MOLECULAR MEDICINE REPORTS 25: 120, 2022

Expression of *SOX2OT*, *DANCR* and *TINCR* long non-coding RNAs in papillary thyroid cancer and its effects on clinicopathological features

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Received December 8, 2021; Accepted January 19, 2022

DOI: 10.3892/mmr.2022.12636

Abstract. Long non-coding RNAs (lncRNAs) are molecules that are >200 base pairs long and do not encode a protein. However, they perform important roles in regulating gene expression. Recent studies have revealed that the changes in the expressions of lncRNAs serve a role in the development and metastases of a number of types of cancer. A number of studies have been published on the association of SOX2 overlapping transcript (*SOX2OT)*, differentiation antagonizing non-protein coding RNA (*DANCR)* and tissue differentiation-induced non-coding RNA (*TINCR)* expression with various types of cancer. However, researchers have not yet studied their roles in papillary thyroid cancer or at least, those roles are not clarified. The aim of the present study was to investigate the expression and clinical significance of *SOX2OT*, *DANCR* and *TINCR* in papillary thyroid cancer (PTC). A total of 102 patients with PTC were included in the present study. Reverse transcription-quantitative PCR method was used to determine the relative gene expression levels of lncRNAs and then the relationship between expressions of lncRNAs and clinical characteristics of the subjects was analyzed in detail. Expression levels of *SOX2OT* (P=0.016) and *DANCR* (P=0.017) increased in the tumor samples in contrast to the normal tissues. No significant difference was observed in the expression level of *TINCR* (P=0.298). In addition, *SOX2OT* expression was associated with micro carcinoma (P<0.001), tumor size (P=0.010) and primary tumor (P=0.006), while *DANCR* expression was associated with age (P=0.030) and micro carcinoma (P=0.004). The findings of the present study indicated that *DANCR* may contribute to the development of PTC while *SOX2OT* may contribute to both the development and progression of PTC.

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Key words: papillary thyroid cancer, long non-coding RNA, SOX2 overlapping transcript, differentiation antagonizing non-protein coding RNA, tissue differentiation-induced non-coding RNA, reverse transcription-quantitative PCR

Introduction

Thyroid cancer is the most widespread form of endocrine cancers worldwide. Its incidence has been reported to have increased in recent years (1). According to histological features, thyroid cancer can be divided into four subgroups: Papillary, follicular, anaplastic and medullary. Among these subgroups, the most common type (~80%) is papillary thyroid cancer (PTC) originating from follicular cells (2,3). Even though current forms of treatment (e.g., surgical intervention and radioactive iodine) can keep this disease under control for a number of patients, it nevertheless has a high morbidity rate; likewise, in some cases, the tumors can be aggressive (4,5). In addition, while it is known that environmental and genetic factors serve a role in development of this disease (6), its pathogenesis is not fully clarified. Therefore, there is a need to elucidate the molecular mechanisms involved in the development of the disease and to discover effective treatment targets, such as biomarkers that can be used in early diagnosis and prognosis prediction.

Long non-coding RNAs (lncRNAs) are molecules that are >200 base pairs long and do not encode a protein. However, they perform important roles in regulating gene expression. LncRNAs can regulate the expression of genes involved in processes such as cell cycle, proliferation, differentiation, stem cell differentiation, apoptosis, invasion, migration and autophagy through different mechanisms such as regulation of chromatin structure, transcription, splicing process, post-transcriptional events and interaction with microRNAs (2,7). The changes in the expression of lncRNAs and impairment of their regulatory functions have been associated with a number of diseases, including numerous types of cancer. It has been reported that several lncRNAs (e.g. *NEAT1, MALAT1, H19, HOTAIR, BANCR, PTCSC3*) are associated with the development and progression of thyroid cancer (3,8).

The SOX2 overlapping transcript (*SOX2OT*) gene is located in the 3q26.3 region and contains the *SOX2* gene in its intronic region. This gene serves a key role in maintaining both pluripotency and self-renewal properties of embryonic stem cells (9,10). Many studies have revealed that the increase in *SOX2OT* expression in cancer cells induces *SOX2* expression and this in turn contributes to tumorigenesis (11-14). The changes in *SOX2OT* expression have been associated with a number of cancers such as lung, breast, esophageal, gastric, hepatocellular and ovarian (10). However, no study has been published so far investigating the relationship between the expression level of *SOX2OT* and thyroid cancer, to the best of the authors' knowledge. Therefore, this association required investigation.

Differentiation antagonizing non-protein coding RNA (*DANCR*), localized on chromosome 4, is 855 base pairs long and helps suppress progenitor cell differentiation (15). The relationship between *DANCR* and tumorigenesis and clinicopathological features of the tumors have been investigated in relation to various types of cancer such as hepatocellular, gastric, colorectal, breast and lung cancer (16-20). It has been reported that *DANCR* is an oncogenic lncRNA that is overexpressed in tumor tissues and is associated with poor prognostic factors (16). In molecular studies, it has been demonstrated that *DANCR* induces proliferation, migration, invasion, metastasis, angiogenesis and drug resistance and inhibits apoptosis (16,21). There are a few studies reporting that its expression decreases in some tumors and even acts as a tumor suppressor (22,23). In the literature, only one study examined the relationship between *DANCR* expression and thyroid cancer and reported that the expression of *DANCR* decreases in thyroid cancer (23). Therefore, the relationship between *DANCR* and thyroid cancer and its clinicopathological features requires investigation.

Tissue differentiation-induced non-coding RNA (*TINCR*), which is ~3.7 kb long, is localized on chromosome 19. *TINCR* regulates the expression of genes that serve a role in epidermal differentiation. The changes in *TINCR* expression have been found in esophageal, breast, colon, lung, prostate and bladder cancers and have associated with tumorigenesis (24,25). Only one study has been published that evaluated *TINCR* expression in thyroid cancer, to the best of the authors' knowledge. In this study, the Cancer Genome Atlas (TCGA) RNA sequencing data for PTC was examined and it was reported that *TINCR* was one of a number of lncRNAs whose expression increased in thyroid tumor tissue (26). Therefore, the relationship between development of thyroid cancer, clinicopathological features and *TINCR* expression needs to be supported by other data.

The aim of the present study was to investigate the relationship between expressions of *DANCR, TINCR, SOX2OT* lncRNAs and development and clinopathological features of PTC.

Materials and methods

Patients. A total of 112 PTC patients who had undergone a thyroidectomy between January 2015 and December 2020 at Giresun University's Faculty of Medicine were included in this study. Of the 102 patients included in this study, 21 were male and 81 were female. Their age range was 23-85 (median 51). The pathology department histopathologically examined the tumor samples and confirmed the diagnosis of PTC. Those samples (tumor and adjacent non-cancerous thyroid tissue) were obtained from formalin-fixed paraffin-embedded (FFPE) tissue blocks that were archived in the pathology department. The patients included in the study upon examination of the patient records were selected from the subjects who had not undergone chemotherapy, radiotherapy, or other cancer treatments before surgery. As a result of spectrophotometric measurements performed after RNA isolation, 10 patients were excluded from the study because RNA quality was not at optimum level (A260/A280 ratio of 1.8-2.1) and the expression analysis was performed on samples taken from the 102 patients. The present study was approved by Giresun University's Faculty of Medicine Clinical Trials Ethics Committee (approval no. 2018-06-10).

RNA isolation from FFPE tissue samples and cDNA synthesis. After removing the excess paraffin, 4-5 5 μm-thick sections were taken from the FFPE tissue blocks of the patients and then transferred into 1.5 ml sterile micro centrifuge tubes. Total RNA isolation was conducted using an RNeasy FFPE kit (Qiagen GmbH), in accordance with the manufacturer's protocol. The quality and the concentration of the RNA samples were assessed by measuring the ratio of the absorbances at 260/280 nm on a NanoDrop One/OneC Microvolume UV-Vis Spectrophotometer (Thermo Fisher Scientific, Inc.). Then the integrity of the RNA samples was analyzed using 1% agarose gel electrophoresis. Then, the samples that had poor RNA quantity and quality were excluded from the study. All samples were kept at -80˚C for further research. The cDNA synthesis was carried out using the RevertAid RT Reverse Transcription kit (Thermo Fisher Scientific, Inc.), in accordance with the manufacturer's protocol.

Reverse transcription-quantitative (RT-q) PCR analysis. Expression analysis of lncRNAs was performed using a LightCycler 480 SYBR-Green I Master (Roche Diagnostics GmbH) and specific primer pairs according to the manufacturer's protocol on the LightCycler 480 Real-Time PCR system (Roche Diagnostics GmbH). β-actin was used as an internal control to normalize the expression of lncRNAs. The primer pairs for RT-qPCR were: *SOX2OT*, Forward 5'-GTAAGG CGATGTGGGTGAAG-3'; Reverse 5'-AGTTGAAGGAGC TTGCAGTT-3'; *DANCR*, Forward 5'-CTGCATTCCTGA ACCGTTATCT-3'; Reverse 5'-GGGTGTAATCCACGTTTC TCAT-3'; *TINCR*, Forward 5'-AGATGACAGTGGCTGGAG TTGTCA-3'; Reverse 5'-TGTGGCCCAAACTCAGGGATA CAT-3'; β-actin, Forward 5'-TCTACAATGAGCTGCGTG TG-3'; Reverse 5'-GGTCTCAAACATGATCTGGGT-3' The thermocycling conditions were: 95˚C for 5 min, 6 cycles of 95˚C for 10 sec and 57˚C for 25 sec, 45 cycles of 95˚C for 10 sec, 57˚C for 10 sec and 72˚C for 10 sec. All samples were tested in triplicate. Relative gene expression levels in lncRNAs were calculated using the $2^{-\Delta\Delta Cq}$ method (27).

Statistical analysis. Statistical analyses were performed using SPSS 15.0 (SPSS, Inc.). Continuous variables were expressed as mean ± standard deviation. The expression levels of *SOX2OT*, *DANCR* and *TINCR* in tumor and adjacent normal thyroid tissues

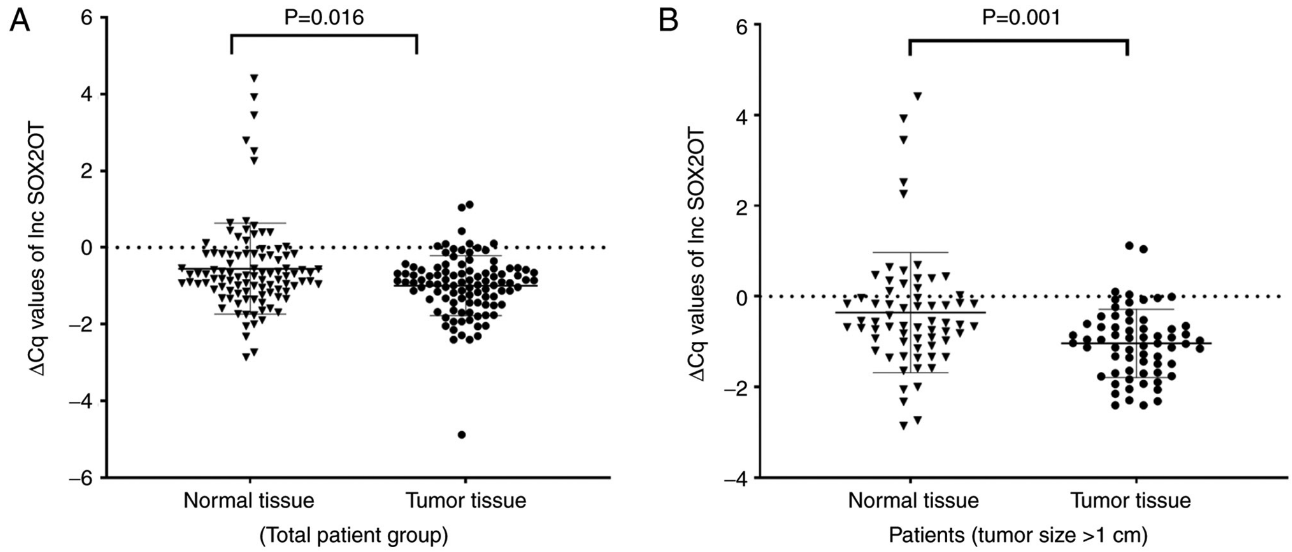

Figure 1. Expression of SOX2OT lncRNA in tumor and normal tissue. (A) The comparisons of lncRNA *SOX2OT* relative expression levels between normal and tumor tissues of the total patient group (n=102). (B) The comparisons of lncRNA *SOX2OT* relative expression levels between normal and tumor tissues of patients whose tumor size was >1 cm (n=66). All of the data are presented as the mean ± standard deviation. Wilcoxon signed-rank test was used for statistical analysis. P<0.05 was considered to indicate a statistically significant difference. lnc, long non-coding.

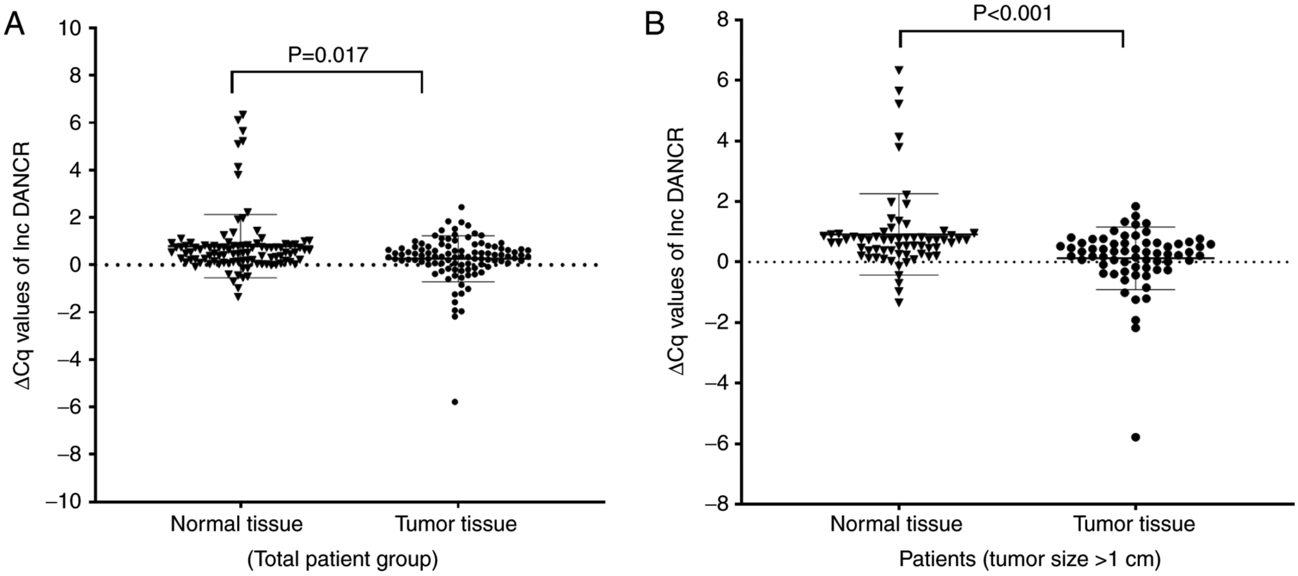

Figure 2. Expression of DANCR lncRNA in tumor and normal tissue. (A) The comparisons of lncRNA *DANCR* relative expression levels between normal and tumor tissues of the total patient group (n=102). (B) The comparisons of lncRNA *DANCR* relative expression levels between normal and tumor tissues of patients whose tumor size is larger than 1 cm (n=66). All of the data are presented as the mean ± standard deviation. Wilcoxon signed-rank test was used to analyze the statistics. P<0.05 was considered to indicate a statistically significant difference. lnc, long non-coding.

were compared using the Wilcoxon signed-rank test. The patient group was divided into two subgroups (low expression and high expression) according to median lncRNA expression values to determine the relationship between the lncRNA expressions and clinicopathological features in more detail. Categorical data were compared using the chi-square test. P<0.05 was considered to indicate a statistically significant difference.

Results

Expression levels of SOX2OT, DANCR and TINCR in PTC tissues. RT-qPCR was performed to determine the expression of *SOX2OT, DANCR* and *TINCR* lncRNAs in tumor and adjacent noncancerous thyroid tissue samples. The data revealed that *SOX2OT* (fold change: 2.03, P=0.016, Z=-2.405) and *DANCR* (fold change: 3.23, P=0.017, Z=-2.392) expression significantly increased in the tumor tissues compared with adjacent noncancerous thyroid tissues (Figs. 1A and 2A). On the other hand, no significant change was observed in the expression of *TINCR* (fold change: -1.39, P=0.298, Z=-1.040; Fig. 3A).

The relationship between expression levels of SOX2OT, DANCR and TINCR and the clinicopathological features

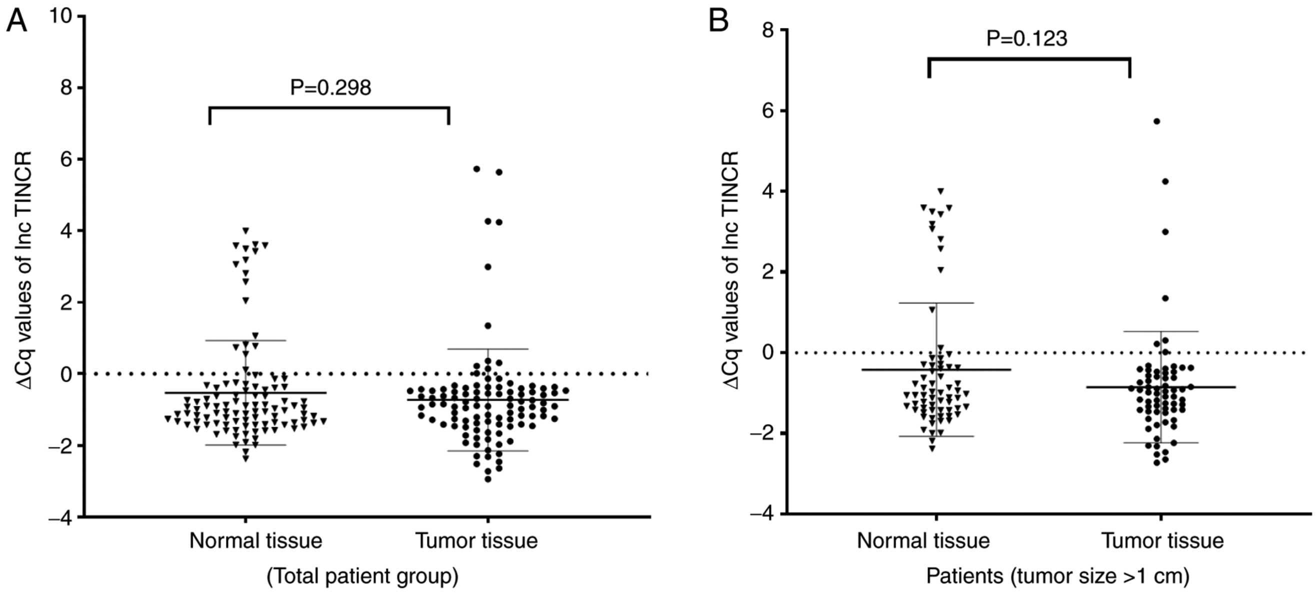

Figure 3. Expression of TINCR lncRNA in tumor and normal tissue. (A) The comparisons of lncRNA *TINCR* relative expression levels between normal and tumor tissues of the total patient group (n=102). (B) The comparisons of lncRNA *TINCR* relative expression levels between normal and tumor tissues of patients whose tumor size larger is than 1 cm (n=66). All of the data are presented as the mean ± standard deviation. Wilcoxon signed-rank test was used for statistical analysis. P<0.05 was considered to indicate a statistically significant difference. lnc, long non-coding.

of PTC. The patient group was divided into high and low expression subgroups according to the median expression values of lncRNAs in order to assess the relationship between clinicopathological features and expressions of lncRNAs. Accordingly, there was a statistically significant correlation between the *SOX2OT* expression level and microcarcinoma (P<0.001), tumor size (P=0.010), primary tumor (P=0.006; Table I) and between the *DANCR* expression level and age (P=0.030), microcarcinoma (P=0.004) (Table II). A significant correlation was found between the expression level of *TINCR* and primary tumor (P=0.029; Table III). Since the findings of the present study revealed a significant correlation between the expression levels of lncRNAs and tumor size, a separate evaluation was carried out only in patients who had either papillary microcarcinoma or papillary carcinoma with a tumor size >1 cm. There was no significant difference in the expression levels of *SOX2OT* (P=0.198, Z=-1.288), *DANCR* (P=0.162, Z=-1.398), or *TINCR* (P=0.637, Z=-0.471) between the tumor and adjacent normal tissues in the patients with papillary microcarcinoma. On the other hand, when evaluating only papillary carcinoma samples (tumor size >1 cm), it was observed that differences in *SOX2OT* (P=0.001, Z=-3.440) and *DANCR* (P<0.001, Z=-3.830) expression levels between tumor and normal tissues became more significant (Figs. 1B and 2B). Again, no significant change was observed in the expression level of *TINCR* (P=0.123, Z=-1.543; Fig. 3B).

Discussion

Although prognosis of PTC is good, diagnostic biomarkers and prognostic predictors are still required (28). Recent studies have focused on lncRNAs as one of the most important molecular biomarkers for various types of cancer and revealed that lncRNAs have important roles in the development and progression of certain cancers (29,30). However, there are few studies specifically investigating the relationship between thyroid cancer and lncRNAs (23,26,28). In the present study, the relationship between the development and clinicopathological features of PTC and expression levels of *SOX2OT, DANCR* and *TINCR* was assessed. To the best of the authors' knowledge, the present study is the first attempt to investigate the association between expression level of *SOX2OT* and PTC.

Some studies have indicated that *SOX2OT* lncRNA contributes to tumorigenesis via different mechanisms (11,31). For example, it regulates the expression of the *SOX2* gene, which is located in its intronic region and has a key role in regulating the pluripotency properties of embryonic stem cells (32). In addition, *SOX2OT* acts as a miRNA sponge. In a number of studies, it has been reported that it regulates the expression of genes involved in tumorigenesis, especially by binding to tumor suppressor miRNAs (12,14,33). Li *et al* (11) reported that *SOX2OT* interacts with epigenetic regulators or directly binds to the transcription factors and destabilizes them. Studies have revealed that *SOX2OT* is an oncogene and its expression increases in esophageal squamous cell, bladder, nasopharyngeal, prostate, non-small-cell lung, gastric and hepatocellular cancer alongside glioma (10,11). Similarly, the present study revealed that *SOX2OT* expression increased significantly in the PTC tumor samples compared with the adjacent normal tissues. This suggested that the increase in the expression of *SOX2OT* may be associated with the tumorigenesis process of PTC.

Expression of miR-204 is downregulated in PTC, breast and cervical cancer and it acts as a tumor suppressor (34). On the other hand, *SOX2OT* contains a binding site for miR-204 and acts as a sponge for this miRNA (11). Similarly, some studies have reported that miR-132 and miR-122 have tumor suppressor roles in PTC by suppressing *FOXA1* and *DUSP4* expression, respectively (35,36). *SOX2OT* binds to these two miRNAs and suppresses their expression (11). When the results of the present study were compared with those of the aforementioned studies,

Table I. Association between expression level of *SOX2OT* and clinicopathological characteristics of patients with PTC. Low/high decided by the median expression of *SOX2OT*. Chi square test was used for statistical analysis.

		SOX2OT expression			
Characteristics	Number of patients	Low	High	P-value	Odds ratio (95% CI)
Age, years				0.759	1.143 (0.490-2.680)
<45	30	16	14		
≥45	72	36	36		
Sex					
Female	81	42	39	0.730	1.185 (0.450-3.100)
Male	21	10	11		
Microcarcinoma				<0.001[a]	0.203 (0.080-0.500)
No	66	25	41		
Yes	36	27	9		
Histological type				0.129	
Classical PTC	17	1	10		
Follicular PTC	62	31	31		
Classical-Follicular PTC	5	1	4		
Unknown	18	13	5		
Tumor size (cm)				0.010[a]	2.940 (1.290-6.720)
<2	62	38	24		
≥2	40	14	26		
Lymphovascular invasion				0.391	1.551 (0.570-4.250)
No	83	44	39		
Yes	19	8	11		
Primary tumor				0.006[a]	
T1	78	46	32		
T2	20	6	14		
T3	4	0	4		
TNM stage				0.114	0.475 (0.390-0.580)
I	99	52	47		
II	3	0	3		
Lymph node metastasis				1.000	0.510 (0.050-5.810)
No	50	49			
Yes	2	1			
Extrathyroidal extension				0.488	0.489 (0.120-2.070)
No	93	46	47		
Yes	9	6	3		
Multicentricity				0.238	1.629 (0.720-3.68)
No	65	36	29		
Yes	37	16	21		
Multifocality				0.712	1.160 (0.530-2.550)
No	31	28			
Yes	21	22			

[a]P<0.05 was considered to indicate a statistically significant difference. PTC, papillary thyroid cancer.

it was hypothesized that *SOX2OT* might be involved in the papillary thyroid carcinoma process as it acts a miRNA sponge for miRNA-204, miRNA-122 and miRNA-132.

Xue *et al* (37) reported that the enhancer of zestehomolog 2 *(EZH2)* expression was higher in tumor samples of PTC patients compared with the normal thyroid tissue samples.

Table II. Association between expression level of *DANCR* and clinicopathological characteristics of patients with PTC. Low/high decided by the median expression of *DANCR*. Chi square test was used for statistical analysis.

		DANCR expression			
Characteristic	Number of patients	Low	High	P-value	Odds ratio (95% CI)
Age				0.030[a]	2.645 (1.090-6.450)
<45	30	20	10		
≥45	72	31	41		
Sex				0.221	1.839 (0.690-4.910)
Female	81	43	38		
Male	21	8	13		
Microcarcinoma				0.004[a]	0.286 (0.120-0.680)
No	66	26	40		
Yes	36	25	11		
Histological type				0.562	
Classical PTC	17	10	7		
Follicular PTC	62	31	31		
Classical-Follicular PTC	5	1	4		
Unknown	18	9	9		
Tumor size (cm)				0.685	1.179 (0.530-2.610)
<2	62	32	30		
≥2	40	19	21		
Lymphovascular invasion				0.799	1.138 (0.420-3.090)
No	83	42	41		
Yes	19	9	10		
Primary tumor				0.693	
T1	78	41	37		
T2	20	8	12		
T3	4	2	2		
TNM stage				1.000	2.041 (0.180-23.240)
I	99	50	49		
II	3	1	2		
Lymph node metastasis				1.000	2.041 (0.180-23.240)
No	99	50	49		
Yes	3	1	2		
Extrathyroidal extension				0.487	0.469 (0.110-1.990)
No	93	45	48		
Yes	9	6	3		
Multicentricity				0.837	0.919 (0.410-2.060)
No	65	32	33		
Yes	37	19	18		
Multifocality				0.160	0.567 (0.260-1.270)
No	59	26	33		
Yes	43	25	18		

[a]P<0.05 was considered to indicate a statistically significant difference. PTC, papillary thyroid cancer.

SOX2OT is known to recruit EZH2 which induces H3K27me3 and downregulates *PTEN* expression (10). Therefore, *SOX2OT* may downregulate the expression of *PTEN* by upregulating *EZH2* and thus might contribute to tumorigenesis of PTC. *SOX2OT* expression in various types of cancer has also been found to be associated with the clinicopathological

Table III. Association between expression level of *TINCR* and clinicopathological characteristics of patients with PTC. Low/high decided by the median expression of TINCR. Chi square test was used for statistical analysis.

		TINCR expression			
Characteristic	Number of patients	Low	High	P-value	Odds ratio (95% CI)
Age, years				0.385	1.462 (0.620-3.450)
<45	30	17	13		
≥45	72	34	38		
Sex				0.807	0.887 (0.340-2.320)
Female	81	40	41		
Male	21	11	10		
Microcarcinoma				0.214	0.595 (0.260-1.350)
No	66	30	36		
Yes	36	21	15		
Histological type				0.148	
Classical PTC	17	5	12		
Follicular PTC	62	31	31		
Classical-Follicular PTC	5	4	1		
Unknown	18	11	7		
Tumor size (cm)				0.417	0.719 (0.320-1.600)
<2	62	29	33		
≥2	40	22	18		
Lymphovascular invasion				0.075	2.566 (0.890-7.400)
No	83	45	38		
Yes	19	6	13		
Primary tumor				0.029[a]	
T1	78	35	43		
T2	20	15	5		
T3	4	1	3		
TNM stage				1.000	2.041 (0.180-23.240)
I	99	49	50		
II	3	2	1		
Lymph node metastasis				1.000	2.041 (0.180-23.240)
No	50	49			
Yes	1	2			
Extrathyroidal extension				0.160	3.900 (0.770-19.760)
No	93	49	44		
Yes	9	2	7		
Multicentricity				0.837	0.919 (0.410-2.060)
No	65	32	33		
Yes	37	19	18		
Multifocality				0.547	0.785 (0.360-1.730)
No	59	28	31		
Yes	43	23	20		

[a]$P<0.05$ was considered to indicate a statistically significant difference. PTC, papillary thyroid cancer.

characteristics of the patients. A study by Teng *et al* (38) correlated exosomal *SOX2OT* expression with tumor size, lymph node metastasis and TNM stage in patients with lung squamous cell carcinoma. Shi and Teng (39) determined that *SOX2OT* expression was associated with histological grade, tumor number and vein invasion status in hepatocellular carcinoma. In the present study, it was found that elevated *SOX2OT* expression was associated with tumor type, tumor size and primary tumor. When the expression of *SOX2OT* was assessed only in micropapillary carcinoma samples (tumor size ≤1 cm), no difference was found between the tumor and normal tissues. On the other hand, when the papillary carcinoma samples (tumor size >1 cm) were examined, it was found that the expression difference between the tumor and normal tissues was greater than in the total sample. Therefore, it might be concluded that the expression of *SOX2OT* increased significantly in PTC; however, the increase in tumors of 1 cm or below was insignificant.

In recent years, an increasing number of studies have reported abnormal *DANCR* expression in a number of types of cancer (18-20). It has been suggested that this abnormal expression is also associated with clinicopathological features (19,21). Almost all of the studies conducted on other types of cancer (e.g. osteosarcoma, nasopharyngeal, glioma, non-small cell lung, cervical, ovarian, bladder, prostate, breast, colorectal, gastric) have reported that *DANCR* expression increased in tumor tissues compared with adjacent normal tissues and this elevated expression level was associated with poor prognosis (21,40-43). For example, in patients with breast and colorectal cancer, DANCR expression is higher in tumor compared with normal tissue and high DANCR expression is associated with poor prognosis (19,20). In the current study, *DANCR* expression increased significantly in the tumor samples of the 102 patients with PTC compared with their adjacent normal tissue. In addition, *DANCR* expression was associated with age and presence of microcarcinoma. Findings of the present study are compatible with those of the literature.

In some studies, it was found that expressions of *miRNA-138, miRNA-199a, miRNA-335-5p* and *miRNA214* were decreased in PTC tumor samples compared with adjacent normal tissue samples and these miRNAs were identified as tumor suppressor miRNAs that served a role in suppressing proliferation, inducing apoptosis and mediating suppression of migration and invasion (34,44). On the other hand, it is known that *DANCR* acts as a sponge for these miRNAs and reduces their expression (21). Therefore, it is hypothesized that *DANCR* may be involved in PTC tumorigenesis process by acting as a competing endogenous RNA (ceRNA) for these miRNAs.

Abnormal activation of the Wnt pathway and increased expression of β-catenin may cause PTC to develop (45). Some studies have suggested that *DANCR* may even have an oncogenic role by activating the β-catenin signaling pathway in various cancers (16,18,43,46). AXL, a receptor tyrosine kinase, shows an oncogenic activity by helping regulate a number of different processes related to cancer development and progression (47). *AXL* is highly expressed in various types of cancer including thyroid cancer (48). In some studies it has been reported that *DANCR* activates the PI3K/AKT/NF-κB signaling pathway by upregulating *AXL* (16). Therefore, *DANCR* may be involved in the development and progression of PTC by interacting with genes in the Wnt and PI3K/AKT pathways.

A few studies have suggested that low *DANCR* expression may be associated with the development of cancer (16,21). Zhang *et al* (23) reported that *DANCR* expression is downregulated in tumor samples compared with the adjacent normal tissues in 76 patients with PTC and they found that *DANCR* expression was correlated with T grade and TNM stage. The findings of the present study are not compatible with that data. It is known that the roles of lncRNAs can differ from one form of cancer to another (16,49). In addition, there are studies in the literature reporting different results in the same cancer type (16,24). Furthermore, expressions of lncRNAs may differ in different populations (50). However, the relatively small number of patients included in such studies may also be the reason for the inconsistency between the results. In the present study, similar to *SOX2OT*, when the expression of *DANCR* was analyzed only in micropapillary carcinoma samples (tumor size ≤1 cm), no difference was observed between the tumor and normal tissues. However, when only papillary carcinoma samples (tumor size >1 cm) were examined, it was observed that the difference between the tumor and normal tissues was greater compared with the total sample evaluation. Based on findings of the present study, it may be concluded that the expression of *DANCR* had increased significantly in PTC, but it did not significantly increase in tumors of ≤1.

Numerous studies have reported abnormal *TINCR* expression in hepatocellular, colon, breast, bladder, lung, prostate, gastric, esophageal and oral squamous cell cancer (25,51-54). The expression of *TINCR* has been defined at the last stage of epidermal differentiation and regulates the expression of *ALOXE3, FLG, LOR* and *ALOX12B*, all of which have important roles in differentiation at the post-transcriptional level (55). Signaling pathways such as Wnt/β-catenin, ERK1/2-SP3 and MAPK have been determined to be the targets of *TINCR* in different types of cancer (24). In addition, *TINCR* expression increases in esophageal, breast, bladder and gastric cancer, but decreases in retinoblastoma, glioma and prostate cancer (24,52,54,56-59). On the other hand, it has been reported that *TINCR* expression is upregulated in certain studies while it is downregulated in others conducted in the same cancer type (24,54,57-59). In their RNA sequencing study, You *et al* (26) found that *TINCR* expression increased in PTC tumor tissues compared with adjacent normal tissues. The findings of the present study however, showed that *TINCR* expression did not show a significant difference between the tumor and adjacent normal tissue samples. You *et al* (26) used TCGA data in their study and determined expression levels by RNA sequencing analysis. In addition, the patient group in the aforementioned study consisted of stage I, II, III and IV patients. In the present study, the patient group mostly consisted of stage I patients. Therefore, the fact that the patients included in the studies were at different stages, the use of different expression analysis methods and the limited number of patients may have led to the difference in the results.

In conclusion, results of the current study demonstrated that the expression levels of *SOX2OT* and *DANCR* increased in PTC tissues compared with their adjacent normal tissue. No significant change was observed in the expression level of *TINCR*. However, when the micropapillary thyroid carcinoma was examined, no significant increase was observed in the expression levels of *SOX2OT* or *DANCR*. In addition, the expression level of *SOX2OT* was associated with tumor size and primary tumor. In the light of the results of the present study, it is concluded that *DANCR* may contribute to the development of PTC and *SOX2OT* may contribute to both the development and progression of PTC. One of the limitations of the present study is that the results of the expression analysis were not confirmed by functional studies. Another limitation is that the number of patients was relatively low. Future studies with a larger number of patients in PTC and other subgroups of thyroid cancer alongside *in vitro* functional studies will facilitate supporting the data of the present study.

Acknowledgements

Not applicable.

Funding

This study was supported by the Scientific Research Projects Committee of Giresun University (project numbers: SAĞ-BAP-A-150219-39).

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

FMI, EAk and DS contributed to conception and design of the study. FMI, EAk, DS and EAl performed experiments and data collection. FMI, AO and EAl performed data analysis and interpretation. FMI and AO drafted the paper. All authors contributed to the manuscript revision and read and approved the final version. FMI and EAl confirm the authenticity of all the raw data.

Ethics approval and consent to participate

This study was approved by Giresun University's Faculty of Medicine Clinical Trials Ethics Committee (approval no: 2018-06-10). All patients provided written informed consent prior to the study.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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