Downregulation and the Diagnostic Value of RMST in Patients with Papillary Thyroid Cancer

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Research

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Abstract

Background: Previous studies have demonstrated that rhabdomyosarcoma 2-associated transcript (RMST) is an indispensable factor in promoting neuronal differentiation. Gene Expression Omnibus datasets showed that long noncoding RNA RMST was downregulated in papillary thyroid cancer (PTC). Further experiments were conducted to detect the expression and diagnostic value of RMST in PTC.

Materials and methods: Quantitative reverse-transcription polymerase chain reaction was applied to uncover the expression of RMST in PTC tissues. Chi-square (χ^2) analysis was employed to evaluate the association between RMST and clinical features. The area under the curve (AUC) and receiver-operating characteristic curves were used to evaluate the feasibility of using RMST to predict PTC, lymph node metastases and the tumor–node–metastasis (TNM) stage.

Result: RMST expression was significantly in PTC tissues than in adjacent noncancerous thyroid tissues ($P<0.001$). We also found that low tissue RMST expression was related to the TNM stage ($P=0.046$), and lymph node metastasis ($P=0.002$). The AUC value of PTC vs. adjacent noncancerous tissues was 0.7243 [95% confidence interval (CI)=0.6411-0.8076, $P<0.0001$] and AUC values of patients with lymph node metastasis vs. patients with out lymph node metastasis and stage I/II PTC vs. stage III/IV PTC were 0.7148 (95%CI =0.6018-0.8351, $P=0.0012$) and , 0.7024 (95%CI= 0.5817-0.8231, $P=0.0066$), respectively.

Conclusion: In conclusion, RMST is associated with PTC progression and may serve as a diagnostic biomarker for predicting PTC, lymph node metastasis, and TNM stage.

1. Background

Thyroid cancer (TC) is one of the most common endocrine malignancies; moreover, the incidence of TC has been increasing in the past three decades (1,2). Currently, the incidence and mortality rates of TC in China are 0.32% and 0.03%, respectively (3). Fortunately, most of the cases of TC are of papillary thyroid cancer (PTC), which is a relatively indolent thyroid malignancy with excellent long-term survival rates [4]. Although most patients with PTC have a good prognosis, the risks of cancer recurrence and metastasis make it impossible to ignore these patients [5]. Although the aggressive characteristics and mechanism of PTC are unclear, the molecular regulatory mechanisms may provide novel insights for patients with PTC. Therefore, new molecular markers may contribute to the diagnosis of aggressive PTC.

Long non-coding RNAs (lncRNAs) are a class of non-coding RNAs that are more than 200 nucleotides in length and play critical roles in epigenetic, transcriptional and post-transcriptional regulation (6,7). Increasing evidence has demonstrated that lncRNAs are involved in the development, migration, and apoptosis of many malignancies, including PTC (8). For example, Yoon et al. identified a novel noncoding RNA, NAMA, which is downregulated in PTC and is associated with growth arrest (9). Increased expression of NEAT1_2 in PTC is associated with aggressive clinicopathological characteristics, such as the tumor–node–metastasis (TNM) stage and tumor size. Moreover, knocking down the expression of

NEAT1_2 inhibits aggressive PTC behavior by downregulating microRNA(miR)-106b-5p to regulate the expression of ATAD2 (10). Previous studies have indicated that lncRNA LINC01061 upregulation is a predictor of poor prognosis and promotes PTC progression (11). Using integrative computational methods, You et al. found a three-lncRNA signature (PRSS3P2, KRTAP5-AS1, and PWAR5) which could effectively predict the prognosis of PTC (12). Therefore, it is worth while to explore lncRNAs in PTC.

Rhabdomyosarcoma 2-associated transcript (RMST), a long non-coding RNA, plays a crucial role in neurogenesis by aiding in the association between the Sox2 transcription factor and its target promoters, which is indispensable for neuronal differentiation (13, 14). A recent study demonstrated that downregulated RMST may promote triple-negative breast cancer (TNBC) cell proliferation, invasion, and migration (15). However, few studies have been conducted to determine the functions of RMST in PTC. In the current study, we measured RMST expression in PTC samples and paired noncancerous samples of Gene Expression Omnibus (GEO) datasets and clinical patients. We then evaluated the association between RMST expression and clinicopathological characteristics in patients with PTC. Finally, we performed receiver-operating characteristic (ROC) curve to analysis the diagnostic value of RMST in predicting PTC, lymph node metastasis, and TNM stage.

2. Materials And Methods

2.1 Patients and samples

A total of 83-pairs of PTCs and adjacent noncancerous samples were collected from patients who had undergone surgical resection of TC between July 2017 and June 2018 at the Hunan Cancer Hospital of Central South University, China. No patients had been treated with radiotherapy or chemotherapy before the surgery. The diagnosis of PTC was established performed by the Pathology Department of Hunan Cancer Hospital, according to the TNM stage standard. The samples were immediately stored in liquid nitrogen before use. The study was approved by the Clinical Research Ethics Committee of Hunan Cancer Hospital of Xiangya Medical School, Central South University. All patients provided written informed consent.

2.2 Quantitative real-time polymerase chain reaction

Total RNA was isolated from tissue samples using TRIzol reagent (TAKARA) according to the manufacturer's protocol. The quality of RNA was evaluated using a NanoDrop Spectrophotometer (Shimadzu Biotech, Beijing China). Only when the A260/A280 ratio was 1.8 to 2.1, we purified the extracted RNA. The isolated RNA was then reverse-transcribed into cDNA using the PrimeScript™ RT reagent kit with gDNA Eraser (RR047A; Takara, Dalian, China). Real-time quantitative reverse-transcription PCR (qRT-PCR) was performed using the Light Cycle®480 II system(Roche, Basel, Switzerland). The qRT-PCR amplification procedure was performed as follows: 95°C for 5 s, 55°C for 30 s and 72°C for 30 s for a total of 45 cycles. The relative expression of RMST was normalized to the endogenous gene expression of β -actin and calculated using the $2^{-\Delta\Delta C_t}$ method. All reactions were performed repetitively. The primer sequences of RMST and β -actin were synthesized by Sangon Biotech (Shanghai, China).

The primer sequencing results were as follows:

RMST sense: 5'- AGAGACAGAACAGCACAG-3';

RMST antisense: 5'- CATAGAACCACGGAGACTT - 3';

β -actin sense: 5'- CCTGGCACCCAGCACAAT - 3';

β -actin antisense: 5'-GGGCCGGACTCGTCATAC - 3'.

2.3 Expression analysis from the GEO database

Two expression profiles [GSE33630 (16) and, GSE66783 (17)] were downloaded from the GEO using the following search terminologys: (papillary thyroid cancer) and (normal OR adjacent). The following filters were then used to specify the results: [Entry type] Series, [study type] expression profiling by array, [Organism] Homo sapiens. Finally, GSE33630 and GSE66783 were used in this study. For GSE33630, 49 PTC and 45 noncancerous thyroid tissues were analyzed. For GSE66783, 5 PTC specimens and their paired adjacent noncancerous thyroid tissue samples were obtained.

2.4 Statistical analysis

SPSS version 21.0 software (IBM Corp, Chicago, IL, USA) was used to analyze the data, and GraphPad Prism V.7.00 software (GraphPad Software, La Jolla, CA, USA) was used to draw figures. Comparisons between groups were performed using the Student t-test or non-paired Mann–Whitney U test. The chi-square (χ^2) test was applied to determine the association between RMST expression and the clinicopathological features of PTC. Multivariable logistic regression analysis was conducted and data are expressed as odds ratios (ORs) and 95% confidence intervals (95% CIs). ROC curves were used to evaluate the diagnostic value of RMST. P-values < 0.05 were considered statistically significant.

3. Results

3.1 Downregulation of RMST expression in tissues from patients with PTC

As shown in Fig. 1A, the expression of RMST in PTC tissue was significantly downregulated compared to that in the paired noncancerous tissues ($P < 0.001$). To further confirm the expression of RMST in PTC samples, two GEO datasets (GSE33630, GSE66783) were used to obtain the expression of RMST. As shown in Fig. 1B and 1C, the expression of RMST was significantly decreased in PTC tissues compared to that in non-tumoral thyroid tissues in these datasets ($P < 0.001$). These results were consistent with the analysis of clinical PTC samples. Moreover, we found that the expression of RMST was significantly decreased in stage III/IV TC compared with that in I/II TC and noncancerous tissues in 83-paired samples (Fig. 2).

3.2 Correlation between RMST and clinicopathological features

The clinical characteristics of the 83 patients with PTC are listed in Table 1. Among all the patients with PTC, 49 had lymph node metastasis (59.04%), and 20 patients had in advanced- stage PTC (25.10%). Other clinical features, such as age, sex, and tumor size, are also listed in Table 1. To analyze the correlation between RMST expression and clinicopathological features, patients with PTC were divided into two groups based on the median expression value (0.68) of RMST. As shown in Table 2, our data demonstrated that the expression of RMST was significantly associated with the TNM stage ($P = 0.046$) and lymph node metastases ($P = 0.002$). However, no significant correlation was found between RMST expression and other clinical features such as gender, age, and tumor size ($P > 0.05$). Furthermore, to investigate whether RMST is an independent protective factor of lymph node metastases ($B < 0$, $OR < 1$), multivariable analysis was employed. As shown in Table 3, RMST was an independent protective factor of lymph node metastases after adjustments for age, sex, body mass index, serum thyroid-stimulating hormone levels, and tumor size ($OR = 0.094$, $P = 0.002$).

Table 1
General characteristics of papillary thyroid cancer

Characteristics	Number (n = 83)	Percentage (100%)
Age (years)		
< 45	41	49.39
≥ 45	42	50.61
Gender		
Male	17	20.48
Female	66	79.52
Tumor size (cm)		
< 2	46	55.42
≥ 2	37	44.58
Lymph node metastases		
Positive	34	40.96
Negative	49	59.04
T stage		
T1	48	57.83
T2	28	33.73
T3	5	6.02
T4a/b	2	2.42
TNM staging		
I/II	63	75.90
III/IV	20	24.10
Extrathyroidal extension		
Positive	45	54.22
Negative	38	45.78

Table 3
Association between lymph node metastasis and RMST expression by multivariate analysis

RMST			
	OR	95%CI	P
Lymph node metastasis			
Model 1	0.242	0.095–0.612	0.003
Model 2	0.148	0.049–0.451	0.001
Model 3	0.094	0.021–0.416	0.002
Notes: Model 1, not adjusted; Model 2, adjusted for sex, age and BMI; Model 3, adjusted for sex, age, BMI, pre-operation serum TSH level and tumor size.			

3.3 Diagnostic value of RMST in patients with PTC

To investigate whether RMST may serve as a diagnostic marker for predicting PTC, lymph node metastasis and the TNM stage, ROC curves and areas under the ROC curves (AUCs) were employed. Our results illustrated significant difference between PTC and adjacent noncancerous tissues, with an AUC of 0.7243 (95%CI = 0.6411–0.8076, $P < 0.0001$; Fig. 3A) for RMST in 83-paired samples when the cutoff value was 1.398, with a sensitivity and specificity of 61.64% and 78.08%, respectively. The AUC of thyroid cancer vs. healthy controls for GSE33630 was 0.7859 (sensitivity, 93.88%; specificity, 55.56%; Fig. 3B). Moreover, RMST may serve as a diagnostic biomarker for predicting lymph node metastasis and the TNM stage with AUCs of 0.704 (sensitivity, 80.65%; specificity, 65.22%; Fig. 3C) and AUC 0.7024 (sensitivity, 70% ; specificity, 68.25%), respectively.

4. Discussion

Recently, the diagnostic value of lncRNAs in PTC has been evaluated in many studies; for example, GAS8-AS1 and LPAR4 may serve as potential diagnostic and therapeutic targets (18). However, the diagnostic value of RMST in PTC remains unclear.

In the present study, we first uncovered downregulated expression of RMST in PTC tissues compared that in adjacent noncancerous tissues. Further analysis revealed that down-regulated RMST was associated with the TNM stage and lymph node metastasis. Moreover, we demonstrated that RMST could predict PTC, lymph node metastasis and the TNM stage using ROC curves and AUCs.

Increasing evidence has indicates that the differential expression of lncRNAs is closely related to the development and progression of cancers, including PTC (19). For instance, lncRNA MALAT1, which actes as a key regulator of proliferation and invasion in several cancers, is upregulated in PTC and promotes

the proliferation and invasion of TC cells (20). A previous study showed that LncRNA HOXA-AS2 is upregulated in PTC and modulates the miR-15a-5p/HOXA3 axis to promote tumorigenesis and the progression of PTC (21). Ding et al. (22) revealed that lncRNA NONHSAT129183 is significantly upregulated in PTC tissues when compared with adjacent noncancerous thyroid tissue. Moreover, silencing NONHSAT129183 significantly suppressed cell proliferation, migration, and invasion in PTC cell lines. The downregulation of lncRNA PTCSC3 has been demonstrated to contribute to drug resistance in Anaplastic TC (23). These studies indicate that differentially expressed lncRNAs are involved in carcinogenesis and proliferation in PTC and may provide novel diagnostic and prognostic biomarkers for patients with PTC.

In this study, we identified that lncRNA RMST is downregulated in PTC tissues; moreover, its expression is correlated with lymph node metastasis, and the TNM stage in patients with PTC. Ng et al. (14) pointed out that RMST is indispensable for neuronal differentiation. Yu et al. (24) found that transspliced RMST inhibits embryonic stem cell differentiation by targeting the epithelial-to-mesenchymal transition pathway. Wang et al. (25) found that RMST expression is low in TNBC tissues and cells; moreover, they demonstrated that RMST overexpression restrains the invasion and migration abilities of TNBC cells. The present study found that RMST was downregulated in PTC tissues compared to that in adjacent noncancerous tissues and associated with some aggressive features, such as lymph node metastasis. Finally, we found that RMST may serve as a diagnostic biomarker for predicting lymph node metastasis and the TNM stage, with AUCs of 0.7243, 0.704, and 0.7024, respectively. However, some limitations need to be addressed in further studies. For instance, the underlying mechanism of the involvement of RMST in tumor suppression in PTC must be elucidated. In addition, large-scale studies and long-term follow-up for verification are also needed.

In conclusion, we demonstrated low expression of RMST in PTC tissues. RMST may be employed as a potential diagnostic biomarker for PTC detection.

Declarations

Ethics approval

This study was approved by the Ethics Committee of Hunan Cancer Hospital and the Affiliated Cancer Hospital of Xiangya School of Medicine, Central South University.

Consent

Written informed consent for publication of their clinical details was obtained from the patient.

Competing interests

The authors declare that they have no competing interests.

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Author contributions

Zhiwei Xiong and Yuyu Peng have drafted the paper, made a substantial contribution to research design, analysis or interpretation of data; Qibing Zhou, Huiqiong Xiao, and Sheng Xu have revised it critically; Jiaqing Hu and Xiaojun have given approval of the submitted and final versions.

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Availability of data and materials

Not applicable.

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26. The expression. of RMST in different stage of PTC patients and the diagnosis value of RMST between malignant and benign.

27. Notes. The data is analyzed by student's t-test.

Figures

Figure 1

Expression of RMST in clinical patients' tissues and GEO datasets. Notes: analyzed by using the Student's t-test * $P < 0.05$

Figure 2

Expression of RMST in noncancerous tissues and different TNM stages of patients with PTC. Notes: Noncancerous controls (n=83) and all thyroid cancers including stages I/II (n=63) and, III/IV (n=20). Data were analyzed using the nonparametric Mann–Whitney U-test.

Figure 3

ROC curve analysis of RMST as a diagnostic marker for papillary thyroid cancer (A–D). Predictive value of RMST for papillary thyroid cancer, GSE33630, lymph node metastasis, and the TNM stage in patients with papillary thyroid cancer, respectively.