RESEARCH ARTICLE

Overexpression miR-211-5p hinders the proliferation, migration, and invasion of thyroid tumor cells by downregulating SOX11

Lei Wang¹ | Yan-feng Shen² | Zhi-min Shi¹ | Xiao-juan Shang³ | Dong-ling Jin¹ | Feng Xi¹

¹Department of Pathology, Medical College of Hebei University of Engineering, Handan, Hebei Province, China

²Department of Oncology, Affiliated Hospital of Hebei University of Engineering, Handan, Hebei Province, China

³Microscope Room, Medicine College of Hebei University of Engineering, Handan, Hebei Province, China

Correspondence

Feng Xi, Department of Pathology, Medical College of Hebei University of Engineering, Handan, Hebei Province, China.
Email: wahahaxd@126.com

Purpose: This study was aimed to investigate the relationship between miR-211-5p and SOX11, and the effects of their interaction on the proliferation, viability, and invasion of human thyroid cancer (TC) cells.

Methods: We used quantitative real-time PCR (qRT-PCR) to determine the expression of miR-211-5p and SOX11 mRNA in the thyroid tumorous and the adjacent tissues. The target relationship between miR-211-5p and SOX11 was confirmed using dual luciferase reporter gene assay. Flow cytometry, colony formation assay, Transwell assay, and MTT assay were performed to determine the cell-cycle progression, cell apoptosis, proliferation and invasion, respectively. In addition, the tumor formation assay in nude mice was done to assess the effect of miR-211-5p on TC development in vivo.

Results: MiR-211-5p was underexpressed, whereas SOX11 was overexpressed in TC. The overexpression of miR-211-5p inhibited the expression of SOX11. The cell cycle was arrested and the proliferation as well as invasiveness was suppressed by exogenous miR-211-5p in TC cell line. The antitumor role of miR-211-5p was proved by the animal experiment.

Conclusion: MiR-211-5p affected the viability, proliferation and invasion of TC by negatively regulating SOX11 expression.

KEYWORDS

in vivo experiments, miR-211-5p, SOX11, thyroid cancer (TC)

1 | INTRODUCTION

Thyroid cancer (TC) is reported to be one of the most common malignancies.¹ It may be formed from follicular or para follicular thyroid cells and they would develop to papillary or follicular cancer and anaplastic thyroid cancer with the former one, papillary thyroid cancer (PTC) make up 80%-90% of them.^{2,3} Although TC may occur in childhood, a higher prevalence among adults in middle age and a female preference were observed by clinical practice.⁴ In general, the development of the cancer may be rapid and the prognosis of PTC may be poor due to the insufficient understanding of the cancer pathogenesis.⁵⁻⁹ Therefore, it is of highly importance to research the developing mechanism of PTC

in order to improve the prognosis of PTC patients and provide theoretical support to novel therapeutics.

MicroRNAs (miRNAs) are a set of endogenous and non-coding RNAs consist of 18-25 nucleotides.¹⁰ Frequently, these miRNAs are proved to be related to many human cancers, including colorectal cancer,¹¹ liver cancer,¹² bladder cancer,¹³ etc. It has also been reported that several human miRNAs were dysregulated in PTC, such as miR-146-5p, miR-155, and miR-219-5p, etc.^{6,14-16} MiR-211 has been confirmed to be related to melanoma,¹⁷ pancreatic cancer,¹⁸ and colorectal cancer.¹⁹

SOX11 (SRY-related HMG box) encodes a neural transcription factor and is widely expressed in the developmental nervous system,

which indicates its role in neurogenesis, neural cell survival and neurite outgrowth.²⁰ SRY (sex-determining region Y) is located on the Y chromosome. SRY proteins interact with DNA by binding to HMG box type of DNA-binding domain, combined with SOX genes consist a subgroup of HMG, and²¹ SOX11 has been reported to play a key role in progresses in many cancers, such as prostate cancer,²² mantle cell lymphoma,²³ fibromatosis,²⁴ and gastric cancer.²⁵ Various genes including MET,²⁶ CXCL12,²⁷ and AKT3²⁸ have been proved to play a key role in the progresses of PTC development. However, the relationship between SOX11 and PTC still remained to be explored.

Having done the database research and literature review, we believe that a research on the interaction of miR-211-5p and SOX11 in PTC may contribute to the mechanism research of PTC. We thus designed a set of biological experiments to investigate the interplay between miR-211-5p and SOX11 in PTC.

2 | MATERIALS AND METHODS

2.1 | Human tissues

Forty pairs of the thyroid cancer and para-carcinoma tissues which were 3 cm from the thyroid cancer edge were obtained from patients who underwent surgeries in the Affiliated Hospital of Hebei University of Engineering during August 2014 and May 2016. The routine examination information of the patients is shown in Table S1. All the samples were placed immediately in freezing tubes and stored in liquid nitrogen with -80°C after removal.

2.2 | Cell culture

The sterile medium with Roswell Park Memorial Institute (RPMI-1640) containing 10% fetal bovine serum (FBS) was used to culture the human papillary thyroid cancer (PTC) cell lines (K1/BCPAP/TPC-1) and normal thyroid cell line (Nthy-ori 3-1). All the cell lines were purchased from American Type Culture Collection. Then, the culture was put in the incubator (37°C and 5% CO_2).

2.3 | Cell transfection and groupings

2.3.1 | The overexpression of miR-211-5p

The cells were set into three groups containing the blank control group, the miR-mimics group (the cells transfected with miR-211-5p

mimics), and the negative control (NC) group (the cells transfected with miR-Negative Control duplex). MiR-211-5p mimics and Negative Control duplex (miR-NC) were bought from the GenePharman company (Shanghai, China) and transfected into the K1 cells using the Lipofectamine™ 2000 reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions.

2.3.2 | The downexpression of SOX11 mRNA

In order to downregulate the expression of SOX11, the Lipofectamine™ 2000 reagent was used to transfect the siRNA SOX11 (GenePharman) to K1 cells, conforming to the manufacturer's instructions. The cells were also divided into three groups including the blank control group, the si-SOX11 group (K1 cells transfected with si-SOX11), and the NC group (K1 cells transfected with siRNA-NC).

2.4 | Quantitative real-time PCR (qRT-PCR)

The RNA in samples was extracted using TRIzol and was stored in the liquid nitrogen according to the manufacturer's instructions of the RT kit (Item numbers: #K1622, Fermentas, Glen Burnie, MD, USA) and the PCR kit (Item numbers: C10572-014, Invitrogen, USA). Sequence of primers for qRT-PCR was detailed in Table 1. The RNAs and miRNAs were reverse transcribed. The cDNAs were amplified using PCR. The normalized internal controls of miR-211-5p and SOX11 were U6 and GAPDH, respectively. The relative expression levels were calculated using $2^{-\Delta\Delta C_T}$ method.

2.5 | Western blot

The protein in the cells which were transfected for 48 hours was extracted and the concentration of that was measured. Then, the samples were subjected to electrophoresis using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and subsequently transferred to a polyvinylidene difluoride (PVDF) membrane. Then, the membrane was blocked with 5% defatted milk and shook for 2 hours. After the membrane was washed with PBST, the primary antibodies (1:1000) were added to the membrane and incubated at the room temperature for 2 hours and then the antibodies were discarded. The membrane washed by PBST was incubated at the incubator at 37°C with the secondary antibodies (1:1000), which were discarded 2 hours later. Chemiluminescence was used to measure signals of SOX11 protein following the development of the membrane in darkroom.

TABLE 1 Sequence of primers for qRT-PCR

cDNA	Forward primer	Reverse primer
MiR-211-5p	5'-ACACTCCAGCTGGGCAAGTAGCATCACTA-3'	5'-TGGTGTCTGGAGTCG-3'
Wt-SOX11-3'UTR	5'-CTCCGCACGAGACCCAG-3'	5'-GAAGCTGTAGTAGAGGCGGC-3'
Mut-SOX11-3'UTR	5'-CCGAGAGACCCAG-3'	5'-GAGTGTAGTAGAGC-3'
U6	5'-CTCGTTCGGCAGCACA-3'	5'-AACGCTTCACGAATTTGCGT-3'
GAPDH	5'-ACAACCTTTGGTATCGTGAAGG-3'	5'-GCCATCACGCCACAGTTTC-3'

2.6 | 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay

The diluted cells with final concentration of 1×10^4 /mL were seeded into the 96-well plates, 100 μ L per well, and then cultured in breeding place with 37°C and 5%CO₂. After culture for 12, 24, 48, 72, and 96 hours, each well was added with 20 μ L of MTT solution (5 mg/mL, sigma, St. Louis, MO, USA), respectively. Then, the supernatant was discarded 4 hours later. The plates were vibrated for 10 minutes following 150 μ L DMSO (sigma, USA) was placed into each well. A microplate reader was used to calculate the absorbance at 570 nm in each well.

2.7 | Colony formation assay

The proliferation ability of cells was examined by colony formation assay. The logarithmic phase cells were resuspended in the DMEM containing 10% fetal calf serum (FCS), which was then diluted into different grads' multiple. The cells were seeded in the culture plates and incubated at 37°C in the humid atmosphere with 5% CO₂ for about 2 or 3 weeks until the cloned cells were seen macroscopically. Then, the washed cells were fixed with 4% paraformaldehyde which was discarded after 15 minutes. GIMSA was employed to dye the cells for approximately 15 minutes and then discarded. After the cells were dried, the clones' number was counted under the microscope, the clone rate was also calculated.

2.8 | Transwell assay

The transwell assay was used to assess the invasion ability of K1 cells. Range of 60-80 μ L diluted Matrigel was coated on polycarbonate membrane of the upper chambers and placed in the condition with 37°C. Pancreatin was used to digest the cells transfected for 48 hours. Then, the cells were washed with PBS, resuspended in serum-free medium, and seeded in the upper chambers, while the lower chambers added in DMEM with 10% FBS. After the cells in transwell chambers were cultured in 37°C for 24 hours, the cells were blocked by 95% ethanol and dyed with hematoxylin. Finally, the invasive cells were counted in three random fields under a microscope.

2.9 | The flow cytometry (FCM)

2.9.1 | Cell cycle was tested by the FCM

Ethanol (70%) was used to blend with cells in logarithmic phase, and then they were put at 4°C all night. The cells were collected and mixed with 70% ethanol at 4°C overnight. The cells were washed and centrifuged after discarding ethanol. PBS, 1% BSA solution, and 3 μ L PI were added in the cells whose cell cycle was then assessed by the FCM.

2.9.2 | Cell apoptosis was assessed via the FCM

Pancreatin (0.25%) was applied to digest the logarithmic phase cells which were then washed with PBS. The cells were resuspended by the Annexin V (the staining solution consists of 10 μ L 10×Binding Buffer,

Annexin V-FITC 5 μ L, and dh2o 85 μ L). Each sample was blended with 10 μ L PI and incubated in the dark room for 15 minutes and then analyzed using the FCM.

2.10 | Dual luciferase reporter gene assay

PCR was employed to amplify the wild-type SOX11 3'-UTR sequences (585-595 section, GenePharman) and the mutated SOX11 3'-UTR sequences. Then pmirGLO vectors, eukaryotic expression vector with the firefly luciferase gene, were used to construct recombinant plasmids. Then, the Rellina Luciferase plasmids (pRL-CMV) and the pmirGLO-SOX11 3'UTR plasmids were cotransfected with either miR-211-5p or miR-NC into the K1 cells using Lipofectamine™ 2000 reagent based on the manufacturer's instructions. According to the manufacturer's instructions, Firefly Luciferase Assay Kit was applied to examine the luciferase activities of the K1 cells.²⁹

2.11 | Tumor formation in nude mice

Forty-eight SPF nude mice (4 weeks old) were divided into two groups in random. Cells with stable overexpression of miR-211-5p were obtained as previously described in the Cell transfection and groupings section. QRT-PCR was used to confirm the stable overexpression. The cells with stable overexpression of miR-211-5p and those transfected with miR-NC were washed and resuspended. 2×10^6 cells were injected subcutaneously into mice in either group. The tumor was visible 7 days later, then tumor volume was measured every 3 days (Tumor Volume = $0.5 \times D \times d^2$, D represents the long diameter of the tumor, while d is the short diameter of the tumor).³⁰

2.12 | Statistical analysis

All the data and schematic diagram were analyzed by using SPSS 21.0 (Armonk, NY, USA) GraphPad Prism 6.0. Student's *t*-test and one-way ANOVA were employed to determine the statistical significance of the differences between two groups and among several groups, respectively. *P*-value < .05 was designed statistically significant.

3 | RESULTS

3.1 | The expression of miR-211-5p and SOX11 in thyroid tumor tissues and cells

Figure 1A shows the representative HE and IHC staining results of normal and tumorous thyroids, demonstrating the pathological features of the thyroids and SOX11 expression in normal and tumorous thyroids. To examine the expression levels of miR-211-5p and SOX11 of the thyroid cancer, we used qRT-PCR assay to measure miR-211-5p and SOX11 mRNA, the results were showed in schematic diagram (Figure 1B-C). The expression of miR-211-5p was significantly less in thyroid tumor tissues than the normal tissues (*P* < .05), while the expression of SOX11 was obviously in a higher level in carcinoma tissues than the normal tissues (*P* < .05). And then the expression level of

FIGURE 1 The expression of 11-5p and SOX11 in thyroid tumor samples. (A) The HE and IHC staining results showing the pathological features of thyroid and SOX11 expression in thyroid tissues, respectively. (B) The expression of miR-211-5p was obviously more in adjacent tissues than in thyroid tumor tissues (** $P<.01$, compared with adjacent tissues). (C) The expression of SOX11 mRNA was significantly less in adjacent tissues than in thyroid tumor tissues (** $P<.01$, compared with adjacent tissues). (D) The expression of miR-211-5p in thyroid tumor cells is substantially less than in Nthy-ori 3-1 cells (** $P<.01$, compared with Nthy-ori 3-1 cell line). (E) The SOX11 mRNA level in thyroid tumor cells was obviously more than Nthy-ori 3-1 cells (** $P<.01$, compared with Nthy-ori 3-1 cell line). IHC, immunohistochemical staining

FIGURE 2 Overexpression of miR-211 inhibits tumor formation in vivo. (A) HE and IHC staining results showing the pathological features of thyroid and SOX11 expression in thyroid tissues, respectively. (B) The relative miR-211-5p expression in nude mice in two groups, the mice injected with miR-mimics expressed obviously more miR-211-5p than the control group. (C) Tumor in the mice with upregulated miR-211-5p developed dramatically more slowly than the control group, which was based on the volume of tumor detected in different time after injection in mice. * P values <.05, ** P values <.01 were considered statistically significant in comparison with miR-NC group

miR-211-5p in the K1, BCPAP, and TPC-1 cell lines was also lower than that of Nthy-ori 3-1 cell lines (Figure 1D) ($P<.05$), while the expression of SOX11 in those three cell lines was higher than that of Nthy-ori 3-1 cell lines (Figure 1E) ($P<.05$). Therefore, K1 cell line was chosen as the following experimental subject because of its lowest expression of miR-211-5p and highest expression of SOX11 among the three cells.

3.2 | Validation of the antitumor function of miR-211-5p on the tumor formation in nude mice

Figure 2A shows the representative HE staining results of the thyroids of xenograft models in miR-NC and miR-mimics groups. The

miR-mimics treatment resulted in less active and more apoptotic of the thyroid tissues. We made the tumor formation in nude mice to determine the function of miR-182-5p on hindering the growth of thyroid tumor *in vivo*. After the injection of the transfected cells for 36 hours, the expression level of miR-211-5p in nude mice injected with cells transfected with miR-mimics was distinctly higher than that of the control mice, which was showed in Figure 2B. What is more, we found that the tumor in the control group grew obviously faster and bigger than the tumor in the mice with overexpressed miR-211-5p mimics ($P<.01$) (Figure 2C), which certified that the up-regulation of miR-211-5p inhibited the growth of the thyroid tumor *in vivo*.

FIGURE 3 MiR-211-5p inhibited the expression of SOX11. (A) The sequences of miR-211-5p and SOX11 3'UTR. (B) The relative luciferase activities of the cells cotransfected with miR-mimics and pmirGLO-SOX11-3'UTR-wt or miR-mimics and pmirGLO-SOX11-3'UTR-mut (** $P < .01$, compared with miR-NC group). (C) QRT-PCR analysis of the relative expression of miR-211-5p after the transfection of miR-211-5p in K1 cells (** $P < .01$, compared with miR-NC group). (D) The relative expression of SOX11 mRNA in different groups (* $P < .05$, compared with miR-NC1 (miR-mimics NC) group, # $P < .05$, compared with the miR-NC2 (miR-inhibitors NC) group). (E) Western Blot analysis of the SOX11 protein in each group, GAPDH was used as the internal standard

3.3 | MiR-211-5p directly targets SOX11

Targetscan database was used to predict the binding site between miR-211-5p and SOX11 3'UTR (Figure 3A). The targeting function relationship between miR-211-5p on SOX11 was confirmed by dual luciferase reporter gene system. Luciferase activities of the cells cotransfected with miR mimics and pmirGLO-SOX11-3'UTR-wt were significantly lower than that of the cells in the control group ($P < .05$) (Figure 3B), which indicated that miR-211-5p directly targets SOX11. Furthermore, we found that miR-211-5p inhibited the expression of SOX11 mRNA, whereas the inhibition of miR-211-5p significantly resulted in the increase in SOX11 mRNA expression (Figure 3C,D). Figure 3E showed that cells in the miR-mimics group expressed significantly less SOX11 in comparison with cells in the miR-NC (mimics) group, whereas the cells in the miR inhibitors group expressed significantly more SOX11 compared with those in the miR-NC (inhibitor) group.

3.4 | MiR-211-5p affected the proliferation and invasion abilities of thyroid tumor by targeting SOX11

The cells were divided into five groups: the blank control group, miR-NC group, miR-mimics group, si-NC group, and si-SOX11 group. The expression of SOX11 protein was assessed by Western Blot (Figure 4A) and we found that cells in the si-SOX11 group expressed significantly less SOX11 than in the si-NC group, which certified that si-RNA downregulated the SOX11 expression in K1 cells. Similarly,

the MTT assay confirmed that the knock-out of SOX11 by si-SOX11 transfection resulted in severe K1 cell viability. Also the transfection of miR-mimics led to significant compromised K1 cell viability (Figure 4B). Figure 4C-D shows the colony formation and cell invasion results, respectively. The proliferation and invasiveness of K1 cells in pcDNA3.1-SOX11 group were stronger than in pcDNA3.1-NC group. The proliferation and invasiveness of K1 cells in the miR-211 inhibitor+pcDNA3.1-SOX11 group were much stronger than in miR-NC (inhibitor)+pcDNA3.1-NC group. However, there was no significant difference between the miR-NC (mimics)+pcDNA3.1-NC and miR-mimics+pcDNA3.1-SOX11 groups in regards of K1 cell proliferation and invasion.

3.5 | The effect of the overexpression of miR-211-5p or the downexpression of SOX11 on cell cycle and apoptosis

K1 cells in pcDNA3.1-SOX11 group were less arrested in G0/G1 phase than in pcDNA3.1-NC group. The G0/G1 phase arrest of K1 cells in the miR-211 inhibitor+pcDNA3.1-SOX11 group was less stronger than in miR-NC (inhibitor)+pcDNA3.1-NC group. Similarly, there was significant difference between the miR-NC (mimics)+pcDNA3.1-NC and miR-mimics+pcDNA3.1-SOX11 groups in regards of K1 cell arrest in G0/G1 phase (Figure 5A). As to K1 cell apoptosis, K1 cells in pcDNA3.1-SOX11 group were less apoptotic than in pcDNA3.1-NC group. The apoptotic K1 cells in the miR-211 inhibitor+pcDNA3.1-SOX11 group were much less than in

FIGURE 4 MiR-211-5p affected the proliferation and invasion of thyroid tumor by targeting SOX11. (A) Western Blot analysis was conducted to confirm the efficiency of si-SOX11 transfection. GAPDH was used as the internal standard. (B) The viability of the cells after transfected miR-mimics and si-SOX11. (C) The proliferation was measured by colony formation assay in each group. (D) Transwell analysis of the cell invasion of each group. **P* values < .05, ***P* values < .01 were considered statistically significant in comparison with the miR-NC group; #*P* values < .05, ##*P* values < .01 were considered statistically significant in comparison with si-NC group

miR-NC (inhibitor)+pcDNA3.1-NC group. Similarly, there was significant difference between the miR-NC (mimics)+pcDNA3.1-NC and miR-mimics+pcDNA3.1-SOX11 groups in regards of cell apoptosis (Figure 5B).

4 | DISCUSSION

MicroRNAs (miRNAs) are a group of around 22 nucleotide non-coding small RNAs, and it is confirmed that they have a close association with many cancers.^{31,32} In the meantime, as one of the tumor antigenic

precursor proteins (TAPP), SOX11 has also been confirmed to be related with many cancers.³³⁻³⁵ According to the prediction of miR-base and our microarray assays, we predicted the target relationship between SOX11 and miR-211-5p. Their interplay in TC was investigated in this study, which may eventually contribute to a novel treatment of TC.

Like many other miRNAs, miR-211 has been identified to be related to many human cancers, however, there has been no report on miR-211 and TC yet.³⁶⁻³⁸ The role that miR-211 plays in cancer development is still controversial and may regulate tumorigenesis in a cell-specific manner.³⁹ Its role differs in different cancers. For example,

FIGURE 5 The effect of miR-211-5p and SOX11 on both cell cycle and apoptosis (A) The cell cycle was arrested in G0/G1 phase by the overexpression of miR-211-5p and less arrested by the overexpression of SOX11. *, #, & $P < .05$, compared with pcDNA3.1-NC, miR-NC(mimics)+pcDNA3.1-NC, and miR-NC(inhibitor)+pcDNA3.1-SOX11 groups, respectively. (B) The apoptosis rate increased in cells transfected with miR-211-5p mimics, but decreased significantly in cells transfected with pcDNA3.1-SOX11. *, #, & $P < .05$, compared with pcDNA3.1-NC, miR-NC(mimics)+pcDNA3.1-NC, and miR-NC(inhibitor)+pcDNA3.1-SOX11 groups, respectively

it can be a cancer suppressor miR in melanoma but an onco-miR in head and neck squamous cell cancer.^{19,40-42} Despite the varied role of miR-211, its aberrant expression not only suggests the tumorigenesis but also provides possible treatment method for cancers. In this

study, we confirmed that miR-211-5p is downregulated in TC, implying the occurrence of TC and its possible tumor suppressor in TC. This was confirmed by a series of experiments. The exogenous miR-211-5p suppressed the proliferation and invasiveness of TC cells. The

regulation between miR-211-5p and TC has not been reported yet and for the first time we found it may act as a suppressor in TC.

Similarly, there has also been no report on the regulation relationship between SOX11 and TC. For the first time we found it is at high-level expression in TC. According to the present studies, SOX11, belonging to the SOX (SRY-related HMG-box) family of transcription factors, mainly exerts its function by interaction with other proteins.⁴³⁻⁴⁵ Among the members in the SOX family, SOX4 and its involvement in carcinogenesis and neurodevelopment has been thoroughly studied. However, SOX11 has also been asserted to be closely associated with the pathogenesis of cancers. For instance, Keith et al. revealed that SOX11 was overexpressed in aggressive fibromatosis cells and could contribute to the classification of aggressive fibromatosis.²⁴ Besides, Ge et al.³³ also claimed that SOX11 was at high expression level in human glioma cells and its underexpression suppressed the proliferation and invasion abilities of glioma cells. Here, we came to a similar conclusion with these former studies that SOX11 is upregulated in TC cells, which may be helpful for deeper understanding of the role that SOX11 and SOX proteins play in the human tumor development process. And our results also agreed with the identification that SOX11 is one of the TAPPs, which may be an important resource of the natural characteristic molecular, as an obvious signal for cancer cells.³³ However, the exact targeting point or, in other words, its target is still unknown in this research. Further studies are needed to confirm the exact region that SOX11 binds with in TC cells and that will be a great guidance for the clinical therapy against TC.

We confirmed this negative target relationship between miR-211 and SOX11 for the first time. No relevant studies have been reported before from a point of penetration of the regulation of miR-211-5p towards SOX11 and this may be a supplement for our present understanding of the mechanism for both miR-211-5p and SOX11. Interestingly, both the miR-211 and SOX11 were reported to play an important role during the process of neuronal and glial maturation, implying the target relationship may not only exist in TC but also other parts organs and other physiological process.^{46,47} A thorough understanding of the involvement of miR-211-5p and SOX11 in one cancer requires a thorough exploration of all the factors that participate in the miR-211-5p/SOX11 signaling.

In all, the inhibition of TC development can be due to the inhibition of SOX11 expression by miR-211-5p. The possible mechanism may be that miR-211-5p binds to the 3'UTR of SOX11 and therefore inhibits the translation of SOX11. SOX11 is overexpressed in TC tissues, indicating its oncogenic role in TC. So when miR-211-5p can be upregulated in TC tissues/cells, which then downregulated SOX11 expression, less oncogenic activity would happen in thyroid tissue, and cancer development could be reduced.

However, although we revealed this regulating relationship and these two important molecules' role in the process of TC for the first time, there are still some limitations in our study. Firstly, a larger number of cohort would be recruited in our further studies. Besides, miR-211-5p is obviously not the only miRNA that regulates the process of TC by regulating SOX11 and further study should be carried out to reach a comprehensive understanding of the miR-SOX11 regulating

mechanism. Lastly, the variation in tissue samples caused by cancer differentiation was not in our consideration and thus its effects on the study results are not clear.

In brief, we carried out a set of experiments and confirmed the target relationship between miR-211-5p and SOX11. Besides, we detected that miR-211-5p was upregulated and SOX11 was downregulated in TC tissues and cell lines. Furthermore, we revealed that miR-211-5p suppressed the expression of SOX11. By inhibiting SOX11, miR-211-5p suppressed the proliferation and invasion of the TC cells. Our findings may provide guidance for clinical treatment against TC and improve the prognosis of the TC patients.

REFERENCES

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2015. *CA Cancer J Clin*. 2015;65:5-29.
2. Lundgren CI, Hall P, Dickman PW, Zedenius J. Clinically significant prognostic factors for differentiated thyroid carcinoma: a population-based, nested case-control study. *Cancer*. 2006;106:524-531.
3. Carling T, Udelsman R. Thyroid cancer. *Annu Rev Med*. 2014;65:125-137.
4. Chen J, Liu C, Yin L, Zhang W. The tumor-promoting function of ECRG4 in papillary thyroid carcinoma and its related mechanism. *Tumour Biol*. 2015;36:1081-1089.
5. Wang Z, Zhang H, Zhang P, Dong W, He L. MicroRNA-663 suppresses cell invasion and migration by targeting transforming growth factor beta 1 in papillary thyroid carcinoma. *Tumour Biol*. 2016;37:7633-7644.
6. Deng X, Wu B, Xiao K, et al. MiR-146b-5p promotes metastasis and induces epithelial-mesenchymal transition in thyroid cancer by targeting ZNRF3. *Cell Physiol Biochem*. 2015;35:71-82.
7. Ulbrich C, Pietsch J, Grosse J, et al. Differential gene regulation under altered gravity conditions in follicular thyroid cancer cells: relationship between the extracellular matrix and the cytoskeleton. *Cell Physiol Biochem*. 2011;28:185-198.
8. Wang F, Wang P, Wang B, et al. Association between TP53 Arg72Pro polymorphism and thyroid carcinoma risk. *Tumour Biol*. 2014;35:2723-2728.
9. Wang H, Li YP, Chen JH, et al. Prognostic significance of USP22 as an oncogene in papillary thyroid carcinoma. *Tumour Biol*. 2013;34:1635-1639.
10. Lai EC. Micro RNAs are complementary to 3' UTR sequence motifs that mediate negative post-transcriptional regulation. *Nat Genet*. 2002;30:363-364.
11. Zhang XF, Li KK, Gao L, et al. miR-191 promotes tumorigenesis of human colorectal cancer through targeting C/EBPbeta. *Oncotarget*. 2015;6:4144-4158.
12. Lu Z, Zhang W, Gao S, et al. MiR-506 suppresses liver cancer angiogenesis through targeting sphingosine kinase 1 (SPHK1) mRNA. *Biochem Biophys Res Commun*. 2015;468:8-13.
13. Zhou J, Dai W, Song J. miR-1182 inhibits growth and mediates the chemosensitivity of bladder cancer by targeting hTERT. *Biochem Biophys Res Commun*. 2016;470:445-452.
14. Li D, Jian W, Wei C, et al. Down-regulation of miR-181b promotes apoptosis by targeting CYLD in thyroid papillary cancer. *Int J Clin Exp Pathol*. 2014;7:7672-7680.
15. Zhang X, Li M, Zuo K, et al. Upregulated miR-155 in papillary thyroid carcinoma promotes tumor growth by targeting APC and activating Wnt/beta-catenin signaling. *J Clin Endocrinol Metab*. 2013;98:E1305-E1313.
16. Huang C, Cai Z, Huang M, et al. miR-219-5p modulates cell growth of papillary thyroid carcinoma by targeting estrogen receptor alpha. *J Clin Endocrinol Metab*. 2015;100:E204-E213.

17. Xu Y, Brenn T, Brown ER, Doherty V, Melton DW. Differential expression of microRNAs during melanoma progression: miR-200c, miR-205 and miR-211 are downregulated in melanoma and act as tumour suppressors. *Br J Cancer*. 2012;106:553-561.
18. Giovannetti E, van der Velde A, Funel N, et al. High-throughput microRNA (miRNAs) arrays unravel the prognostic role of MiR-211 in pancreatic cancer. *PLoS One*. 2012;7:e49145.
19. Cai C, Ashktorab H, Pang X, et al. MicroRNA-211 expression promotes colorectal cancer cell growth in vitro and in vivo by targeting tumor suppressor CHD5. *PLoS One*. 2012;7:e29750.
20. Lu TX, Li JY, Xu W. The role of SOX11 in mantle cell lymphoma. *Leuk Res*. 2013;37:1412-1419.
21. Lovell-Badge R, Hacker A. The molecular genetics of Sry and its role in mammalian sex determination. *Philos Trans R Soc Lond B Biol Sci*. 1995;350:205-214.
22. Yao Z, Sun B, Hong Q, et al. The role of tumor suppressor gene SOX11 in prostate cancer. *Tumour Biol*. 2015;36:6133-6138.
23. Kuo PY, Leshchenko VV, Fazzari MJ, et al. High-resolution chromatin immunoprecipitation (ChIP) sequencing reveals novel binding targets and prognostic role for SOX11 in mantle cell lymphoma. *Oncogene*. 2015;34:1231-1240.
24. Skubitz KM, Skubitz AP. Gene expression in aggressive fibromatosis. *J Lab Clin Med*. 2004;143:89-98.
25. Qu Y, Zhou C, Zhang J, et al. The metastasis suppressor SOX11 is an independent prognostic factor for improved survival in gastric cancer. *Int J Oncol*. 2014;44:1512-1520.
26. Wang G, Cai C, Chen L. MicroRNA-3666 regulates thyroid carcinoma cell proliferation via MET. *Cell Physiol Biochem*. 2016;38:1030-1039.
27. Dong S, Jin M, Li Y, Ren P, Liu J. miR-137 acts as a tumor suppressor in papillary thyroid carcinoma by targeting CXCL12. *Oncol Rep*. 2016;35:2151-2158.
28. Li R, Liu J, Li Q, Chen G, Yu X. miR-29a suppresses growth and metastasis in papillary thyroid carcinoma by targeting AKT3. *Tumour Biol*. 2016;37:3987-3996.
29. Qian Y, Wang X, Lv Z, et al. MicroRNA126 is downregulated in thyroid cancer cells, and regulates proliferation, migration and invasion by targeting CXCR4. *Mol Med Rep*. 2016;14:453-459.
30. Zhu H, Fang J, Zhang J, et al. miR-182 targets CHL1 and controls tumor growth and invasion in papillary thyroid carcinoma. *Biochem Biophys Res Commun*. 2014;450:857-862.
31. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell*. 2004;116:281-297.
32. Kozubek J, Ma Z, Fleming E, et al. In-depth characterization of microRNA transcriptome in melanoma. *PLoS One*. 2013;8:e72699.
33. Ge L, Cornforth AN, Hoa NT, et al. Differential glioma-associated tumor antigen expression profiles of human glioma cells grown in hypoxia. *PLoS One*. 2012;7:e42661.
34. Emruli VK, Olsson R, Ek F, Ek S. Identification of V-ATPase as a molecular sensor of SOX11-levels and potential therapeutic target for mantle cell lymphoma. *BMC Cancer*. 2016;16:493.
35. Teng Y, Fan YC, Mu NN, Zhao J, Sun FK, Wang K. Serum SOX11 promoter methylation is a novel biomarker for the diagnosis of Hepatitis B virus-related hepatocellular carcinoma. *Neoplasma*. 2016;63:419-426.
36. Yin Z, Cui Z, Li H, et al. Polymorphisms in miR-135a-2, miR-219-2 and miR-211 as well as their interaction with cooking oil fume exposure on the risk of lung cancer in Chinese nonsmoking females: a case-control study. *BMC Cancer*. 2016;16:751.
37. Chen YF, Yang CC, Kao SY, Liu CJ, Lin SC, Chang KW. MicroRNA-211 enhances the oncogenicity of carcinogen-induced oral carcinoma by repressing TCF12 and increasing antioxidant activity. *Cancer Res*. 2016;76:4872-4886.
38. Wise J. NICE approves innovative treatment for moderate to severe heart failure. *BMJ*. 2016;353:i2402.
39. Chu TH, Yang CC, Liu CJ, Lui MT, Lin SC, Chang KW. miR-211 promotes the progression of head and neck carcinomas by targeting TGFbetaRII. *Cancer Lett*. 2013;337:115-124.
40. Mazar J, DeYoung K, Khaitan D, et al. The regulation of miRNA-211 expression and its role in melanoma cell invasiveness. *PLoS One*. 2010;5:e13779.
41. Sakurai E, Maesawa C, Shibasaki M, et al. Downregulation of microRNA-211 is involved in expression of preferentially expressed antigen of melanoma in melanoma cells. *Int J Oncol*. 2011;39:665-672.
42. Chang KW, Liu CJ, Chu TH, et al. Association between high miR-211 microRNA expression and the poor prognosis of oral carcinoma. *J Dent Res*. 2008;87:1063-1068.
43. Ambrosetti DC, Basilico C, Dailey L. Synergistic activation of the fibroblast growth factor 4 enhancer by Sox2 and Oct-3 depends on protein-protein interactions facilitated by a specific spatial arrangement of factor binding sites. *Mol Cell Biol*. 1997;17:6321-6329.
44. Nishimoto M, Fukushima A, Okuda A, Muramatsu M. The gene for the embryonic stem cell coactivator UTF1 carries a regulatory element which selectively interacts with a complex composed of Oct-3/4 and Sox-2. *Mol Cell Biol*. 1999;19:5453-5465.
45. Kamachi Y, Uchikawa M, Tanouchi A, Sekido R, Kondoh H. Pax6 and SOX2 form a co-DNA-binding partner complex that regulates initiation of lens development. *Genes Dev*. 2001;15:1272-1286.
46. Fan C, Wu Q, Ye X, et al. Role of miR-211 in neuronal differentiation and viability: implications to pathogenesis of Alzheimer's disease. *Front Aging Neurosci*. 2016;8:166.
47. Schepers GE, Teasdale RD, Koopman P. Twenty pairs of sox: extent, homology, and nomenclature of the mouse and human sox transcription factor gene families. *Dev Cell*. 2002;3:167-170.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

How to cite this article: Wang L, Shen Y-F, Shi Z-M, Shang X-J, Jin D-L, Xi F. Overexpression miR-211-5p hinders the proliferation, migration, and invasion of thyroid tumor cells by downregulating SOX11. *J Clin Lab Anal*. 2018;32:e22293. <https://doi.org/10.1002/jcla.22293>