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ORIGINAL ARTICLE

Down-Regulation of APTR and it's Diagnostic Value in Papillary and Anaplastic Thyroid Cancer

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Abstract

APTR has been employed as a potential biomarker attributing to it was involved in carcinogenesis and malignancy's progression. However, the roles of APTR in papillary thyroid cancer (PTC) and anaplastic thyroid cancer (ATC) are unclear. In the present study, we aimed to explore the relative expression of APTR in PTC and ATC tissues and the relation between APTR expression and PTC clinicopathological features. We analyzed APTR expression in PTC and ATC by investigating data obtained from the Gene Expression Omnibus (GEO) database. Then, we tested 76-pair PTC and adjacent normal samples by qRT-PCR, and the result was in accordance with the analysis in GEO datasets. Chi-square ($\chi 2$) analysis was employed to evaluate the association between APTR and PTC clinical features. These results showed that APTR was negatively related to TNM stages, distant metastasis. In addition, we further evaluated the feasibility of using APTR to detect PTC and ATC patients by the receiver operating characteristic (ROC) and the area under curve (AUC). These findings implied that down-regulation of APTR is correlated with tumorigenesis, also indicated that the potential diagnostic value of APTR for detecting PTC and ATC patients.

Keywords APTR · Papillary thyroid cancer (PTC) · Anaplastic thyroid cancer (ATC) · ROC · Diagnosis

Introduction

Thyroid carcinoma is one of the most common endocrine malignancy worldwide [1]. The incidence of thyroid carcinoma has increased with 2.4-times incidence over the past three decades [2]. Fortunately, most of patients suffer from PTC which is a relatively indolent and excellent long-term survival thyroid malignancy [3]. Although most patients with PTC have a good prognosis, the risk of cancer recurrence and metastasis is still impossible to ignore for patients [4].Furthermore, the disease-specific mortality of ATC can approach 100% [5]. At present, conventional treatments and chemotherapy such as thyroidectomy, radio-iodine treatment, doxorubicin mono-therapy or doxorubicin combine with cisplatin changes the effectiveness of thyroid cancer with a poor response to radioiodine [6], however, primary or secondary drug resistance eventually leads to treatment failure in patients as their diseases progress. Therefore, new biomarkers of predicting the progression and diagnosis for PTC and ATC need to be further identified.

Long non-coding RNAs (lncRNAs) are currently defined as non-coding protein transcripts with more than 200 nucleotides [7]. LncRNAs have various biological functions, for instance, silencing or activating gene expression and controlling chromatin states at distantly located genes [8]. Dysregulation of lncRNAs has been demonstrated that associated with cancer phenotypes such as proliferation, growth suppression, motility, immortality, angiogenesis, and viability [9]. Recent studies displayed that lncRNAs also exert critical effects in thyroid cancer. Many lncRNAs have been identified that associated with thyroid cancer cell proliferation, migration, and invasion, such as LncRNA TNRC6C-AS1 [10], LncRNA BISPR [11] and lncRNA GAS8-AS1 [12],by sponging miRNA or other mechanisms, to regulate genesis and progression of cancer.

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Moreover, increasing studies have demonstrated that body fluid lncRNAs could act as potential diagnostic markers for tumors [13]. Zhang et al. found downregulation of GAS8-AS1 was associated with lymph node metastasis [14], Liao et al. identified BLACAT1 was downregulated in PTC and could serve as a prognosis biomarker for PTC [15]. Meanwhile, lncRNAs also involved in drug resistance, for example, lncRNA PTCSC3 suppressed drug resistance of ATC to doxorubicin through negatively regulating STAT3 and INO80 [16]. LncRNA Alu-mediated p21 transcriptional regulator (APTR), acts in trans to repress the CDKN1A/p21 promoter, was identified that related to the cell proliferation in several cancers, such as human glioblastomas, hepatic stellate cells [17, 18]. However, the potential diagnostic ability of APTR to distinguish PTC and ATC patients from normal is unclear. In current study, we detected the expression of APTR in PTC and ATC and then evaluated the feasibility of using the APTR to detect PTC and ATC patients in GEO dataset. By analyzing GEO datasets, we identified that APTR is significantly decreased in PTC and ATC patients. Then, we detected APTR expression levels in PTC tissues and adjacent normal tissues, and investigated the association between APTR expression and clinical features. Finally, we evaluated the feasibility of using the DANCR to detect PTC and ATC patients.

Materials and Methods

Expression Analysis in GEO Database

We downloaded the expression of APTR from Gene Expression Omnibus (GEO) (http://www.ncbi.nlm.nih.gov/geo/) datasets. Three datasets were obtained for current analysis, including GSE65144 [19] (11 ATC and 12 unmatched normal thyroid tissues and 2 matched normal thyroid tissues were enrolled into investigation), GSE66783 [20] (analysis of 5 cases of PTC and their paired adjacent noncancerous thyroid tissue samples), GSE33630 [21] (11ATC samples, 49 PTC samples and 45 normal samples were collected for microarray analysis). Detailed information of these series are listed in Table 1. GEO2R was used to analyze the expression of APTR and profile graph was employed to visualize the expression of APTR.

Patients and Tissue Samples

96-pair PTC tissues and their corresponding adjacent normal tissues were collected from 2016 to 2017 at the Hunan Cancer Hospital of Xiangya medical school, Central South University. Physicians from the Pathology Department of Hunan Cancer Hospital diagnosed and graded these PTC tissues according to the TMN stage standard. According to medical history, only 76 patients have been enrolled into this study. No patients had been treated with radiotherapy or chemotherapy before surgery. All tissues were frozen at liquid nitrogen until RNA was extracted for qRT-PCR. The present study was approved by the ethics committee of Hunan Cancer Hospital of Xiangya medical school, Central South University. Informed consent form (IFC) was signed by all each patient involved in the study. We collected age, gender, TNM stage and other clinical characteristics from the case management system in Hunan Cancer Hospital.

RNA Extraction and Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)

We extracted total RNA from tissue specimens using Trizol reagent (Takara) according to the manufacturer's protocol. NanoDrop Spectrophotometer (Shimadzu Biotech, Beijing China) and gel electrophoresis were employed to measure the quality and quantity of extracted RNA. We would to pure the extracted RNA when the A260/A280 ratio is between 1.8 to 2.1 [22]. The isolated RNA concentration was calculated and normalized with RNase-free water and then reverse-transcribed into cDNA using PrimeScript™ RT reagent kit with gDNA Eraser (RR047A; Takara, Dalian, China). All cDNA samples were stored at −80 °C until use. Light Cycle@480 II (Roche, Basel, Switzerland) was employed to conduct qRT-PCR by using TB GReen™ Premix Ex Taq™ II(Tli RNaseH Plus) (Code No: RR820A, Takara Bio Inc.) according to the manufacturer's protocol. The qRT-PCR amplification was performed as follows: an initial denaturation at

Table 1 Detailed information of the GEO datasets in the present study

Series	Accession	Cancer detail and number	Type platform
GSE66783	Homo sapiens	Five PTC specimens and paired normal controls	GPL19850 Agilent-060228 Human LncRNA v5 4X180K
GSE65144	Homo sapiens	11 ATC specimens and 13 unmatched normal	GPL570[HG-U133_Plus_2] Affymetrix Human Genome U133 Plus 2.0 Array
GSE33630	Homo sapiens	11 ATC samples, 49 PTC samples, 45 normal samples	GPL570 [HG-U133_Plus_2] Affymetrix Human Genome U133 Plus 2.0 Array

GEO, Gene Expression Omnibus

95 °C for 30 s, followed by 40 cycles at 95 °C for 5 s, 60 °C for 30 s. The $2^{-\Delta\Delta Ct}$ method was used to quantify the fold change of APTR expression in tumor samples versus normal control samples as shown in reference [23]. β-actin was used as an internal control, and all reactions were performed in duplicate. The primer sequences of lncRNA APTR and β-actin were synthesized by Sangon Bio-tech (Shanghai, China).

The primer sequences were as follows:

APTR sense: 5′- AGTAGCAGGAGACAGCAT-3′;
APTR antisense: 5′- TGACAGCCTTCCACAATC-3′;
β-actin sense: 5′- CCTGGCACCCAGCACAAT -3′;
β-actin antisense: 5'-GGGCCGGACTCGTCATAC -3′.

Statistical Analysis

All data are presented as mean ± SD and analyzed by GraphPad Prism V.7.00 software(GraphPad Software,La Jolla, CA,USA), and SPSS version 19.0 software(IBM Corp, Chicago, IL,USA). Comparison between patients' and healthy tissues for statistical significance was performed with two-tailed Student's t test. Based on the mean value, 76 PTC patients were divided into two groups; one is low APTR expression, and another is high APTR expression. Chi-square test was applied to evaluate the association between APTR expression and the clinical features of PTC. P value <0.05 was considered to be statistically significant. Receiver operating characteristic (ROC) curve and area under the ROC curve (AUC) were used to evaluate the feasibility of using the APTR to detect PTC and ATC patient.

Results

Expression of APTR in GEO Datasets

We employed GSE66783 which contains 5 PTC samples and paired adjacent normal tissues and GSE33630 which includes 49 PTC samples and 45 normal samples to evaluate the relative expression of APTR in PTC. As shown in Fig. 1a, b APTR expression was downregulated in PTC tissues when compared with adjacent normal tissues ($P = 0.0079$, $P < 0.001$, respectively). Furthermore, based on the samples from GSE33630 and GSE65144, we evaluated the expression of APTR in ATC and normal tissues. As shown in Fig. 1c, d, APTR expression was significant decreased in ATC tissues compared with normal tissues ($P < 0.001$, $P < 0.0001$, respectively).

APTR Expression Is Downregulated in PTC Patients

As shown in Fig. 1, the expression levels of APTR decreased in ATC and PTC patients when compared with normal thyroid

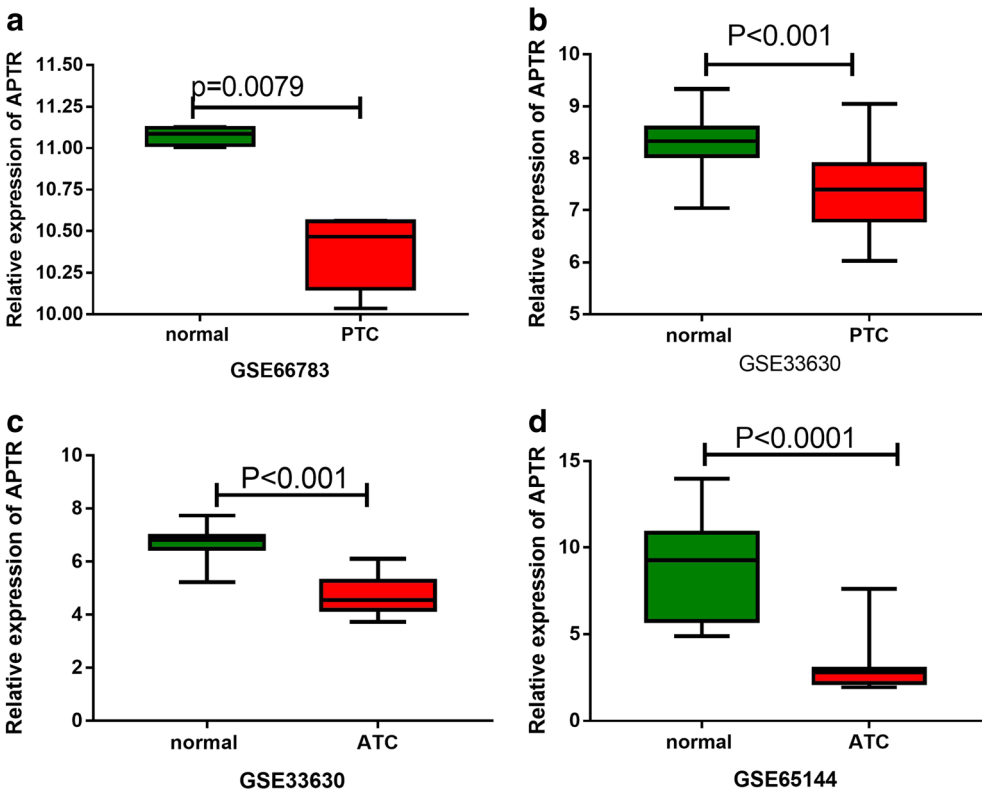

Fig. 1 The box plot of APTR relative expression in GEO dates, (**a**) GSE66783, (**b**) GSE33630, (**c**) GSE33630, (**d**) GSE65144. Notes: Data were analyzed by Student's t test

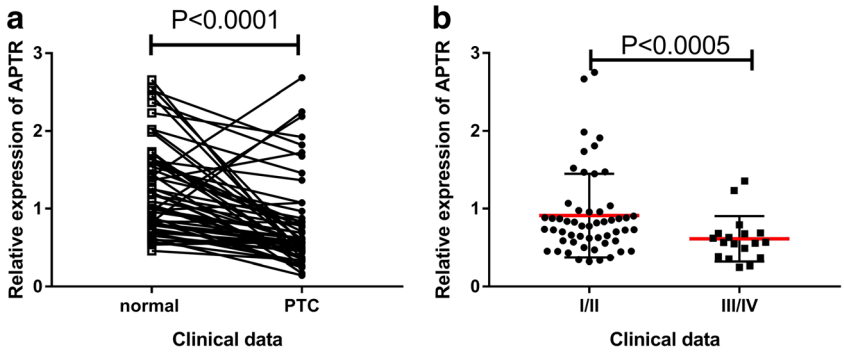

Fig. 2 The scatter plot of comparison of the APTR expression between normal and PTC (**a**) and the different TNM stage (**b**). Notes: *P*-values were calculated using the Student's test. Data are expressed as the means ± SD

tissues from the GEO datasets. Then, qRT-PCR was employed to detect 76-pair PTC and adjacent normal samples. As shown in Fig. 2a, the relative expression of APTR in the adjacent normal thyroid tissues was higher than in PTC tissues, which was consistent with the analysis of GEO datasets. Moreover, we also found the expression of APTR was statistically down-regulated in III/IV stage when compared with I/II stage ($P < 0.005$, Fig. 2b).

Association of APTR Expression and Clinicopathological Characteristics in PTC

To evaluate whether APTR expression associated with clinicopathological characteristics of PTC. We divided patients into two groups based on median value (0.68) and then employed chi-square test to analyze the relationship. As shown in Table 2, the gender ($P = 0.521$), tumor size ($P = 0.484$) and T stage ($P = 0.054$) have no significant association with APTR expression, but the expression of APTR was significantly associated with the age ($P = 0.038$), distant metastasis ($P = 0.025$) and TNM stage ($P = 0.031$).

Evaluation of APTR in Tissue as Novel Tumor Marker for PTC

To examine whether APTR is a potential tumor marker for PTC and ATC or not, we first evaluated the feasibility of using the APTR to detect PTC and ATC patients in GEO datasets through using ROC curves and AUC. The AUC of APTR for PTC tissues vs. normal counterparts in GSE33630 was 0.853 (95% CI 0.775–0.9309; Fig. 3a). Then, we tested the diagnostic value of APTR in 76-pair PTC patients, and the results showed that APTR had an AUC value of 0.88 (95% CI 0.8015–0.9585; Fig. 3b) for PTC. We further assessed the

Table 2 The relationship between APTR expression and clinicalpathological factors in thyroid Cancer

Characteristics	Number	Expression of lncRNA APTR		X^2	P
		Low expression (%)	High expression (%)		
Age (years)					
< 45	41	25(60.98)	16(39.02)	4.290	0.038*
≥ 45	35	14(40)	21(60)		
Gender					
Male	14	6(42.86)	8(57.14)	1.302	0.521
Female	62	32(51.61)	30(48.39)		
Tumor size(cm)					
< 2	44	22(50)	22(50)	1.453	0.484
≥ 2	32	16(50)	16(50)		
T stage					
T1-T2	59	26(44.07)	33(55.93)	3.713	0.054
T3-T4	17	12(70.59)	5(29.41)		
M					
M0	68	31(49.33)	37(50.67)	5.029	0.025*
M1	8	7(87.5)	1(12.5)		
TNM stage					
I/II	58	25 (45.59)	33 (54.41)	4.659	0.031*
III/IV	18	13(72.22)	5(27.78)		
Lymph node metastasis					
N0	35	15(42.86)	20(57.14)	1.324	0.250
N1	41	23(56.09)	18(43.91)		

Data were analyzed by chi-square e ($\chi 2$) test and *by student's t test, given as mean ± SD

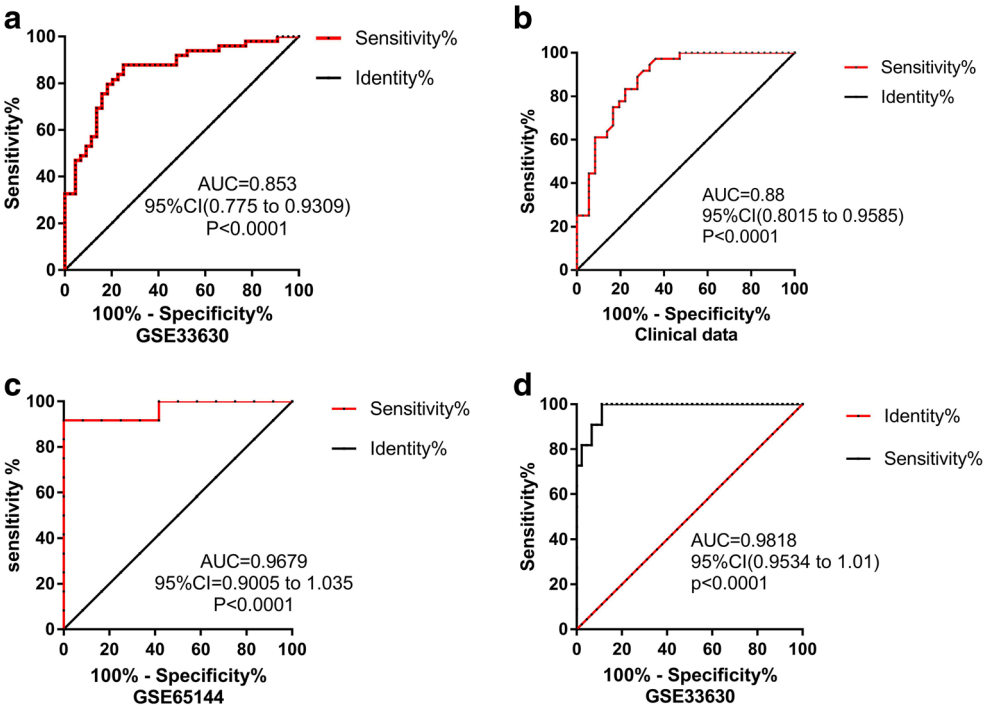

Fig. 3 ROC of APTR expression for differentiating TC tissue from normal tissue in different GEO data and in validated clinical data. Notes: The diagnostic value of APTR for (A-B) PTC patients and (C-D) ATC patients

diagnostic potential of APTR in ATC tissues, the diagnostic value of APTR in GSE65144 and GSE33630 was 0.9653(95%CI 0.8926–1.038; Fig. 3c), 0.9818(95%CI 0.9534–1.01; Fig. 3d) respectively. The sensitivity, specificity and Youden index of APTR for distinguishing PTC and ATC from healthy thyroid tissues were shown in Table 3. Together, these results indicate that APTR may be exploited as a biomarker of PTC and ATC diagnosis.

Discussion

As we have aforementioned in introduction section, LncRNAs participated in various various biological functions, such as cell proliferation, differentiation, and apoptosis [7]. Many studies have been demonstrated that APTR plays a crucial role in tumor development and prevention, for instance, APTR dysregulated in glioblastomas, hepatic stellate cells and can employed as a prognostic marker for cirrhotic patients [17, 18, 24], yet the role of APTR in thyroid cancer is unclear. In current study, we demonstrated that APTR was downregulated in PTC and ATC samples. In addition, the expression of APTR was negatively associated with TNM stage and distant metastasis. Furthermore, based on the results from ROC and AUC analysis, APTR was considered as a candidate diagnostic biomarker of PTC and ATC. (AUC = 0.96, AUC = 0.853, respectively in GSE33630).

Many studies have demonstrated that APTR aberrantly (particularly elevated) expressed in various cancers compared with normal tissues. However, contrary to previous studies, in our research, we found that APTR expression was decreased in both GEO datasets and 76-pair samples, while APTR upregulated in hepatic stellate cells and "promotes the activation of hepatic stellate cells as well as the progression of liver fibrosis" [18]. There are some reasons may describe this difference. APTR, Alu-mediated p21 transcriptional regulator, which repress p21 transcription by epigenetic silencing mechanism [17]. Although many researches have demonstrated that p21 suppress tumor progression, other studies found p21 serve as tumor-promoting function [25]. Because "P21 can promote oncogenesis independently of its anti-apoptotic activity by promoting the assembly of complexes of cyclin D with

Table 3 Performance of APTR in the differential diagnosis of TC from healthy tissues

Subtypes	Series	Sensitivity (%)	Specificity (%)	Youden index (%)
PTC	GSE33630	87.76	75	62.76
	76 tissue samples	88.89	72.22	61.11
ATC	GSE33630	88.89	100	88.89
	GSE65144	91.67	100	91.67

CDK4 or CDK6 without inhibiting their kinase activity" [26].In Adrienne et al. study, they found p21 expression is higher in malignant thyroid cancer than in benign lesions and p21 could serve as a poor clinical outcomes prognosticator for thyroid cancer individuals [27]. Therefore, APTR may serve as a tumor suppressor in thyroid cancer, however, the underlying mechanism of APTR suppresses thyroid cancer needs future investigation.

However, there are some limitations in our study, including:

1. We just enrolled PTC and normal controls samples into investigation, we should also take benign nodules take into consideration.
2. The diagnostic value of APTR should base on large clinical samples, therefore, in our future experiments, we need to expand the sample size and continue to verify its value.
3. In vitro and in vivo experiments needed to be conducted to further validation the biological function of APTR in thyroid cancer. Understanding the underlying mechanism of APTR in TC will be helpful to establish the novel diagnostic biomarker.
4. In conclusion, our findings demonstrated that APTR expression was decreased in PTC and ATC, and APTR was negatively associated with TNM stage and distant metastasis. Finally, APTR can be used as potent tumor biomarker for PTC and ATC detection.

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Compliance with Ethical Standards

Ethical Approval This study was approved by the ethics committee of Hunan Cancer Hospital of Xiangya medical school, Central South University (License No. SBQLL-2016-015).All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Conflict of Interest The authors declare that they have no conflict of interest.

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