



Original Article

Evaluation of the Long noncoding RNA *NONHSAT037832* Gene Expression Changes in Patients with Thyroid Carcinoma

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Abstract

Background & Objective: Thyroid cancer is the most common endocrine disorder in the world. One of the main causes of this cancer is genetic changes, in particular mutations in the tumor and oncogene inhibition genes. In addition to mutations, some non-encoding RNAs called lncRNAs play an important role in the progression of tumors. NONHSAT is a lncRNA and has recently been referred to the role of this gene in various types of cancer. This study aimed to evaluate the *lncRNA-NONHSAT037832* gene expression changes in patients with thyroid carcinoma.

Materials & Methods: A cross-sectional study was conducted on 140 homogeneous samples (70 cancer tissue homogeneous samples and 70 homogeneous healthy tissue samples) from patients with thyroid cancer referred to Alzahra Hospital in Tabriz. Using the Real-Time PCR, changes in the expression of the *lncRNA-NONHSAT037832* gene were evaluated. In addition, the relationship between *lncRNA-NONHSAT037832* gene expression changes and clinical parameters was investigated.

Results: The results showed that the expression of *lncRNA-NONHSAT037832* gene expression in the tissues of cancer was significantly higher than healthy tissues ($P < 0.001$) and a significant relationship with MTN ($P = 0.001$) and node metastasis Lymphoma was shown ($P = 0.30$).

Conclusion: According to the results, the *lncRNA-NONHSAT037832* gene may be used as a prognostic biomarker for thyroid cancer.

Keywords: lncRNA, *lncRNA-NONHSAT037832*, Thyroid carcinoma

Introduction

Thyroid cancers are the most common type of endocrine cancers. However, many thyroid nodes that are detected accidentally by the patient, medical examinations, or neck imaging are benign (1). Thyroid cancer accounts for 90% of all endocrine cancers (2).

The spread of this disease over recent years is on the rise in some countries, and the increase can be assigned to changes in lifestyle or environmental factors including iodine deficiency or exposure to radiation (3). In general, thyroid cancers are divided into two major categories, namely those derived from follicular cells, and the ones derived from parafollicular cells. The first category includes follicular thyroid cancer (FTC), papillary carcinoma cancer (PTC),

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and anaplastic thyroid cancer (ATC), and the second category includes medullary thyroid cancer (4). Papillary carcinoma (PTC) is the most common type of thyroid cancer making up about 80% of all cases of thyroid cancer (5).

Studies on changes in gene expression have been conducted on PTC where an increase has been observed in the expression of Angiopoitin (Ang-), cytokeratin19 (CK-19), and tissue inhibitor metalloproteinase1 (TIMP-1) (6). Another prognostic factor concerning PTC is the mutation in BRAF and TERT genes (7, 8). The decrease in bcl-2 expression in the initial stages of cancer and decrease in the let-7s gene expression was observed in PTC tumors (9, 10). Mutations also have a role in FTC that is the second most common thyroid cancer including point mutation in RAS gene (11). In medullary thyroid cancer, the activating mutations in RET gene are present in germ cells (12). Anaplastic thyroid cancer (ATC) is uncommon and aggressive which usually causes death (13). Moreover, the mutation in P53 gene changes the shapes of PTC and FTC cells to that of ATC (14). In ATC, mutations in RAS and BRAF, β -catenin, PIK3CA and Auxin, APC, PTEN and MET, CDKN2A, FOSL 1, UBE2C have been reported too (15). In animal cells, a major part of RNA products is the non-protein-coding RNAs or ncRNA that apparently do not code any protein (16). These non-coding RNAs mainly consist of circular RNAs, micro RNAs, and long non-coding RNA (17). lncRNAs are longer consisting of more than 200 nucleotides, and they are not broken down into smaller RNA cells (18). lncRNAs are involved in different gene expression levels, both in transcriptional levels and post-transcriptional levels. One of the post-transcriptional regulations is the cooperation with mRNAs (19). There are extensive molecular relationships between lncRNAs and miRNAs, which are of considerable importance in controlling gene expression and production of genes in cells (20). miRNAs are the short RNA cells, about 19-23 nucleotides in length that function through controlling the expression of different genes as main

regulators of an extensive range of biological processes including initial evolution, cell differentiation, reproduction, and Apoptosis (21). Hence, the increase or decrease in the amounts of these RNAs is observed in many patients, in particular in the patients suffering from cancer (22, 23). Thyroid cancers are detected by the disruptions in regulations of a set of (miRNA) microRNAs, and the expressions of different types of these RNAs increase or decrease in thyroid cancers (16).

Studies show that there are some disruptions in lncRNA regulation in some cancers including intestine, bladder, breast, and colorectal cancers. This report shows that some lncRNAs have a practical role in causing cancer, thus, they can be used as biomarkers to detect cancer (24). A new lncRNA called *NONHSAT037832* was detected through an investigation into a new *NONHSAT037832* and was detected that to a considerable extent, it made an decrease in expression compared to the non-aggressive tissues of the thyroid (25). PGF (Placental Growth Factor) is one of the potential *NONHSAT037832* genes. PGF is an angiogenic protein of the vascular endothelial growth factor (VEGF) family that can stimulate endothelial cell growth, and vascular migration, enhancement, and permeability (26, 27). PGF expression has been reported for different cancers, and it is connected with tumor stage and metastasis (28, 29). Therefore, *NONHSAT037832* may assume a role by regulating PGF expression in genetic tumors. Considering the study conducted on the *NONHSAT037832* gene in 2016 in China, examining the negative expression regulation of this gene in PTC (25), this paper aims to examine the changes in the expression levels of this gene in some samples suffering from thyroid carcinoma in Tabriz to show the importance of *NONHSAT037832* prognosis in patients compared to healthy individuals. Researches show that there are about 240 lncRNAs with genetically changed expression of PTC. The search for the relationship between *NONHSAT037832* and thyroid cancer indicates the lack of a study on this issue, and the



reason for not addressing the issue is that *NONHSAT037832* has been detected recently. A limited number of lncRNAs are also involved in thyroid tumors, thus this study aimed to evaluate the changes in *NONHSAT037832*-lncRNA gene expression level in patients with thyroid carcinoma.

Materials & methods

Tissue samples

This study was conducted in Alzahra Hospital on 140 thyroids (70 cancer tissues and 70 marginal and healthy tissues) collected from patients suffering from thyroid cancer who visited Alzahra Hospital in the period 2016-2017. These samples were collected by endocrine glands specialists and pathologists after making a definitive diagnosis with an endoscopy. The samples were collected after obtaining consent forms from all patients. In this study, the subjects met a particular age criterion and had already been diagnosed with malignancy by pathologists. Being treated by surgeries or undergoing chemotherapy were the exclusion criteria.

RNA extraction from samples and examination of its quality and quantity

The extraction of RNA from thyroid tissue samples by specialized extraction kit was performed by the kit offered by manufacture catalogue No. 740971.50. After RNA extraction, it was necessary to determine the quantity and quality of the extracted RNA. Two methods including spectrophotometry and electrophoresis were applied on agarose gel to evaluate the quality

and quantity of extracted RNA. The results of spectrophotometry showed that the quality of the samples was good in most cases i.e., the ratio ranging from A260 to A280 was appropriate, and it was between 1.9 and 2. According to the instructions proposed by the manufacturing company, at first, the RNAs of the samples were extracted and they were converted to cDNA using reverse transcriptase (RT) after being treated with DNAs enzymes.

cDNA synthesis

cDNA synthesis is performed by reverse transcriptase that is a reverse transcribing enzyme used in some viral systems like that of AIDS. The reverse transcriptase (RT) places DNA against RNA by exactly using the complementary DNA sequencing, in other words, it creates the DNA strand representing the same RNA sequencing. The evaluation of synthesized cDNA can represent the same amount of produced RNA or the expression of the given gene.

Real Time PCR REACTION to SYBER GREEN

In this method, *GAPDH* gene method was used to normalize gene expression levels in comparison with the control group and patient group. The sequencing of primers has been mentioned in table (1) (25).

1) Initial denaturation stage was performed at 94°C for 3 minutes, 2) Denaturation stage was performed at 94°C for 30 seconds, 3) Annealing stage was performed at 60°C for 40 seconds, 4) Extension was performed at 72°C for 45 seconds.

Table 1. Primers used in Real Time PCR (25)

Primer	Sequences
<i>NONHSAT.F</i>	5'-GCCAATGTCACCATGCACTC-3'
<i>NONHSAT.R</i>	5'-GCTGAGAGAACGTCAGCTCC-3'
<i>GAPDH.F</i>	5'-GCCGTCTAGAAAAACCTGCC-3'
<i>GAPDH.R</i>	5'-ACCACCTGGTGCTCAGTGTA-3'

Statistical analysis

In the information analysis section, first the relationship between *lncRNA-NONHSAT037832* gene expression and demographic and clinical characteristics of patients with the accepted chi-square test was investigated. Then, gene expression level and the significance of the probable differences of this gene in cancer tissues and adjacent tissues were investigated using Real-Time PCR. In addition, a t-test was

used to compare *NONHSAT* gene expression between cancer tissues and their adjacent tissues. This analysis was conducted by SPSS 22.

Results

The results of Real-Time PCR indicated that the *NONHSAT* gene expression levels in cancer tissues were significantly greater than those of adjacent tissues ($P=0.0004$). In other words, this gene underwent overexpression in cancer tissues (Chart 1).

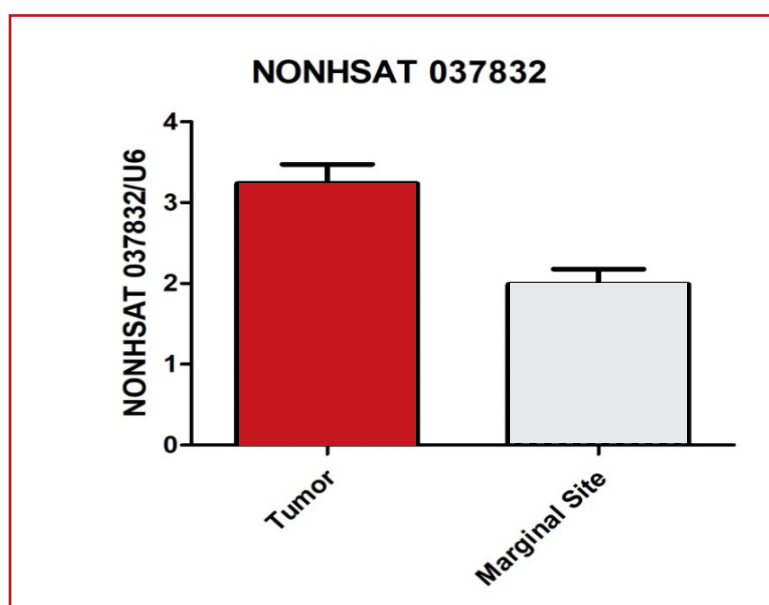


Chart 1. The relative *NONHSAT037832* gene expression in cancerous and non-cancerous tissues in patients with thyroid cancer

The results of the investigation into the data on subjects showed that 40 individuals (57.1%) of patients were male and the rest were female (Table 2). The disease progression in subjects was observed as follows: most subjects, that is, 25 individuals (35.7%) were in N1a stage, the next 18 individuals (25.7%) were in M1 stage, 12

individuals (17.1%) were in N1b stage, and there was only 1 individual (1.4%) in T2 stage (Table 3). Furthermore, the types of thyroid cancer the subjects suffered from were as follows: 26 subjects (37.1 %) with follicular thyroid cancer, 8 subjects (11.4%) with medullary thyroid cancer, and 1 subject (1.4%) with anaplastic thyroid cancer (Table 4).

Table 2. Gender of subjects

Gender	Number	Percentage
Male	40	57.1
Female	30	42.9
Total	70	100



Table 3. Tumor stages of thyroid cancer

Stage	Number	Percentage
Stage M1	18	25.7
Stage N1a	25	35.7
Stage N1b	12	17.1
Stage T1a	8	11.4
Stage T1b	6	8.6
Stage T2	1	1.4
Total	70	100

Table 4. Types of thyroid cancer in subjects

Types of thyroid cancer	Number	Percentage
Papillary	35	50.0
Follicular	26	37.1
Medullary	8	11.4
Anaplastic	1	1.4
Total	70	100.0

Changes in *NONHAST* gene expression levels in tissues that were examined were as follows: greater than normal in 34

samples (48.6%), normal in 32 samples (45.7%), less than normal only in 4 samples (5.7%) (Table 5).

Table 5. Changes in *NONHAST* gene expression level in examined tissues

Type	Number	Percentage
Normal	32	45.7
Excessive	34	48.6
Less than normal	4	5.7
Total	70	100



Relationship between *NONHAST* gene expression and patients' demographic and clinical characteristics

The results of this study on the relationship between *NONHAST* gene expression with patients' demographic and clinical characteristics indicated that *NONHAST* gene expression from among some variables such as progression stages of TMN and *NONHAST* gene expression had a significant relationship with lymph node metastasis and stages

of cancer. This was the case such that we observed significant *NONHAST* overexpression in 36% of the samples undergoing lymph glands involvement, and we also observed *NONHAST* underexpression in 11% of the samples undergoing lymph glands involvement. However, in 31% of the samples that cancer was not complicated with lymph glands involvement, we observed *NONHAST* underexpression and *NONHAST* overexpression in 22% of these samples (Table 5).

Table 6. The relationship between *NONHAST* with demographic characteristics

8Factors	NONHAST LOW	High	p
Age at diagnosis (Years)	55.7±1.6	58.59±1.9	0.126
T-Stage (%)			
T1	32	15	0.302
T2-T4	36	17	
Lymph node metastasis (%)			
Yes	11	36	0.030
No	31	22	
TNM staging (%)			
M1-N1b	16	20	0.001
T1a-T2	21	43	
5ysr (%)			
overall five-year survival rate positive	25	33	0.017
overall five-year survival rate negative	14	28	



Table 6 investigates the relationship between *NONHAST* gene expression, on the one hand, and disease diagnosis age, progression stages of disease (TMN), and five-year survival, on the other hand. Considering the significance level of the test, it can be said that *NONHAST* gene expression has a significant relationship with lymph node metastasis and stages of cancer.

Discussion

Research shows that there are about 240 lncRNAs with the changed genetic expression of PTC which are present in the thyroid tumors (30). However, the lncRNA's relationship with cancer and cancer detection has not been discovered exactly. In this study, *NONHSAT037832* expression level in PTC tissue was investigated and the relationship between its expression and the clinical-pathological characteristics of the patients with PTC was taken into account. The results of the study on the relationship between *NONHAST* gene expression and patients' clinical-pathological characteristics of patients indicated that *NONHAST* gene expression from among some variables such as disease progression stages (TMN) and *NONHAST* gene expression had a significant relationship with lymph node metastasis and stages of cancer (TMN). In addition, the results on the staging of cancer indicated that the level of *NONHAST* increased significantly along with the progression of cancer. Moreover, the comparison made between *NONHAST* gene expression among cancer tissues and its adjacent tissues indicated that the average *NONHAST* gene in cancer tissues was significantly greater than the average *NONHAST* gene in adjacent tissues. In other words, it underwent overexpression in cancer tissues.

Kim et al. (31) conducted a study to investigate lncRNAs expression level. They found that LOC 100507661 in anaplastic thyroid cancers and thyroid gland cancers underwent overexpression. In addition, patients suffering these particular cancers undergoing overexpression of this RNA, experienced increased lymphatic metastasis, and

BRAF V600E mutation. They concluded that LOC 100507661 has an important role in causing thyroid cancers, and it can be used as a prognostic marker. Similar research was conducted by Du et al. (30) where the RNA sequencing profile in patients with PTC was used, and the sequencing was compared to those of healthy people. The results of the comparison showed that about 240 lncRNAs underwent changes in their expression levels. Concerning *lncRNANONHSAT037832* sequencing, its expression has been investigated recently by Lan, et al. (25) in some cell lines and 87 PTC samples. The results showed that PTC *NONHSAT037832* tissues underwent downregulation compared to the normal tissues. The researchers found in their studies that in two PTC cell lines, namely K1 and IHH4, this type of RNA underwent downregulation compared to NTHy-ori 3-1 cell line (a normal thyroid cell line). However, there was no marked difference between BCPAP and normal cells. In this study, *NONHSAT037832* downregulation was significantly related to LNM and motor size. However, it had no relationship with other clinical characteristics. In this study, Lan et al. (25) reported that *NONHSAT037832* was most probably a tumor suppressor lncRNA.

The result of this study was inconsistent with that of Lan et al. (25) because as mentioned, in their study this gene was accompanied by downregulation (underexpression) in cancer tissues. The reason for this inconsistency may be that the study conducted by Lan et al (25) only addressed PTC while all types of thyroid cancers were examined in this study and this can affect the findings. lncRNA *BANCR* produced different results in two different studies on thyroid cancer. The difference was that in the study conducted by Wang et al. in 2014 (32) on cancer tissues, *BANCR* underwent overexpression while it underwent downregulation in cancer tissues in the study conducted by Liu et al. in 2017 (33).

Although it has been determined by the studies that *NONHSAT037832* has an important role in producing tumors by PGF expression regulation, the relevant mechanism has not been exactly



discovered, and there is a need for further studies on this. Discovering if lncRNAs are the key regulators involved in deforming towards cancer and its progression has gained some landscapes towards the application of these molecules for diagnostic and therapeutic purposes. Many lncRNAs like ANRIL, HOTAIR, etc. are expressed in a way limited to a particular tissue and are specific to a particular cancer, and they can be used as helpful prognostic markers. Some lncRNAs that underwent downregulation and upregulation in previous studies on thyroid cancer are: lncRNAs of ANRIL (34), NEATI (35), LOC100507661 (30), HOTAIR (36), H19 (37), ENST00000537266 (38), were accompanied by overexpression of thyroid cancer, and some lncRNAs including AK023948/PTCSC1 (39), MEG3 (40), NAMA (41), PTCSS2 (42), PTCSC3 (43), BANC1 (33) underwent downregulation in thyroid cancer. In general, the results of this study indicate that the changes in NONHAST gene expression may have an important role in the oncogenesis of the thyroid gland. Of course, extensive evaluations will be required in future investigations to confirm the results of previous studies.

General conclusion

Considering the results of this study, it can be said that NONHAST may be used as a helpful prognostic marker of cancer.

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Conflict of interests

Authors announce that there is no conflict of interests in this study.

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