assay showed that SIRT3 was a direct target of miR-1225-5p. (E) The SIRT3 mRNA expression after overexpression of miR-1225-5p. (F) The SIRT3 protein expression after overexpression of miR-1225-5p. * $P < 0.05$ and ** $P < 0.01$ compared to controls.

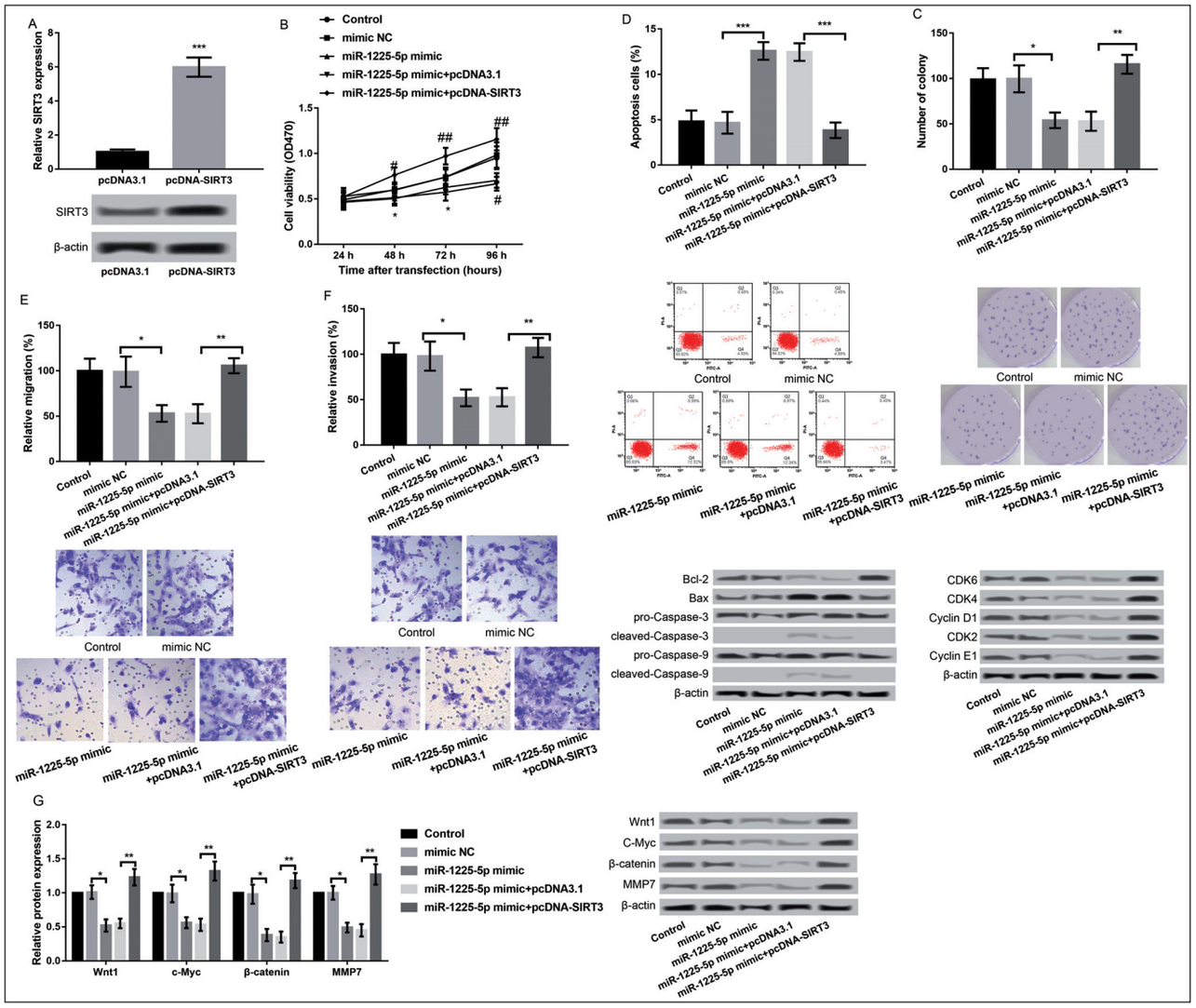

Fig. 4: miR-1225-5p regulated the behaviors of thyroid cancer cells through targeting SIRT3. (A) The SIRT3 mRNA and protein expression after transfection with pcDNA-SIRT3. (B) TPC-1 cell viability after overexpression of miR-1225-5p and or overexpression of SIRT3. (C) Colony formative ability of TPC-1 cells and the expression of cell cycle-related proteins including CDK4, CDK6, CDK2, Cyclin D1, and Cyclin E1 after overexpression of miR-1225-5p and or overexpression of SIRT3. (D) TPC-1 cell apoptosis and the expression of Bax/Bcl-2, cleaved/pro-caspase-3 and cleaved/pro-caspase-9 after overexpression of miR-1225-5p and or overexpression of SIRT3. (E) TPC-1 cell migration after overexpression of miR-1225-5p and or overexpression of SIRT3. (F) TPC-1 cell invasion after overexpression of miR-1225-5p and or overexpression of SIRT3. (G) The expression of wnt 1, c-Myc, β-catenin, and MMP7 after overexpression of miR-1225-5p and or overexpression of SIRT3. Overexpression of miR-1225-5p inhibited the activation of Wnt/β-catenin pathway, which was remarkably reversed after overexpression of SIRT3.* $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$ compared to controls.

proteins including CDK4, CDK6, CDK2, cyclin D1, and cyclin E1 (Fig. 2D). These data indicate that overexpression of miR-1225-5p inhibits TPC-1 cell proliferation. Moreover, we found that overexpression of miR-1225-5p markedly promoted TPC-1 cell apoptosis ($P < 0.001$, Fig. 2E) and obviously increased the expression of Bax/Bcl-2, cleaved/pro-caspase-3 and cleaved/pro-caspase-9 (Fig. 2E). Furthermore, overexpression of miR-1225-5p distinctly suppressed migration and invasion of TPC-1 cells ($P < 0.05$, Fig. 2F-G).

2.3. *SIRT3 is verified as a functional target of miR-1225-5p*

As shown in Fig. 3A-B, SIRT3 expression was overtly increased in thyroid cancer tissues and cell lines compared to that in matched normal tissues and Nthy-ori 3-1 cells ($P<0.05$), respectively. Inversed expression between miR-1225-5p and SIRT3 in thyroid cancer was found, we thus investigated the regulatory relationship between them. Based on the information of Targetscan, there were complementary pairings between miR-1225-5p and SIRT3 (http://www.targetscan.org/cgi-bin/targetscan/vert_71/view_gene.cgi?rs=ENST00000382743.4&taxid=9606&members=miR-1225-5p&showcnc=0&shownc=0&shownc_nc=&showncf1=1&showncf2=1&subset=1, Fig. 3C). Dual-luciferase reporter assay showed that SIRT3 was a direct target of miR-1225-5p (Fig. 3D). Besides, qPCR and western blot assays further revealed that overexpression of miR-1225-5p resulted in a remarkable decrease in SIRT3 expression ($P < 0.01$, Fig. 3E-F), confirming the negative regulatory relationship between them.

2.4. *miR-1225-5p regulates the behaviors of thyroid cancer cells through SIRT3*

To verify whether the role of miR-1225-5p in thyroid cancer cells is related to targeting SIRT3, we overexpressed SIRT3 expression through transfection with pcDNA-SIRT3 and observed the combined effects of miR-1225-5p overexpression and SIRT3 overexpression on the proliferation, apoptosis, migration and invasion of thyroid cancer cells. As presented in Fig. 4A, SIRT3 expression was significantly upregulated in TPC-1 cells after transfection with pcDNA-SIRT3 compared to pcDNA3.1 transfection ($P < 0.001$). Moreover, the results showed that miR-1225-5p overexpression and SIRT3 overexpression at the same time could significantly

reverse the effects of miR-1225-5p overexpression alone on TPC-1 cell viability ($P < 0.05$, Fig. 4B), colony formative ability ($P < 0.01$, Fig. 4C) and the expression of cell cycle-related proteins (Fig. 4C), cell apoptosis ($P < 0.001$, Fig. 4D) and the expression of Bax/Bcl-2, cleaved/pro-caspase-3 and cleaved/pro-caspase-9 (Fig. 4D), and cell migration and invasion ($P < 0.01$, Fig. 4E-F), indicating that miR-1225-5p may regulate the behaviors of thyroid cancer cells through targeting SIRT3.

2.5. Overexpression of miR-1225-5p inhibits the activation of Wnt/β-catenin pathway

We further explored the association between miR-1225-5p and Wnt/β-catenin pathway. As shown in Fig. 4G, we found that overexpression of miR-1225-5p resulted in the decreased expression of wnt 1, c-Myc, β-catenin, and MMP7, indicating that overexpression of miR-1225-5p inhibited the activation of Wnt/β-catenin pathway. However, the above effects were remarkably reversed after overexpression of SIRT3.

3. Discussion

Dysregulation of miRNAs is a common feature of malignancy, including thyroid cancer (Aragon et al. 2015; Sondermann et al. 2015; Yoruker et al. 2016). MiRNA can play a role similar to that of oncogenes or tumor suppressors, modulating tumor growth, apoptosis, invasion and metastasis (Aurora and Slack 2006; Hata and Lieberman 2015). Previous studies have revealed that miR-1225-5p can inhibit tumor growth and metastasis in gastric carcinoma and glioblastoma *via* targeting insulin receptor substrate-1 (Li et al. 2018a; Zheng et al. 2016); and upregulation of miR-1225 impaired the proliferation and survival of laryngeal carcinoma cells, as well as induced G1/S cell cycle arrest (Sun et al. 2019). These data imply that miR-1225-5p behaves as a tumor suppressor in multiple cancers. Moreover, miR-1225-5p is reported to be downregulated in aggressive papillary thyroid carcinoma tissues compared to that in non-aggressive tissues (Yang et al. 2013). In this study, miR-1225-5p expression was downregulated in thyroid cancer tissues and cell lines. Overexpression of miR-1225-5p significantly inhibited TPC-1 cell proliferation, increased cell apoptosis, and suppressed migration and invasion. These data suggest that miR-1225-5p may also play a tumor suppressive role in thyroid cancer progression, and may therefore serve as a promising therapeutic target for the treatment of this disease.

Identification of the target gene of miR-1225-5p is important for understanding its role in tumorigenesis and tumor progression. In this study, SIRT3 was verified as a functional target of miR-1225-5p. SIRT3 is a key mitochondria protein, playing a key role in preventing cell aging and transformation through regulating mitochondrial metabolic homeostasis (Cui et al. 2015). Previous studies have confirmed that SIRT3 is increased expressed in gastric cancer (Cui et al. 2015), esophageal cancer (Zhao et al. 2013), node-positive breast cancer (Ashraf et al. 2006), and oral cancer (Alhazzazi et al. 2011), and its enhanced expression is associated with the malignant phenotypes of these cancers. Additionally, Rodney et al. (2013) revealed that SIRT3 expression was increased in thyroid carcinomas. Consistent with these findings, this study showed that SIRT3 expression was overtly increased in thyroid cancer tissues and cell lines compared to that in matched normal tissues and Nthy-ori 3-1 cells respectively. Overexpression miR-1225-5p and SIRT3 at the same time could significantly reverse the effects of miR-1225-5p overexpression alone on the malignant phenotypes of thyroid cancer cells. Therefore, we hypothesise that SIRT3 may function as an oncogene in thyroid cancer, and miR-1225-5p may inhibit thyroid cancer development and progression through targeting SIRT3.

Furthermore, another important finding of this study was that overexpression of miR-1225-5p inhibited the activation of the Wnt/β-catenin pathway. The Wnt/β-catenin pathway drives increased cyclin D1 levels that are linked to lymph node metastasis in papillary thyroid cancer (Zhang et al. 2012). Upregulation of miR-155 is known to promote tumor growth in papillary thyroid carcinoma by activating Wnt/β-catenin signaling (Zhang et al. 2013). In addition, SIRT3 can increase FOXO3A expression through suppressing the Wnt/β-catenin pathway, thereby inhibiting prostate cancer metastasis (Li et al. 2018b). In this study, overexpression of miR-1225-5p significantly inhibited the activation of the Wnt/β-catenin pathway, which was remarkably reversed after overexpression of SIRT3. We thus speculate that miR-1225-5p/SIRT3 axis regulate the thyroid cancer development and progression through Wnt/β-catenin pathway.

In conclusion, our findings reveal that miR-1225-5p is downregulated in thyroid cancer tissue samples and cell lines. Downregulation of miR-1225-5p may promote tumor cell proliferation and metastasis in thyroid cancer by targeting of SIRT3 directly. Activation of the Wnt/β-catenin pathway may be a key mechanism to mediate the role of miR-1225-5p/SIRT3 axis in thyroid cancer. miR-1225-5p may serve as a potential anti-cancer target for thyroid cancer treatment.

4. Experimental

4.1. Tumor tissue collection

In total, 26 paired papillary thyroid carcinoma tissues and matched normal tissues were obtained from 26 papillary thyroid carcinoma patients (10 males and 16 females; age range, 42–66 years) during surgery. The diagnosis of papillary thyroid carcinoma was confirmed by a pathologist. All collected tissues were immediately stored in liquid nitrogen. This study was approved by the local ethics committee of our hospital. Patients provided written informed consent.

4.2. Cell culture

Human thyroid cancer cell lines TPC-1, IHH-4, FTC133, and 8505C were obtained from the Cell Resource Center, Life Science Research Institute, University of Chinese Academy of Sciences, and normal human thyroid follicular epithelial Nthy-ori 3-1 cell line was obtained from the European Collection of Cell Cultures (Wiltshire, UK). They were cultured in the Roswell Park Memorial Institute (RPMI)-1640 containing 10 % fetal bovine serum (FBS), 1 % penicillin–streptomycin, 1 mM sodium pyruvate, and 0.1 mM non-essential amino acids and maintained in a 37 °C humidified incubator with 5% CO_2.

4.3. Cell transfection

For overexpression of miR-1225-5p and SIRT3, 100,000 to 200,000 TPC-1 cells were transfected with hsa-miR-1225-5p mimic (90 pmol, Qiagen, Hilden, Germany), mimic control, pcDNA-SIRT3, and pcDNA3.1 using Lipofectamine® RNAiMAX Transfection Reagent (Life technologies, Carlsbad, California, USA) following the manufacturer's protocol.

4.4. Cell viability assay

Cell viability was detected using the 3-(4,5-dimethyl-2-thiazolyl)-2, 5-diphenyltetrazolium bromide (MTT) assay. Briefly, after transfection for 24 h, TPC-1 cells were plated in 96-well plates at a density of 5×10^3 cells/well. After incubation for 24 h, 20 µL of MTT was added into each well for another 4 h incubation. Consequently, 150 µL of dimethyl sulfoxide (DMSO) was added to incubate the cells for 10 min. For the cell number calculation, absorbance of each well at 570 nm was observed under an absorption spectrophotometer (Olympus, Japan).

4.5. Clonogenic assay

Clonogenic assay was also performed to detect the colony formative ability. In brief, after cell transfection, 100 TPC-1 cells were plated into the 60-mm culture dishes in triplicate, and cultured in RPMI-1640 containing 10 % FBS for 14 days. Subsequently, TPC-1 cells were fixed, stained with Diff-Quick, air-dried. The formative colonies that contained at least 30 cells were counted under microscope (IX83, Olympus).

4.6. Apoptosis analysis

After cell transfection, TPC-1 cells were trypsinized and washed with cold PBS. Then cells were fixed in 70 % ethanol, stained with fluorescein isothiocynate (FITC)-conjugated Annexin V and propidium iodide (PI) using the Annexin V-FITC kit (Biosea Biotechnology Co., Beijing, China) in the light of the manufacturer's instructions. After incubated at room temperature in the dark for 1 h, the apoptotic cells were detected by flow cytometry using a FACS can (Beckman Coulter, Fullerton, CA, USA).

4.7. Quantitative PCR (qPCR)

Total RNA was extracted from thyroid tissues and cells using TRIzol Reagent (Invitrogen, CA, USA) and then treated with RNase-free DNase I (Promega Biotech, USA). After detecting the quality of the isolated RNA with SMA 400 UV-vis (Merinton, Shanghai, China), complementary DNA (cDNA) synthesis was conducted using the

reverse transcriptase PrimerScript First Strand cDNA Synthesis Kit (Invitrogen). After that, qPCR was performed to detect the expression levels of targets based on a standard protocol of the SYBR ExScript qRT-polymerase chain reaction (PCR) Kit (Takara, China). Phosphoglyceraldehyde dehydrogenase (GAPDH) was chosen as the internal control, and the $2^{-\Delta\Delta Ct}$ method was used to calculate the relative gene expression levels.

4.8. Cell migration and invasion assay

Cell migration and invasion were detected using Transwell chambers (8 μm pore size; Costar, Switzerland). For invasion assay, the inserts of Transwell chambers were coated with 1 mg/ml BD Matrigel Matrix (BD Biosciences, USA). The Transwell chambers were prepared for an initial equilibrium After 48 h transfection, 8×10^4 TPC-1 cells were suspended in 200 μL of fresh serum-free medium and then added to the inserts. Subsequently, 600 μL of RPMI-1640 medium containing 10 % FBS was added into the lower compartment as a chemoattractant. After cultivation for 24 h, the cells on the lower compartment of the inserts were fixed and stained with 0.1 % crystal violet. The number of cells in five randomly selected visual fields was counted under a light microscope.

4.9. Dual-luciferase reporter assay

To create the wide-type (WT) luciferase reporter construct, the full-length 3'-UTR of SIRT3 was amplified and cloned into the EcoRI and XhaI sites of the pGL3-BS vector (Promega Corporation, Madison, WI, USA). The mutant (MUT) luciferase reporter construct of SIRT3 3'-UTR was generated using a Quick Change mutagenesis kit (Stratagene, Heidelberg, Germany) and also inserted into the pGL3-BS vector. After that, TPC-1 cells were cotransfected the luciferase reporter vectors and miR-1225-5p mimics or mimic NC using Lipofectamine 2000 (Invitrogen Life Technologies). After 48 h of incubation, dual-luciferase activity was detected using the Dual-Luciferase® reporter assay system (Promega Corporation).

4.10. Western blot assay

After 48 h transfection, total protein for western blot were extracted from cells by lysing with radioimmunoprecipitation (RIPA) buffer (Sangon Biotech) containing phenylmethanesulfonyl fluoride (PMSF) (Sigma). After brief sonication and centrifugation, the protein supernatants were collected and quantified using a BCA protein assay kit (Pierce, Rockford, IL). Total 20 μg protein per cell lysate was subjected to a 10% SDS-PAGE and transferred to PVDF membrane (Millipore, Bedford, MA, USA). After blocked for 1 h at room temperature, the membranes were incubated with first antibodies toβ-actin, CDK4, CDK6, CDK2, cyclin D1, cyclin E1, Bcl-2, Bax, pro-caspase-3, cleaved caspase-3, pro-caspase-9, cleaved caspase-9, SIRT3, wnt 1, c-Myc, β-catenin, and MMP7 (1:1000 dilution, Abcam, Cambridge, UK) at 4 °C overnight, followed by incubation with horseradish peroxidase-labeled secondary antibody (1:1000 dilution) for 1 h at room temperature. After that, the members were incubated with a chromogenic substrate, and the protein signals in the members were detected using the enhanced chemiluminescence (ECL) method. β-actin served as the internal control.

4.11. Statistical analysis

All experiments were conducted independently for three times. Data are presented as the mean±standard deviation (SD), and data significance between groups was analyzed by independent sample t test or one-way ANOVA followed by Post-hoc Tukey test. The statistical analyses were conducted using Graph Prism 5.0 software (GraphPad Prism, San Diego, CA) with $P < 0.05$ as statistically significant result in comparison.

Conflicts of interest: None declared.

References

Abba ML, Patil N, Leupold JH, Moniuszko M, Utikal J, Niklinski J, Allgayer H (2017) MicroRNAs as novel targets and tools in cancer therapy. Cancer Lett 387: 84-94.

Adams BD, Kasinski AL, Slack FJ (2014) Aberrant regulation and function of microRNAs in cancer. Curr Biol 24: R762-R776.

Alhazzazi TY, Pachiyappan K, Nam J, Jing-Yi H, Eric V, D'Silva NJ, Kapila YL (2011) Sirtuin-3 (SIRT3), a novel potential therapeutic target for oral cancer. Cancer 117: 1670-1678.

Aragon HP, Weng CH, Khawaja HT, Nagarajan N, Schneider EB, Umbricht CB, Witwer KW, Zeiger MA (2015) MicroRNA expression and association with clinicopathologic features in papillary thyroid cancer: a systematic review. Thyroid 25: 1322-1329.

Ashraf N, Zino S, Macintyre A, Kingsmore D, Payne AP, George WD, Shiels PG (2006) Altered sirtuin expression is associated with node-positive breast cancer. Br J Cancer 95: 1056-1061.

Aurora EK, Slack FJ (2006) Oncomirs - microRNAs with a role in cancer. Nature Rev Cancer 6: 259-269.

Bartel DP (2009) MicroRNAs: Target recognition and regulatory functions. Cell 136: 215-233.

Cui Y, Qin L, Wu J, Qu X, Hou C, Sun W, Li S, Vaughan AT, Li JJ, Liu J (2015) SIRT3 enhances glycolysis and proliferation in SIRT3-expressing gastric cancer cells. Plos One 10: e0129834.

Davies L, Welch H (2006) Increasing incidence of thyroid cancer in the united states, 1973-2002. JAMA 295: 2164-2167.

Dong S, Meng X, Xue S, Yan Z, Ren P, Liu J (2016) microRNA-141 inhibits thyroid cancer cell growth and metastasis by targeting insulin receptor substrate 2. Am J Transl Res 8: 1471-1481.

Esin YE, Duygu T, Serkan T, Ezel UF, Ugur G, Nejat D (2016) MicroRNA Expression profiles in papillary thyroid carcinoma, benign thyroid nodules and healthy controls. J Cancer 7: 803-809.

Hata A, Lieberman J (2015) Dysregulation of microRNA biogenesis and gene silencing in cancer. Science Signaling 8: re3.

Haugen BR, Alexander EK, Bible KC, Doherty GM, Mandel SJ, Nikiforov YE, Pacini F, Randolph GW, Sawka AM, Schlumberger M, Schuff KG, Sherman SI, Sosa JA, Steward DL, Tuttle RM, Wartofsky L (2016) 2015 American Thyroid Association management guidelines for adult patients with thyroid nodules and differentiated thyroid cancer. Thyroid 26: 1-133.

Hay ID, Thompson GB, Grant CS, Bergstralh EJ, Dvorak CE, Gorman CA, Maurer MS, Mciver B, Mullan BP, Oberg AL (2002) Papillary thyroid carcinoma managed at the Mayo Clinic during six decades (1940-1999): temporal trends in initial therapy and long-term outcome in 2444 consecutively treated patients. World J Surg 26: 879-885.

Hollenbeak CS, Boltz MM, Schaefer EW, Saunders BD, Goldenberg D (2013) Recurrence of differentiated thyroid cancer in the elderly. Eur J Endocrinol 168: 549-556.

Li D, Chi G, Chen Z, Jin X (2018a) MicroRNA-1225-5p behaves as a tumor suppressor in human glioblastoma via targeting of IRS1. OncoTargets Ther 11: 6339-6350.

Li R, Quan Y, Xia W (2018b) SIRT3 inhibits prostate cancer metastasis through regulation of FOXO3A by suppressing Wnt/β-catenin pathway. Exp Cell Res 364: 143-151.

Lodewijk L, Prins AM, Kist JW, Valk GD, Kranenburg O, Rinkes IH, Vriens MR (2012) The value of miRNA in diagnosing thyroid cancer: a systematic review. Cancer Biomarkers 11: 229-238.

Qiu W, Yang Z, Fan Y, Zheng Q (2016) MicroRNA-613 inhibits cell growth, migration and invasion of papillary thyroid carcinoma by regulating SphK2. Oncotarget 7: 39907-39915.

Rodney S, Sharon H, Katherine H, Asim AM, Emad K, Domenico C (2013) Nicotinamide phosphoribosyltransferase and SIRT3 expression are increased in well-differentiated thyroid carcinomas. Anticancer Res 33: 3047-3052.

Sondermann A, Andreghetto FM, Moulatlet ACB, Victor EDS, Castro MGD, Nunes FD, Brandão LG, Severino P (2015) MiR-9 and miR-21 as prognostic biomarkers for recurrence in papillary thyroid cancer. Clin Exp Metastasis 32: 521-530.

Sun P, Zhang D, Huang H, Yu Y, Yang Z, Niu Y, Liu J (2019) MicroRNA-1225-5p acts as a tumor-suppressor in laryngeal cancer via targeting CDC14B. Biol Chem 400: 237-246.

Trang P, Weidhaas JB, Slack FJ (2017) MicroRNAs and Cancer. In: (ed.) The Molecular Basis of Human Cancer, pp. 277-286.

Wójcicka A, Kolanowska M, Jażdżewski K (2016) Mechanisms in endocrinology: MicroRNA in diagnostics and therapy of thyroid cancer. Eur J Endocrinol 174: R89-R98.

Yang Z, Yuan Z, Fan Y, Deng X, Zheng Q (2013) Integrated analyses of microRNA and mRNA expression profiles in aggressive papillary thyroid carcinoma. Mol Med Rep 8: 1353-1358.

Yoruker EE, Terzioglu D, Teksoz S, Uslu FE, Gezer U, Dalay N (2016) MicroRNA expression profiles in papillary thyroid carcinoma, benign thyroid nodules and healthy controls. J Cancer 7: 803-809.

Yue K, Wang X, Wu Y, Zhou X, He Q, Duan Y (2016) microRNA-7 regulates cell growth, migration and invasion via direct targeting of PAK1 in thyroid cancer. Mol Med Rep 14: 2127-2134.

Zhang J, Gill AJ, Issacs JD, Atmore B, Johns A, Delbridge LW, Lai R, McMullen TP (2012) The Wnt/β-catenin pathway drives increased cyclin D1 levels in lymph node metastasis in papillary thyroid cancer. Human Pathol 43: 1044-1050.

Zhang X, Li M, Zuo K, Li D, Ye M, Ding L, Cai H, Fu D, Fan Y, Lv Z (2013) Upregulated miR-155 in papillary thyroid carcinoma promotes tumor growth by targeting APC and activating Wnt/β-catenin signaling. J Clin Endocrinol Metabol 98: E1305-E1313.

Zhao Y, Yang H, Wang X, Zhang R, Wang C, Guo Z (2013) Sirtuin-3 (SIRT3) expression is associated with overall survival in esophageal cancer. Ann Diagn Pathol 17: 483-485.

Zheng H, Zhang F, Lin X, Huang C, Zhang Y, Li Y, Lin J, Chen W, Lin X (2016) MicroRNA-1225-5p inhibits proliferation and metastasis of gastric carcinoma through repressing insulin receptor substrate-1 and activation of β-catenin signaling. Oncotarget 7: 4647.