Genetic predisposition to papillary thyroid carcinoma is mediated by a long non-coding RNA TINCR enhancer polymorphism

Qiang Wang

West China School of Basic Medicine Sciences & Forensic Medicine, Sichuan University

Hong Huang

Dazhou Vocational and Technical College

Peng Chen

Sichuan University West China Second University Hospital

Xiao Xiao

Sichuan University West China Second University Hospital

Xiaolei Luo

Sichuan University West China Second University Hospital

Yanyun Wang

Sichuan University West China Second University Hospital

Linbo Gao (✉ gaolinbo@scu.edu.cn)

Sichuan University <https://orcid.org/0000-0002-7572-8990>

Lin Zhang

West China School of Basic Medicine Sciences & Forensic Medicine, Sichuan University: Sichuan University West China Hospital

Research Article

Keywords: TINCR long non coding RNA, Polymorphism, Single nucleotide polymorphisms, Enhancer, Transcription factor, Thyroid Cancer, Papillary thyroid carcinoma

Posted Date: November 2nd, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-1023538/v1>

License: This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

Abstract

Single nucleotide polymorphisms (SNPs) in the enhancer region have been demonstrated to confer to altered enhancer activities, aberrant gene expression, and cancer susceptibility. In this study, we aimed to examine the association between an SNP, rs8101923, within terminal differentiation-induced non-coding RNA (TINCR) and the risk of papillary thyroid carcinoma (PTC). Blood samples from 559 patients with PTC and 445 healthy individuals were collected. The rs8101923 was genotyped by using polymerase chain reaction-restriction fragment length polymorphism assay. The impact of the rs8101923 on TINCR expression and enhancer activity was evaluated by quantitative real-time PCR and dual-luciferase reporter assay. The binding of AP-2 α to TINCR enhancer was determined by chromatin immunoprecipitation. The rs8101923 G allele was significantly associated with a higher risk of PTC (adjusted OR = 1.37; 95% CI: 1.15–1.64). Mechanistically, the rs8101923 was related to increased transcriptional levels and enhancer activities ($P < 0.05$). Transcription factor AP-2 α binds to the enhancer region of TINCR containing the rs8101923 locus, and promotes cell proliferation in PTC. These findings suggest the rs8101923 as a risk factor in the pathogenesis of PTC, which provides evidence for explaining the mechanism of the rs8101923 risk allele predisposing to PTC.

Introduction

Thyroid cancer is the most common head and neck cancer. With the development of diagnostic technology, the incidence of thyroid cancer, especially papillary thyroid carcinoma (PTC), has increased significantly worldwide in the past few decades^{1,2}. In 2020, the incidence of thyroid cancer ranks 9th among all cancers globally, with more than 580,000 cases³. A clinical study including 15,000 thyroid cancer patients from China showed that PTC is the most common histological subtype, accounting for about 99% of all cases⁴. It is evident that the occurrence of thyroid cancer is related to multiple risk factors, such as genetic variants, radiation exposure, and iodine intake^{2,3,5}. However, the specific molecular mechanism of its initiation and progression is not completely clear.

Long non-coding RNAs (lncRNAs), a type of non-coding transcripts with the length over 200 nucleotides, are most easily overlooked in research at the beginning due to its non-coding feature. Recent studies have shown that lncRNAs can perform a variety of functions in organisms, such as regulating chromatin function, modulating the assembly and function of membraneless nucleosomes, changing the stability and translation of cytoplasmic mRNA, and interfering with signal pathways⁶. Currently, lncRNAs and lncRNA-related single nucleotide polymorphisms (SNPs) have been proven to contribute to the susceptibility and aggressiveness of various cancers, including PTC^{7–9}. Kim et al. reported that a lncRNA, LOC100507661, was highly expressed in human thyroid cancer, and the over-expression promoted proliferation, migration, and invasion of thyroid cancer cells¹⁰. Jendrzewski et al. reported that a lncRNA, PTCSC3 (PTC susceptibility candidate gene 3), influenced PTC predisposition and carcinogenesis by modulating S100A4 gene expression¹¹. Gou et al. reported that a tumor-suppressive lncRNA, AB074169, inhibited cell proliferation by binding KHSRP (KH-type splicing regulatory protein) and increasing p21 expression in PTC¹². Maruei-Milan et al. reported that SNPs within *ANRIL* (antisense non coding RNA in the INK4 locus) were

associated not only with the risk but also with the severity of PTC¹³. More importantly, enhancer-related SNPs were found to confer to altered enhancer activities, aberrant expression, and cancer susceptibility^{8,14-16}.

Terminal differentiation-induced non-coding RNA (TINCR), a lncRNA with a length of 3.7k bases, was first discovered in 2012 with an important role in human epidermal differentiation¹⁷. Subsequently, TINCR has been found to be associated with the occurrence and progression of a variety of malignant tumors, such as gastric cancer, esophageal cancer, and breast cancer¹⁸⁻²⁰. As mentioned above, SNPs in the enhancer region may play an essential role in the predisposition to PTC^{8,14-16}. In this study, we hypothesized that SNPs within the enhancer region of TINCR may contribute to the risk of PTC. To test this hypothesis, we carried out a case-control study to evaluate the relationship between SNP within the enhancer region of TINCR and the susceptibility of PTC in a Chinese population.

Methods

Study subjects

The study included 559 patients with PTC and 445 healthy controls. The subjects were enrolled from the West China Hospital of Sichuan University and were all unrelated Han nationality. Participants' information such as age, gender, and TNM staging were recorded in detail. Tumor stages were determined according to American Joint Committee on Cancer. The healthy volunteers were selected from the same hospital during the same period as the PTC cases were recruited. All controls were frequency matched to PTC cases based on age and gender. Among them, individuals suffering from thyroid disease or other tumors were excluded. Peripheral blood samples of all subjects were taken before undergoing any medical treatment such as surgery and radiotherapy. Furthermore, the fresh PTC clinical specimens and adjacent normal tissues were collected immediately after the operation and placed in liquid nitrogen for later use. Informed consent was obtained from all individuals included in the study. The research protocol was approved by the ethics committees of West China Second University Hospital, Sichuan University.

SNPs selection

We selected SNPs according to the following criteria: (1) SNPs within TINCR enhancer that are annotated by ensemble database (<http://www.ensembl.org/index.html>); (2) SNPs with minor allele frequency more than 10% in Han Chinese. Only one SNP, rs8101923, followed the criteria and was selected for further analysis.

DNA extraction and SNP genotyping

According to the manufacturer's protocol, we used TIANamp Genomic DNA Kit (TianGen Biotech Co. Ltd., Beijing, China) to extract genomic DNA from blood samples, and used NanoDrop spectrophotometer (ND-1000) to determine the purity and concentration of DNA. The DNA fragment containing the SNP site (i.e., rs8101923) was amplified by polymerase chain reaction (PCR). The amplification primers used in this study were: 5'-AAGGTTGTGTGTGGGAGAGG-3' (forward), 5'-CATGACCTCTGGGGTGTCTT-3' (reverse). The PCR system was set to 10 µl mix: 5 µl 2X Power Taq PCR MasterMix, 10 µM for each primer, 0.5 µl DNA template,

and Milli-Q water as a supplement to 10 µl. The PCR reaction conditions were: 94°C for 4 min, 94°C for 30 s, 60°C for 30 s, 72°C for 30 s, 35 cycles, and 72°C for a final extension of 10 minutes. Subsequently, the PCR product was digested by *Sty*I (New England BioLabs, MA, USA), and genotyped by PCR-restriction fragment length polymorphism assay. After digestion, the G allele produced a 216 bp band, and the A allele produced 123 bp and 93 bp bands. In order to verify the genotyping results, we randomly selected the samples for Sanger sequencing, and the agreement rate was 100%.

Quantitative real-time RT-PCR (qRT-PCR)

TRIzol reagent (Invitrogen, Carlsbad, CA, USA) was used to extract total RNA from 43 pairs of PTC tissues and adjacent normal tissues, and RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific Scientific, Rockford, IL, USA) was used to reverse transcribed total RNA into cDNA. LightCycler® 480 System (Roche Indianapolis, IN, USA) and QuantiNova™ SYBR® Green PCR Kit (Qiagen, Hilden, Germany) were used for determining the relative expression levels of TINCR. The TINCR primers used for qRT-PCR were described as follows¹⁷: forward 5'-TGTGGCCCAAACCTCAGGGATACAT-3' and reverse 5'-AGATGACAGTGGCTGGAGTTGTCA-3'. 18s was used as an internal control and amplified with the following primers: forward 5'-GCAATTATTCCTCATGAACG-3' and reverse 5'-GGCCTCACTAAACCATCCAA-3'. Each amplification reaction was completed in a total volume of 10 µl, which contained 0.7 µl primers, 5 µl Master mix, and 100 ng cDNA. The reaction conditions were set as follows: 95°C for 2 min, 95°C for 5 s, and 60°C for 10 s, 40 cycles.

Cell culture, plasmid construction, and luciferase reporter assay

TPC-1 and BCPAP cells were purchased from GuangZhou Jennio Biotech Co. Ltd. (Guangzhou, China). All cell lines were maintained in RPMI1640 medium supplemented with 10% fetal bovine serum and 1% ampicillin/streptomycin and cultured under 5% CO₂. The TINCR fragment containing the rs8101923 AA or GG was amplified by PCR, and the amplification primers were forward 5'-AGATGACAGTGGCTGGAGTTGTCA-3' and reverse 5'-TGTGGCCCAAACCTCAGGGATACAT-3'. The PCR products were inserted into pmirGLO miRNA target expression vector (Promega) to construct pmirGLO-rs8101923A and pmirGLO-rs8101923G plasmids. For the luciferase reporter gene detection, we used the dual luciferase reporter gene detection system (Promega). Firefly and renilla luciferase activity was measured at 48 h after transfection according to the manufacturer's instructions.

Cell transfection and cell viability assay

The small interfering RNA (siRNA) targeting AP-2α (si1-AP-2α and si2-AP-2α) was constructed by Genepharma (Shanghai, China). The sequences of si1-AP-2α and si2-AP-2α were 5'-GCAAGAUCUUACUCCCACTT-3' and 5'-CCUGCUCACAUCACUAGUATT-3', respectively. When cells reached 90% confluence, the medium was changed to serum-free and antibiotic-free medium, and transfection was performed using Lipofectamine 3000 (Invitrogen) according to the reagent manufacturer's protocol. At 24 h, 48 h, and 72 h after transfection, cell viability assay was performed using the 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method.

Chromatin immunoprecipitation (ChIP)

Cells were grown to approximately 80% to 90% confluence, cross-linked with 1% paraformaldehyde at 37°C for 10 minutes, and quenched in 125 mM glycine. The cell lysates were sonicated under conditions that produced fragments of 200 bp to 1000 bp. The material was clarified by centrifugation, diluted 10 times in dilution buffer, and pre-clarified with protein A-Sepharose beads. The pre-cleared supernatant containing chromatin was used for the immunoprecipitation reaction with the antibody against AP-2 α (Abcam, Cambridge, MA, USA). Anti-mouse IgG was used as a control. After digestion with proteinase K and RNase, the immunoprecipitated genomic DNA was extracted with phenol: chloroform: isoamyl alcohol and precipitated with ethanol. For gene-specific ChIP analysis, PCR and Sanger sequencing were used to determine the DNA sequence enriched by immunoprecipitation.

Statistical analysis

SPSS 20.0 statistical software (SPSS Inc, IL, USA) was used for data analysis. The rs8101923 genotype frequency was obtained by direct counting. The Student's *t*-test used to compare the age of cases and controls. The gender comparison and Hardy-Weinberg equilibrium (HWE) were calculated using Chi-square (χ^2) test. Results of qPCR were analyzed using Wilcoxon rank sum test. The unpaired Student's *t*-test was used to analyze the luciferase data. The difference of the rs8101923 genotype distribution between cases and controls was analyzed using the χ^2 check test. Odds ratio (OR) and 95% confidence interval (CI) were used to assess the correlation between the rs8101923 and PTC risk. *P* < 0.05 was considered statistically significant.

Results

Characteristics of the study population

Totally, 559 patients with PTC and 445 healthy individuals were included in this study. The age of the PTC group and the control group was 43.2 ± 9.4 and 44.5 ± 13.3 years, respectively; while the difference was not statistically significant (*P* = 0.08). The proportion of male subjects in the two groups was 29.7% and 24.7%, respectively, and the difference was not statistically significant (*P* = 0.08). Among PTC patients, there were 42 cases of Tx (7.5%), 173 cases of T1 and T2 (31%), and 344 cases of T3 and T4 (61.5%); there were 343 cases with lymph node metastasis (61.4%) and 216 cases without lymph node metastasis (38.6%); there were 11 patients (2%) who developed distant metastasis (Table 1).

Table 1
Characteristics of the study population in patients with PTC and controls.

Variables	Controls, n = 445	PTC, n = 559	Pvalue
Age, mean $\pm$ SD (years)	43.2 $\pm$ 9.4	44.5 $\pm$ 13.3	0.08
Gender (%)	132 (29.7)	138 (24.7)	0.08
Male	313 (70.3)	421 (75.3)	
Female			
T status (%)		42 (7.5)	
Tx		173 (31.0)	
T1 & T2		344 (61.5)	
T3 & T4			
N status (%)		216 (38.6)	
N0		343 (61.4)	
N1a & N1b			
M status (%)		548 (98.0)	
M0		11 (2.0)	
M1			
Multiplicity of tumor (%)			
No		394 (70.5)	
Yes		165 (29.5)	
PTC, papillary thyroid carcinoma; SD, standard deviation.			

The rs8101923 within the enhancer of TINCR increased PTC risk

The genotype and allele distributions of the rs8101923 in patients with PTC and controls are summarized in Table 2. An increased risk was observed in a codominant model (AG vs. AA: adjusted OR = 1.36; 95% CI: 1.02–1.82; P = 0.03; GG vs. AA: adjusted OR = 1.81; 95% CI: 1.27–2.57; P < 0.001, respectively). The increased risk was also observed in a dominant model (adjusted OR = 1.50; 95% CI: 1.14–1.95; P = 0.003) and in a recessive model (adjusted OR = 1.49; 95% CI: 1.10–2.03; P = 0.01). Furthermore, the allele frequencies of the rs8101923 were significantly different between PTC cases and controls (adjusted OR = 1.37; 95% CI: 1.15–1.64; P < 0.001). However, in stratification analysis, the rs8101923 was not related to clinical status of PTC patients, including T status, N status, and multiplicity (Table 3).

Table 2
Association between the rs8101923 and PTC risk.

Genetic models	rs8101923	Controls, n = 445 (%)	PTC, n = 559 (%)	Logistic regression (crude)		Logistic regression (adjusted)	
				OR (95% CI)	P value	OR (95% CI)	P value
Codominant	AA	164 (36.9)	158 (28.3)	1.00		1.00	
	AG	200 (44.9)	261 (46.7)	1.35 (1.02-1.80)	0.04	1.36 (1.02-1.82)	0.03
	GG	81 (18.2)	140 (25.0)	1.79 (1.26-2.55)	0.001	1.81 (1.27-2.57)	< 0.001
Dominant	AA	164 (36.9)	158 (28.3)	1.00		1.00	
	AG/GG	281 (63.1)	401 (71.7)	1.48 (1.13-1.93)	0.004	1.50 (1.14-1.95)	0.003
Recessive	AA/AG	364 (81.8)	419 (75.0)	1.00		1.00	
	GG	81 (18.2)	140 (25.0)	1.50 (1.10-2.04)	0.009	1.49 (1.10-2.03)	0.01
Allele	A	528 (59.3)	577 (51.6)	1.00		1.00	
	G	362 (40.7)	541 (48.4)	1.37 (1.14-1.63)	< 0.001	1.37 (1.15-1.64)	< 0.001
PTC, papillary thyroid carcinoma; OR, odds ratio; CI, confidence interval.							

Table 3
Association between the rs8101923 and clinical status of PTC patients.

Clinical status	Genotype frequency		Dominant model		Recessive model		Allele comparison	
	n (%)	n (%)	Adjusted OR (95% CI)	P value	Adjusted OR (95% CI)	P value	Adjusted OR (95% CI)	P value
T status	T1 & T2	T3 & T4						
AA	52 (30.1)	93 (27.0)						
AG	75 (43.4)	165 (48.0)						
GG	46 (26.6)	86 (25.0)	1.17 (0.78-1.75)	0.45	0.93 (0.61-1.41)	0.72	1.04 (0.80-1.34)	0.79
N status	N0	N1a & N1b						
AA	61 (28.2)	97 (28.3)						
AG	105 (48.6)	156 (45.5)						
GG	50 (23.1)	90 (26.2)	0.93 (0.63-1.38)	0.72	1.21 (0.80-1.83)	0.36	1.04 (0.81-1.34)	0.74
Multiplicity	No	Yes						
AA	106 (26.9)	52 (31.5)						
AG	181 (45.9)	80 (48.5)						
GG	107 (27.2)	33 (20.0)	0.82 (0.55-1.22)	0.32	0.66 (0.43-1.03)	0.07	0.79 (0.61-1.03)	0.08
PTC, papillary thyroid carcinoma; OR, odds ratio; CI, confidence interval.								

The rs8101923 G increased TINCR levels through increasing enhancer activity

qRT-PCR was used to examine the expression levels of TINCR in 43 pairs of clinical PTC tissue samples. We found that the expression of TINCR in PTC tissues was significantly increased compared to normal tissues ($P < 0.001$) (Fig. 1A). We then examined whether the rs8101923 had an allele-specific effect on the expression of TINCR. As shown in Fig. 1B, the TINCR levels in PTC tissues with the rs8101923 GG genotype

were significantly higher than those with the AA genotype ($P < 0.05$). Next, we used reporter gene analysis to evaluate whether the rs8101923 influenced enhancer activity. As shown in Fig. 1C, TPC-1 and BCPAP cells transfected with the rs8101923 G allele presented a significantly increased enhancer activity compared to the rs8101923 A allele ($P < 0.05$).

AP-2 α enriched the TINCR enhancer region containing the rs8101923 and promoted cell proliferation

Using the TRANSFAC® 7.0 software, we predicted that the transcription factor AP-2 α may be enriched in the enhancer region of TINCR containing the SNP, rs8101923. We then used the CHIP assay to verify the binding site. As shown in Fig. 2A and 2B, AP-2 α specifically bound to DNA sequences in BCPAP cells and PTC-1 cells compared to the IgG control. Sanger sequencing confirmed that AP-2 α binding site contained the rs8101923 (Fig. 2C and 2D). Next, we examined whether knock-down of AP-2 α influenced proliferation in BCPAP and PTC-1 cells (Fig. 2E). As shown in Fig. 2F and 2G, cell viability was significantly reduced at 72h after transfection in both BCPAP cells ($P < 0.01$) and PTC-1 cells ($P < 0.05$). These findings suggested that AP-2 α enriched the enhancer region of TINCR containing the rs8101923, and promoted cell proliferation in PTC.

Discussion

Previous studies have demonstrated that various lncRNAs were differentially expressed in PTC, such as PTCSC3, PTCSC2, maternally expressed gene 3 (MEG3), and BRAF-activated non-protein coding RNA (BANCR), serving as tumor suppressors or oncogenic lncRNAs^{8,21-24}. However, there is no report investigating the role of TINCR in the occurrence of PTC. In this study, we found for the first time that TINCR was highly expressed in PTC tissues. Mechanistically, an SNP, rs8101923, within the enhancer region of TINCR was found to be associated with increased transcriptional levels and enhancer activities, and contributed to the risk of PTC in the Chinese population. Transcription factor AP-2 α bound to the enhancer region of TINCR containing the SNP (i.e., rs8101923) and promoted cell proliferation in PTC. These findings suggest the rs8101923 as a risk factor in the pathogenesis of PTC, which provides evidence for explaining the mechanism of the rs8101923 risk allele predisposing to PTC.

As a key lncRNA required for somatic tissue differentiation, TINCR was first characterized by Markus Kretz et al. in 2013¹⁷. Subsequently, TINCR was emphatically identified as an important regulating molecular in the development of epithelial malignant tumors, such as gastric cancer¹⁹. Xu et al. reported that nuclear transcription factor SP1-induced TINCR was significantly up-regulated in the plasma and tissues of gastric cancer patients, and the high expression of TINCR contributed to tumor occurrence and cancer progression by affecting the stability and expression of *KLF2* (Kruppel like factor 2) mRNA via binding to Staufen1 (STAU1) protein^{19,25}. The expression of TINCR can also be induced by transcription activator E2F1²⁶. Additionally, TINCR can serve as a competing endogenous RNA by sponging miR-375, and promote proliferation and inhibit apoptosis²⁷. The high expression of TINCR and its carcinogenic effect were also observed in epithelial ovarian cancer²⁸, breast cancer¹⁸, cervical squamous cell carcinoma²⁹, hepatocellular carcinoma³⁰, non-small cell lung cancer³¹, nasopharyngeal carcinoma³², and bladder urothelial carcinoma³³, which suggests that TINCR may be a strategic target for cancer treatment. However, the opposite situation was found in lung adenocarcinoma³⁴, colorectal cancer³⁵, prostate cancer³⁶,

melanoma³⁷, oral squamous cell carcinoma³⁸, and skin cancer³⁹, with a down-regulation in these cancers. To date, no reports investigated the expression of TINCR in PTC patients. In this study, we used a qPCR to examine the expression of TINCR in 43 PTC paired tumor/normal tissues and found that the expression of TINCR in PTC tissues was significantly up-regulated compared to normal tissues, suggesting that TINCR might be an oncogenic lncRNA in PTC tumorigenesis.

Previous studies have shown that genetic variants are involved in the tumorigenesis of PTC^{8,14–16,40,41}. For example, rs153109 and rs17855750 in interleukin (*IL*)-27 and rs10877887 in the promoter of *let-7* were identified to be risk factors for PTC occurrence^{40,41}. Notably, SNPs in the enhancer region were found to be functional. Jendrzewski et al. reported that an SNP, rs944289, located in the PTCSC3 enhancer region, inhibited the expression of PTCSC3 and led to susceptibility to PTC⁸. He et al. reported that multiple SNPs in long-range enhancer elements altered enhancer activities and/or transcription factor binding activities, and regulated the expression of forkhead box E1 (FOX E1) and PTCSC2 in thyroid cancer¹⁴. Ge et al. reported that a variant, rs2736100, located in the intronic enhancer region of *TERT* (telomerase reverse transcriptase), was significantly associated with an elevated PTC risk and presented a genotype-specific effect on *TERT* expression¹⁵. Comiskey et al. reported that a variant, rs17134155, located in the enhancer region of *EPB41L4A* (erythrocyte membrane protein band 4.1 like 4A), was significantly associated with PTC predisposition by regulating the expression and splicing of *EPB41L4A* transcript¹⁶. Based on this background, we hypothesized that SNPs within the enhancer region of TINCR might contribute to the risk of PTC. Our findings confirmed this hypothesis. We found that the rs8101923 within the enhancer region of TINCR was associated with a 1.37-fold increased risk of PTC and the risk G allele correlated to a higher enhancer activity and TINCR expression levels. These findings indicate the rs8101923 as an enhancer SNP that affects the occurrence of PTC by modulating the expression of TINCR.

Enhancers are cis-regulatory elements that can be bound by transcription factors to activate gene expression⁴². In this study, the transcription factor AP-2α was predicted to bind the enhancer region of TINCR containing the rs8101923 by using the TRANSFAC® 7.0 software. ChIP results confirmed the prediction. *AP-2α* was found to be highly expressed in PTC tissues, and the high expression of *AP-2α* mRNA was significantly correlated with advanced tumor stage and shorter overall survival⁴³. Knock-down of *AP-2α* inhibited proliferation in PTC cells. Previous findings together with our current work indicate that AP-2α may enrich the TINCR enhancer region containing the rs8101923, and promote cell proliferation.

There are some limitations in this study. All patients with PTC included in this study were collected from the same hospital for a continuous period of time, and thus the selection bias might be inevitable. There was no significant association between the rs8101923 and clinical features of PTC in stratified analysis. This negative result may be caused by limited sample size and insufficient statistical power. What's more, we did not consider the patient's living environment, such as ionizing radiation and daily iodine intake, when collecting samples. The interaction of TINCR and environmental factors on the risk PTC cannot be assessed.

Conclusion

This study provides the first evidence that the rs8101923 within the enhancer region of TINCR was a risk factor for the development of PTC in the Chinese population. Mechanistically, the rs8101923 might increase transcriptional levels and enhancer activities of TINCR. In the future, further research on the correlation between the rs8101923 and other thyroid tumors will help to understand the role of SNP in the treatment, diagnosis, and prognosis of thyroid cancer.

Declarations

Acknowledgements

This work was supported by the National Natural Science Foundation of China (No. 81974226), the Sichuan Science and Technology Program (No. 2021YFS0026) and the Science and Technology Program of Dazhou, Sichuan (No.20YYJC0002).

Author contributions

ZL and GL designed and revised the manuscript. WQ performed the experiments and drafted the manuscript. HH helped to perform the experiments and provided funding assistance. CP and LX collected clinical samples and performed genotyping assay. WY performed statistical analysis.

Conflict of interests

None.

References

1. R. Udelsman, Y. Zhang, The epidemic of thyroid cancer in the United States: the role of endocrinologists and ultrasounds. *Thyroid: official journal of the American Thyroid Association*. **24**(3), 472–479 (2014)
2. V. Drozd, D.I. Branovan, C. Reiners, Increasing Incidence of Thyroid Carcinoma: Risk Factors and Seeking Approaches for Primary Prevention. *ijt*. **13**(2), 95–110 (2020)
3. H. Sung, J. Ferlay, R.L. Siegel et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA: a cancer journal for clinicians*. 2021;71(3):209-249
4. C. Sui, N. Liang, R. Du et al., Time trend analysis of thyroid cancer surgery in China: single institutional database analysis of 15,000 patients. *Endocrine*. **68**(3), 617–628 (2020)
5. M.E. Cabanillas, D.G. McFadden, C. Durante, Thyroid cancer. *Lancet* (London, England). **388**(10061), 2783–2795 (2016)
6. L. Statello, C.J. Guo, L.L. Chen, M. Huarte, Gene regulation by long non-coding RNAs and its biological functions. *Nature reviews Molecular cell biology*. **22**(2), 96–118 (2021)
7. G.J. Goodall, V.O. Wickramasinghe, RNA in cancer. *Nature reviews Cancer*. **21**(1), 22–36 (2021)
8. J. Jendrzewski, H. He, H.S. Radomska et al., The polymorphism rs944289 predisposes to papillary thyroid carcinoma through a large intergenic noncoding RNA gene of tumor suppressor type.

- Proceedings of the National Academy of Sciences of the United States of America. **109**(22), 8646–8651 (2012)
9. K. Jazdzewski, E.L. Murray, K. Franssila, B. Jarzab, D.R. Schoenberg, A. de la Chapelle, Common SNP in pre-miR-146a decreases mature miR expression and predisposes to papillary thyroid carcinoma. Proceedings of the National Academy of Sciences of the United States of America. **105**(20), 7269–7274 (2008)
 10. D. Kim, W.K. Lee, S. Jeong et al., Upregulation of long noncoding RNA LOC100507661 promotes tumor aggressiveness in thyroid cancer. Molecular and cellular endocrinology. **431**, 36–45 (2016)
 11. J. Jendrzewski, A. Thomas, S. Liyanarachchi et al., PTCSC3 Is Involved in Papillary Thyroid Carcinoma Development by Modulating S100A4 Gene Expression. The Journal of clinical endocrinology and metabolism. **100**(10), E1370–E1377 (2015)
 12. Q. Gou, L. Gao, X. Nie et al., Long Noncoding RNA AB074169 Inhibits Cell Proliferation via Modulation of KHSRP-Mediated CDKN1a Expression in Papillary Thyroid Carcinoma. Cancer research. **78**(15), 4163–4174 (2018)
 13. R. Maruei-Milan, Z. Heidari, A. Aryan, M. Asadi-Tarani, S. Salimi, Long non-coding RNA ANRIL polymorphisms in papillary thyroid cancer and its severity. British journal of biomedical science. **78**(2), 58–62 (2021)
 14. H. He, W. Li, S. Liyanarachchi et al., Multiple functional variants in long-range enhancer elements contribute to the risk of SNP rs965513 in thyroid cancer. Proceedings of the National Academy of Sciences of the United States of America. **112**(19), 6128–6133 (2015)
 15. M. Ge, M. Shi, C. An et al., Functional evaluation of TERT-CLPTM1L genetic variants associated with susceptibility of papillary thyroid carcinoma. Scientific reports. **6**, 26037 (2016)
 16. D.F. Comiskey Jr., H. He, S. Liyanarachchi et al., Characterizing the function of EPB41L4A in the predisposition to papillary thyroid carcinoma. Scientific reports. **10**(1), 19984 (2020)
 17. M. Kretz, Z. Siprashvili, C. Chu et al., Control of somatic tissue differentiation by the long non-coding RNA TINCR. Nature. **493**(7431), 231–235 (2013)
 18. S. Xu, D. Kong, Q. Chen, Y. Ping, D. Pang, Oncogenic long noncoding RNA landscape in breast cancer. Molecular cancer. **16**(1), 129 (2017)
 19. T.P. Xu, X.X. Liu, R. Xia et al., SP1-induced upregulation of the long noncoding RNA TINCR regulates cell proliferation and apoptosis by affecting KLF2 mRNA stability in gastric cancer. Oncogene. **34**(45), 5648–5661 (2015)
 20. M. Hazawa, D.C. Lin, H. Handral et al., ZNF750 is a lineage-specific tumour suppressor in squamous cell carcinoma. Oncogene. **36**(16), 2243–2254 (2017)
 21. H. Yoon, H. He, R. Nagy et al., Identification of a novel noncoding RNA gene, NAMA, that is downregulated in papillary thyroid carcinoma with BRAF mutation and associated with growth arrest. International journal of cancer. **121**(4), 767–775 (2007)
 22. H. He, W. Li, S. Liyanarachchi et al., Genetic predisposition to papillary thyroid carcinoma: involvement of FOXE1, TSHR, and a novel lincRNA gene, PTCSC2. The Journal of clinical endocrinology and metabolism. **100**(1), E164–E172 (2015)

23. Y. Liu, P. Yue, T. Zhou, F. Zhang, H. Wang, X. Chen, LncRNA MEG3 enhances (131)I sensitivity in thyroid carcinoma via sponging miR-182. *Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie*. **105**, 1232–1239 (2018)
24. J. Zhang, Y. Du, X. Zhang, M. Li, X. Li, Downregulation of BANCR Promotes Aggressiveness in Papillary Thyroid Cancer via the MAPK and PI3K Pathways. *Journal of Cancer*. **9**(7), 1318–1328 (2018)
25. K. Zhang, H. Shi, H. Xi et al., Genome-Wide lncRNA Microarray Profiling Identifies Novel Circulating lncRNAs for Detection of Gastric Cancer. *Theranostics*. **7**(1), 213–227 (2017)
26. T.P. Xu, Y.F. Wang, W.L. Xiong et al., E2F1 induces TINCR transcriptional activity and accelerates gastric cancer progression via activation of TINCR/STAU1/CDKN2B signaling axis. *Cell death & disease*. **8**(6), e2837 (2017)
27. Z. Chen, H. Liu, H. Yang, Y. Gao, G. Zhang, J. Hu, The long noncoding RNA, TINCR, functions as a competing endogenous RNA to regulate PDK1 expression by sponging miR-375 in gastric cancer. *OncoTargets and therapy*. **10**, 3353–3362 (2017)
28. R. Li, Y. Wang, Y. Xu, X. He, Y. Li, Silencing the long noncoding RNA, TINCR, a molecular sponge of miR-335, inhibits the malignant phenotype of epithelial ovarian cancer via FGF2 suppression. *International journal of oncology*. **55**(5), 1110–1124 (2019)
29. A. Hou, Y. Zhang, Y. Zheng, Y. Fan, H. Liu, X. Zhou, LncRNA terminal differentiation-induced ncRNA (TINCR) sponges miR-302 to upregulate cyclin D1 in cervical squamous cell carcinoma (CSCC). *Human cell*. **32**(4), 515–521 (2019)
30. H. Zhao, Z. Xie, G. Tang, S. Wei, G. Chen, Knockdown of terminal differentiation induced ncRNA (TINCR) suppresses proliferation and invasion in hepatocellular carcinoma by targeting the miR-218-5p/DEAD-box helicase 5 (DDX5) axis. *Journal of cellular physiology*. **235**(10), 6990–7002 (2020)
31. Z.J. Zhu, J.K. He, TINCR facilitates non-small cell lung cancer progression through BRAF-activated MAPK pathway. *Biochemical and biophysical research communications*. **497**(4), 971–977 (2018)
32. Z.Q. Zheng, Z.X. Li, J.L. Guan et al., Long Noncoding RNA TINCR-Mediated Regulation of Acetyl-CoA Metabolism Promotes Nasopharyngeal Carcinoma Progression and Chemoresistance. *Cancer research*. **80**(23), 5174–5188 (2020)
33. Z. Chen, Y. Liu, A. He et al., Theophylline controllable RNAi-based genetic switches regulate expression of lncRNA TINCR and malignant phenotypes in bladder cancer cells. *Scientific reports*. **6**, 30798 (2016)
34. Y.W. Gao, F. Ma, Y.C. Xie et al., Sp1-induced upregulation of the long noncoding RNA TINCR inhibits cell migration and invasion by regulating miR-107/miR-1286 in lung adenocarcinoma. *American journal of translational research*. **11**(8), 4761–4775 (2019)
35. S. Yu, D. Wang, Y. Shao et al., SP1-induced lncRNA TINCR overexpression contributes to colorectal cancer progression by sponging miR-7-5p. *Aging*. **11**(5), 1389–1403 (2019)
36. L. Dong, H. Ding, Y. Li, D. Xue, Y. Liu, LncRNA TINCR is associated with clinical progression and serves as tumor suppressive role in prostate cancer. *Cancer management and research*. **10**, 2799–2807 (2018)
37. M. Melixetian, D. Bossi, M. Mihailovich et al., Long non-coding RNA TINCR suppresses metastatic melanoma dissemination by preventing ATF4 translation. *EMBO reports*. **22**(3), e50852 (2021)

38. Z. Zhuang, J. Huang, W. Wang et al., Down-Regulation of Long Non-Coding RNA TINCR Induces Cell Dedifferentiation and Predicts Progression in Oral Squamous Cell Carcinoma. *Frontiers in oncology*. **10**, 624752 (2020)
39. W. Zhou, S. Zhang, J. Li, Z. Li, Y. Wang, X. Li, lncRNA TINCR participates in ALA-PDT-induced apoptosis and autophagy in cutaneous squamous cell carcinoma. *Journal of cellular biochemistry*. **120**(8), 13893–13902 (2019)
40. Y. Wang, T. Wei, J. Xiong et al., Association Between Genetic Polymorphisms in the Promoter Regions of Let-7 and Risk of Papillary Thyroid Carcinoma: A Case-Control Study. *Medicine*. **94**(43), e1879 (2015)
41. X. Nie, F. Yuan, P. Chen et al., Association between IL-27 gene polymorphisms and risk of papillary thyroid carcinoma. *Biomarkers in medicine*. **11**(2), 141–149 (2017)
42. S.R. Grossman, X. Zhang, L. Wang et al., Systematic dissection of genomic features determining transcription factor binding and enhancer function. *Proceedings of the National Academy of Sciences of the United States of America*. **114**(7), E1291–E1300 (2017)
43. H.R. Wu, J. Zhang, AP-2 α expression in papillary thyroid carcinoma predicts tumor progression and poor prognosis. *Cancer management and research*. **10**, 2615–2625 (2018)

Figures

Figure 1

The rs8101923 influenced TINCR levels and enhancer activities. (A) qRT-PCR examining the expression levels of TINCR in 43 pairs of clinical PTC tissues and normal tissues. (B) The TINCR expression levels in PTC patients carrying the rs8101923 AA, AG, and GG, respectively. (C) Plasmid containing the rs8101923 A or G allele was constructed and transfected into TPC-1 and BCPAP cells. Relative enhancer activity was measured by using the dual luciferase reporter gene detection system. (* $P < 0.05$, *** $P < 0.001$)

Figure 2

AP-2α enriched the TINCR enhancer region containing the rs8101923 and promoted cell proliferation. (A and B) DNA amplified product enriched by chromatin immunoprecipitation with the antibody against AP-2α and IgG in BCPAP cells and PTC-1 cells. (C and D) Sanger sequencing confirmed that AP-2α binding site contained the rs8101923. (E) AP-2α was knocked down using small interfering RNA (si1-AP-2α and si2-AP-2α). (F and G) At 24 h, 48 h, and 72 h after AP-2α knock-down, cell viability was examined using the 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide method. (* $P < 0.05$, ** $P < 0.01$)