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ORIGINAL RESEARCH

The altered expression of long non-coding RNAs: GHET1, BACE1-AS, PANDA, UCA1 associated with non-small cell lung cancer

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Abstract

Objective: Long non-coding RNAs (lncRNAs) are characterized as non-coding transcripts greater than 200 nucleotides. lncRNAs have extensive molecular connections with proteins and microRNAs, which are important in the regulation of gene expression in physiologic and pathologic states including cancer. About 18% of human LncRNAs were recently found to be associated with tumours. Many studies indicated that aberrant expression of LncRNAs play key roles in the progression and metastasis of NSCLC. In this study we evaluated the expression of long non-coding RNAs: GHET1, BACE1-AS, PANDA, UCA1 in non-small cell lung cancer.

Material & Methods: In this study, RNA was extracted from tumor tissues of NSCLC and paired adjacent normal lung tissues. After cDNA synthesis, the relative expression level of lncRNA *GHET1*, *BACE1-AS, PANDA*, and *UCA1* genes was studied by TaqMan Real-Time PCR, and the data were analyzed by $2^{-\Delta\Delta CT}$. The t-test was used to compare the values and P-value < 0.05 was considered statistically significant.

Results: The data of qRT-PCR analysis revealed that the expression level of *GHET1* gene in patients with NSCLC is increased (P= 0.0032) and *BACE1-AS* showed down-regulation (P= 0.0093). There was no significant change in the expression of *PANDA* and *UCA1* genes.

Conclusion: Our study sheds lights on the expression signature of several crucial lncRNAs in human lung cancer. This data not only could be further be utilized for different therapeutic approaches but also reveal the changes in biological processes of human lung tumors.

Keywords: BACE1, GHET1, lncRNA, Lung cancer, PANDA

Introduction

Lung cancer is the most common cause of cancer-related death worldwide. Histologically it is subdivided into two main types: non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC). NSCLC comprises around 85% of all lung cancer cases. Despite progress in diagnosis and treatment, the 5-year survival rate remains 15%. Smoking, air pollution, and genetic background are the main known risk factors (1).

In recent years, researchers have identified extensive regions of the human genome earlier believed to be non-coding and non-functional sequences. The non-coding regions accounted for more than 90% of the genome, encoding a large variety of regulatory RNAs with different properties and functions. These RNAs play a critical role in the regulation of gene expression and biological processes in cancer (2).

Non-coding RNAs are mainly divided into two classes of short non-coding RNAs such as miRNA (~22 nucleotides) and long non-encoding RNAs (lncRNA) with a length of more than 200 nucleotides (3).

Several studies suggest that 18% of the human lncRNAs are associated with cancer, with their altered expression playing a pivotal role in the progression and metastasis of different types of cancer. Based on their role in cancer, lncRNAs are categorized into two groups of tumor-suppressors and oncogens capable of enhancing the tumor prgression as well as the invasion to the extracellular matrix of the cancer cells (4). These molecules could probably serve as new biomarkers for diagnosis, prognosis, metastasis, and prediction of the response to treatment (5). Nowadays, lncRNAs are considered as new therapeutic targets in the metastasis of lung cancer, with most pieces of evidence proving the involvement of lncRNAs in invasion and metastasis (4).

Due to lack of robust markers for early detection of lung cancer and the potential of non-coding RNAs, this study aimed to investigate the expression levels of four distinct lncRNA including *PANDA, GHET1, UCA1*, and *BACE1-AS* in NSCLC patients.

Two different isoforms of *BACE1-AS* transcript have been identified so far. *BACE1-AS* is an antisense transcript for the beta-secretase, fully overlapping with the exon 6 of the *BACE1* gene. *BACE1* is expressed at a far higher level than its antisense strand *(BACE1-AS),* except in Alzheimer's disease in which there is a higher brain *BACE1-AS* expression (6).

The *BACE1-AS* mechanism and function regarding cancer have not been completely characterized. However, comprehending its biological mechanism may contribute to the diagnosis and treatment of the associated diseases such as NSCLC (7).

Considering the genomic studies, *GHET1* is associated with several functions including growth regulation. The gene is activated by H3K27 via the ATF1 factor, inducing chromatin acetylation in hepatocellular carcinoma (8). Also, as an oncogenic lncRNA, *GHET1* is involved in the tumorigenesis and progression of different cancers such as colorectal and gastric cancer (9, 10).

Genomic studies indicate the *PANDA* gene is positioned 5 kb upstream of the *CDKN1A* gene. The *PANDA* gene is evidenced to affect the cell cycle through its interaction with the *CDKN1A* gene. The *PANDA* gene is also involved in cell death and apoptosis. *PANDA* deletion influences the expression of many genes, especially those involved in response to DNA damage (11).

UCA1 has three exons and isoforms of 1.4, 2.2, and 2.7 kb in the length of which the 2.2 kb isoform is the most recognized one (12). *UCA1* is believed to be associated with several activities and functions, such as cell proliferation, oncogenesis, and drug resistance in bladder cancer. Investigations have revealed the increased expression level of this lncRNA in different types of cancer, including gastric, intestinal, hepatic, and breast cancers (13).

This study aimed to examine the *PANDA, GHET1, UCA1,* and *BACE1-AS* gene expression levels in patients with NSCLC.

Materials and Methods

After obtaining informed consent, 30 patients diagnosed with NSCLC from Masih Daneshvari, Atieh, and Khatam-al-Anbya hospitals were included in the study. Samples from the tumor and paired adjacent normal tissue from each patient were taken by the surgeon. Once NSCLC was diagnosed and confirmed by the pathologist, samples were included in the study. The study was approved by the ethics committee (sbmul. REC1394.

112) of the Masih Daneshvari Hospital and written informed consent was taken from all patients before surgical resection. Samples were stored at -70˚C until use.

RNA extraction and cDNA synthesis

RNA was extracted using TRIzol reagent (Qiagen, USA) according to the manufacturer's protocol from the lung tumor and normal adjacent tissues. The concentration of RNA was calculated through the measurement of the absorbance at 260 nm by a nanodrop (Epoc,Biotek, USA). The quality of RNA extracted was determined using the absorbance ratios of 260/280 and 260/230. The GeneALL kit (South Korea) was used to synthesize cDNA from 1000 nanograms of RNA according to the manufacturer's instructions.

Primer sequence and gene expression assessment

In the present study, the TaqMan Real-Time PCR method was employed to assess the expression levels of *PANDA, UCA1, GHET1*, and *BACE1-AS* genes. The sequences of primers and probes are shown in Table 1. The *HPRT1* gene was selected as the reference gene in our study.

The real-time master mix was contained 2X Master Mix (amplicon), cDNA (10ng), 10 picomoles of primers and probes, and double-distilled water were added to make a total reaction volume of 20 microliters. All samples were performed in duplicate, and the Real-Time PCR reaction proceeded using a StepOnePlus device (Applied Biosystems, USA). The temperature program of the system consisted of two steps: in the initial step, a temperature of 95 °C was used for 15 minutes to activate the enzyme. Then, 40 cycles of denaturation at 95°C occurred within 15 seconds followed by 1 minute at 60°C.

Table 1. The sequences of the primers and probes

Primer	Sequence	Length
GHET1-F	AGTCAGCTCCCTACAGAGGTG	94bp
GHET1-R	TCCTTAGGTGGTGGTTTCTGTTC	
Probe	FAM-TCCCACTGCCCAAGATCCCTGCCT-TAMRA	
BACE1-F	GACACTGTACCATCTCTTTTACCC	113bp
BACE1-R	CACCACCAACCTTCGTTTGC	
Probe	FAM-AGTCCACTCACGGAGGAGGCTGCC-TAMRA	
PANDA-F	GTTTTCCTGTTCGTCGATTCTGG	81bp
PANDA-R	GGAAAGCTGAGAGAGACTTTGAAC	
Probe	FAM-CTGGACCACCTCTGAAGGCAGGCA-TAMRA	
UCA1-F	TCTCCATTGGGTTCACCATTCC	100bp
UCA1-R	GCTCTCGGCCTAATCTTGTGG	
Probe	FAM-AGCCATGCCCATCAGACAGCCAGC-TAMRA	
HPRT1-F	AGCCTAAGATGAGAGTTC	88bp
HPRT1-R	CACAGAACTAGAACATTGATA	
Probe	FAM -CATCTGGAGTCCTATTGACATCGC-TAMRA	

The expression level of genes in the tumor tissue compared to the paired adjavent normal tisssue were measured by using the comparative 2-ΔΔCT (fold change) method (14). The statistical significance of relative changes in gene expression between different groups of lung tumors compared to adjacent normal tissue were determined by ANOVA and T-test (P-value≤0.05). The graphs were created by GraphPad PRISM 5.0 software.

Results

In the present study, The TaqMan Real-Time PCR method was used to assess the levels of expression of four lncRNAs (*PANDA, UCA1, GHET1,* and *BACE1-AS*) from 60 NSCLC tissue and paired adjacent normal tissues. Based on the pathology of tumor samples, 33.4% and 66.6% of the patients had squamous cell carcinoma and adenocarcinoma, respectively. In terms of gender, 36.7% of the patients were female, and 63.3% were male. 3.3 % of the patients were under 40, while 96.7% were over 40. 36.7% of the patients were smokers, and 63.3% were non-smokers.

There was a significant decrease in the expression level of the *BACE1-AS* gene in NSCLC tumor samples compared to the normal samples (P= 0.0093) (Figure 1).

In contrast, *GHET1* expression was increased significantly in the NSCLC tumor samples (P= 0.0032) (Figure 2).

There were no significant changes in the expression levels of *PANDA* (P= 0.38), and *UCA1* (P= 0.22) in the tumor samples compared to the normal tissues (Figure 3) (Figure 4).

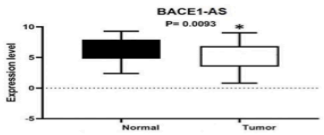

Figure 1. Changes in the expression level of the BACE1-AS gene in the tumor samples compared to the normal samples

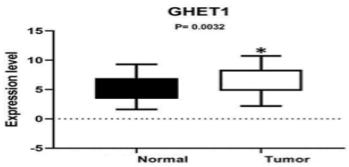

Figure 2. Changes in the expression level of the GHET1 gene in the tumor samples compared to the normal samples

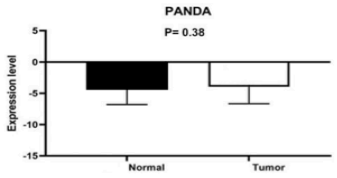

Figure 3. Changes in the expression level of the PANDA gene in the tumor samples compared to the normal samples

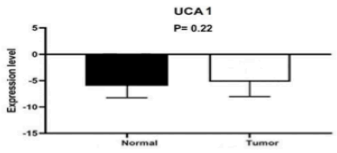

Figure 4. Changes in the expression level of the UCA1 gene in the tumor samples compared to the normal samples

Discussion

According to a newly outlined hypothesis, lncRNAs with more conserved sequences are more functional molecules (15). These long regulatory RNAs act as key molecules in the regulation of the processes such as chromatin remodeling, transcription, post-transcriptional modifications, epigenetic regulation, processing of small RNAs, cell cycle, and apoptosis. They also can serve as scaffolds to hold several proteins together, as well as guiding agents in recruiting proteins for specific areas of the chromatin and affecting the local structure of the chromatin.

Studies have indicated the expression of several lncRNAs regulating the cell cycle in mammalian cells (16).

It is estimated more than 50 proteins coding genes and more than 200 lncRNAs play key roles in the regulation of cell cycle. lncRNAs are dysregulated in different types of cancer, with their expression levels associated with cancer progression, metastasis, and prognosis of cancer. The up regulation of some lncRNAs appears to be able to increase tumor growth and invasion of the extracellular matrix (17). This study investigates the expression of *PANDA*, *UCA1*, *GHET1*, and *BACE1-AS* in NSCLC patients from an Iranian population.

Zhou et al. (2016) investigated the knock down of *GHET1* in patients with colorectal cancer, observing its substantially increased expression in the tumor samples. Considering its oncogenic role, the knockdown of the *GHET1* resulted in inhibited metastasis of colorectal cancer (10).

Guan et al. (2018) found out that the knockdown of lncRNA *GHET1* suppressed cell proliferation, invasion, and LATS1/YAP pathway in NSCLC, revealing that *GHET1* acts as a tumor-promoting gene (18). Additional researches have also reported the oncogenic role of lncRNA-GHET1 in cervical and gastric cancers (19, 20).

As discussed in several investigations, *GHET1* expression is commonly increased in various types of cancer, indicating its oncogenic role. That was also the case in the present study since the *GHET1* expression was increased significantly in cancerous cells of lung tissue (P= 0.0032). Given the genome-wide association studies, *GHET1* is correlated with a variety of regulatory functions, in cancer and growth. However, the primary role of *GHET1* is considered to be the regulation of cell proliferation in normal tissues. *GHET1* induces indirect cell proliferation by influencing the *c-MYC* gene. As a transcription factor, *c-MYC* is known to be a key element in various stages of tumorigenesis in humans. *GHET1* has been shown to affect *c-MYC* through binding to the IGF2BP1 (Insulin-like Growth Factor 2 mRNA Binding Protein 1), increasing the physical interaction between the IGF2BP1 protein and *c-MYC* mRNA as well as enhanced stability and expression of *c-MYC* mRNA, and the induction of growth and proliferation in cancer cells *in vivo* and *in vitro* (19). Identifying the reasons for increased expression of *c-MYC* can help diagnose different types of cancer, such as NSCLC, and serve as a potential therapeutic target.

The other lncRNA examined in the present study was *BACE1-AS*, with few studies conducted on it. Yaghoubi et al. (2019) investigated the expression level of *BACE1* and *BACE1-AS* in breast invasive ductal carcinoma (IDC). They indicated that *BACE1* experienced a decreased level of expression, while *BACE1-AS* expression remained unchanged (21).

Yeganeh et al. (2020) studied the role of *BACE1-AS* as the predictor biomarker in breast cancer, reporting an increased expression level of *BACE1-AS* in patients compared to the normal control group (22). In the present study, the analysis revealed a significant decrease in the expression of the *BACE1-AS* gene in the tumor samples compared to the adjacent normal ones (P= 0.0093).

It is vital to consider the natural role of

BACE1-AS in lung tissue to understand and interpret the outcomes of its decreased level of expression.

Known to be involved in the formation pathway of beta-amyloid, *BACE1-AS* undergoes an increased expression level under cellular damage and stress conditions leading to increased production of beta-amyloid as well as decreased cellular stress. The elevated expression of *BACE1-AS* in response to oxidative stress and other kinds of cellular stressors results in the increased stability of the BACE1 mRNA. In turn, *BACE1* attenuates cellular stress through the increased formation of beta-amyloid plaques as a process occurring in normal tissues (7).

In this study, there was a significant decrease in the *BACE1-AS* expression, which may dysregulate the cell response mechanisms to the oxidative stressors. Studies also suggest that various types of cellular stress stimuli including increased temperature, serum levels, H2O2, and glucose levels can raise BACE1-AS RNA levels as well as the BACE1 protein (23). Nevertheless, the mechanisms by which *BACE1-AS* affects the development and progression of cancer are quite complicated, requiring further investigations.

Peng et al. (2017) studied the expression level of *PANDA* in hepatocellular carcinoma (HCC) under *in-vitro* conditions, indicated the effects of its increased level in HCC patients on inhibiting the senescence-associated inflammatory factor IL8 as an inhibitor of cellular senescence. Unlike previous studies on liver cancer, they revealed an increased *PANDA* expression (24).

In another research, Wang et al. (2017) investigated the function of lncRNA PANDA in human diffuse large B-cell lymphoma (DLBCL). They observed *PANDA* acted as a tumor-suppressive agent through the inactivation of the MAPK/ERK signaling pathway and inhibition of the cell proliferation in DLBCL (11).

Functional studies on *PANDA* and *P53* have shown that *P53* directly induces *PANDA* expression. Then, *PANDA* interacts with another transcription factor called NF-YA to form a complex that binds to the promoter regions of specific genes and impedes their transcription, leading to inhibited apoptotic activity (25).

Probably due to the small number of samples in the present study, no significant increase in *PANDA* expression level was observed in Iranian patients with NSCLC. The different expression levels of lncRNAs in different tissues should also be considered, leading to different outcomes in various types of cancer.

As the last lncRNA studied in this investigation, *UCA1* expression showed no significant changes in NSCLC patients. Wang et al. (2020) studied the interaction between *UCA1* and miR-182-5p in renal cancer. They reported that *UCA1* functioned as an inhibitor sponge, preventing miR-182 from binding to the Delta-like ligand 4 *(*DLL4). Subsequently, it promoted malignant phenotypes of renal cancer cells (26).

Chen et al. (2020) demonstrated the inhibitory influence of *UCA1* on miR-143 in NSCLC patients. They also confirmed an increased expression level of *UCA1* in Gefitinib-resistant NSCLC cells. Through the *UCA1* gene knockdown, the researchers observed an impaired cell proliferation, followed by increased cell sensitivity to Gefitinib (27).

A larger statistical population is required to investigate the effects of *UCA1* on miRNAs involved in NSCLC. Furthermore, the potential role of *UCA1* as a biomarker for the diagnosis, prognosis, and even treatment of lung cancer needs to be scrutinized.

In this study, it has been observed to be a significant increase in the expression level of *GHET1* in the tumor tissue of NSCLC patients, compared to the adjacent normal tissue. *BACE1-AS* expression experienced a decrease in the tumor tissue compared to the adjacent normal tissue. The elevated expression levels of *GHET1*, which subsequently increase *c-MYC* expression, tend to dysregulate and disrupt numerous cellular mechanisms and promote lung cancer. Further functional studies are required to unravel the role of *BACE1-AS* in the development and progression of lung cancer.

Conflict of interest

Author declares no conflict of interest.

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