Expression of CASC9 and LINC01234 in Different Cell Lines

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Abstract. Cancers are multifactorial but the advancement in research in the recent decades have drawn attention towards lncRNAs as they are proven to play important function as biological regulators. LINC01234, CASC9 are important long non coding RNAs (lncRNAs). They are not extensively studied therefore, their role in esophageal cancer as well as in other cancers is still not clear. There are few studies about LINC01234 and CASC9 for their role in esophageal cancer. The objective of the study was to investigate the expression of LINC01234 and CASC9 in different cell lines. Our lab cultured and maintained model esophageal cancer cells which were obtained after transformation of human embryonic esophageal epithelial cells into malignant cells by HPV18 E6E7. After this, elevated expression of LINC01234 was found in Gene Chip showing that it may have some role in causing esophageal cancer. For further verification, qRT-PCR was used for the detection of expression level of LINC01234 in different cell lines. Furthermore, expression of CASC9 was also investigated by qRT-PCR in different cell lines. Expression of LINC01234 and CASC9 was markedly high in esophageal cancer cell lines as compared to other cell lines while high expression of LINC01234 was also found in one lung cancer cell line. These lncRNAs may be used as biomarker and potential therapeutic target for esophageal cancer intervention by further exploration of their functions.

Introduction

Esophageal cancer (EC) is one of the aggressive malignancies of human digestive system and ranks as one of the deadliest cancer in the world due to high mortality [1]. Esophageal squamous cell carcinoma (ESCC) and esophageal Adreno-carcinoma (EAC) are the two subtypes of EC with distinct characters of pathology and aetiology. In China, ESCC is the most frequent subtype of EC with more than 90% incidence [2]. Although, there are improvements
and advancement in the diagnostic techniques and therapeutic strategies but still its initial diagnosis at advance stage, aggressive features of ESCC and deficiencies of effective treatment therapies makes it lethal. Therefore the overall 5 years survival of ESCC patients remains less than 40% [3,4]. Early diagnosis and effective therapeutic modalities are the need of time to reduce the cancer causing deaths. Biological markers are important and can help us to move on to the way of success in the battle against cancer. Some of the bio-markers are in use but have limited use because of the low diagnostic specificity as well as sensitivity. The critical issue for Esophageal cancer is early diagnosis and surveillance for metastasis and recurrences. Therefore, new biological markers with high sensitivity and specificity should be explored for early diagnosis of EC.

Accumulating evidence suggests that human / eukaryotic genome and transcriptome is not as simple as it was thought in past. It is now known that human genome produces a large spectrum of RNA by the process of transcription which includes long protein coding mRNA along with non protein coding RNA. Now mounting evidence indicates that these non coding RNAs are the key regulators in different biological processes and affect the phenotypes of normal and cancerous cells. Still the complete function of non coding RNA is not clearly understood [5,6]. Advancement in the recent decades has shown that the human genome encodes a novel class noncoding RNA in large amount which are long non-coding RNAs (lncRNAs). These molecules are longer than 200 nucleotides and act as biological regulators but our knowledge about their functions is still limited. Temporal and spatial variation in expression level of lncRNAs have been observed is also due to stimuli. Research evidence indicates that lncRNAs act as scaffold at DNA level by chromatin modification, in transcription and translation process. They show epigenetic role by regulating the expression of multiple genes [7,8,9]. LncRNAs have been linked with different human diseases and cancers by exerting specific function. They have shown tissue specific and cancer specific expression. They modulate oncogenic and tumor suppressing pathways and play important role in initiation and progression of tumor and metastasis [9,10]. This advancement has opened new doors for researchers to investigate their role in different biological processes in different diseases.

Some of the important lncRNAs previously studied as prognostic biomarkers are HOTAIR, POU3F3 and SPRY4-IT1 [11,12,13]. One of the study regarding LINC01234 investigated its expression profile alongwith two other lncRNAs in esophageal squamous cell carcinoma (ESCC) tissues and normal esophageal epithelial tissues, and found significant expression difference. This study identified three-lncRNA signature as prognostic marker for ESCC patients [14]. Differential expression of LINC01234 is reported in lung cancer tissues and normal lung tissues as well as in esophageal cancerous (EC) and normal tissues [15]. CASC9 is also observed to have high expression in esophageal cancer tissues and EC cell lines [16, 17, 18]. Future efforts can help to use lncRNAs as therapeutic targets for cancer.

In the present study, we investigated the expression of CASC9 in ESCC cell lines as well as in some other cell lines to see the difference in expression. Human embryonic esophageal epithelial cells were transformed into malignant cells by HPV18 E6E7. After this, upregulated expression of LINC01234 was found in Gene Chip showing that it may have some role in causing esophageal cancer therefore we investigated the expression of LINC01234 in different cell lines for further verification.
Methods

Cell Lines

The human cell lines used were Kyse116, Kyse450, HN6, EC9706, Kyse 180, Caski, 150, Hela, TCA83, EC109, A549, CEC3, CEC2, MCF7. Immortalized cell line used was HaCat. These cell lines were maintained in DMEM (Hyclone, Thermo Scientific) supplemented with 10% fetal bovine serum (Gibco, Life Technologies), 100 units/ml penicillin and 100 mg/ml streptomycin. All the cell lines were cultured in an incubator at 37 °C in a humidified and sterile atmosphere with 5% CO2.

Transformation of Normal Esophageal Epithelial Cells to Cancerous Cells

Human embryonic esophageal epithelial cells were transformed into malignant cells by HPV18 E6E7-AVV in our laboratory (by Prof. Zeng Yi and Prof. Shen). The infection with HPV18 E6E7 was the first step while addition of tumor promoting factor TPA was the second step [19]. Normal esophageal cells were transformed into cancer cells after 76 passages. After this, change in the expression level of LINC01234 was detected by Gene Chip.

RNA Extraction and lncRNA Reverse Transcription

Total RNA from Cell lines was extracted using Trizol (Invitrogen, Life Technologies) according to standard protocol. Then Total RNA was purified and DNA was removed from RNA by using DNase kit (Promega). Reverse Transcription kit (Promega) is used to synthesize cDNA from RNA according to manufacturer’s instructions. The cDNA was amplified by quantitative real-time PCR (qRT-PCR) with SYBR Select Master Mix (Life Technologies) on Applied Biosystems ViiA™ 7 (Life Technologies). 384 well block plate is used for qRT-PCR to detect the target sequence. Reaction volume per well was a total of 8ul (including 2ul cDNA). For qRT-PCR, primers were designed for each lncRNA by using Beacon Designer 7 Software and the primers for each lncRNA is given here. There were two sets of primers sequences used for LINC01234 which were as follows: LINC01234(1) Forward; 5’-ACCACACCTGAGATAAGC-3’ and Reverse; 5’-GAGACAGACAGCAAGAGA-3’. LINC01234(2) Forward; 5’- AAGGAAGGAAGAGAAGGAG -3’ and Reverse; 5’-GGATGGACACTGAAGGAT -3’. The primers sequences used for CASC9 were as follows: Forward; 5’-CAGGTAATCTCAGCAGTCA-3’ and Reverse; 5’-ACAGGTCTCCAAAGGATATAC- 3’. While Beta-actin and GAPDH were used as an endogenous control for the relative expression levels of LINC01234 and CASC9 respectively. The relative expression levels of IncRNAs were measured in terms of the CT. The relative fold change in expression of LINC01234 is analysed by using 2^{-\Delta\Delta CT} method. Each sample was used in triplicates for qRT-PCR. The reactions were amplified by using the following conditions: denaturation at 95 °C for 10 min, followed by 40 PCR cycles at 95 °C for 15s and 60 °C for 1 min.

Location of LINC01234 was also investigated by RNA extraction separately from Cytoplasm and Nucleus by using PARIS™ Kit (Ambion, Life Technologies). U6 was used as an endogenous control. Primer sequences for U6 were as follows: Forward 5’CTCGCTTCGGCAGCACGTA-3’ and Reverse 5’ AACGCTTCACGAATTTGCGT 3’.
Results

Transformation of Normal Esophageal Cells into Cancerous Cells

Normal esophageal epithelial cells transformation into cancerous cells after 76 passages, due to transfection with HPV18 E6E7 by Adeno-associated virus (AAV) showed that HPV may have some role in esophageal cancer. Then change in the expression of LINC01234 in these cancerous cells were detected by Affymetrix gene Chip which reported its upregulation as shown in Fig. 1. This predicts that HPV may have some role in upregulation of LINC01234 and this lncRNA may have some relationship with Esophageal cancer.

Expression of LINC01234 in different Cell Lines

Quantitative RT-PCR was used to measure the expression of LINC01234. CEC2 cell line showed the highest level of LINC01234 expression with both primers so CEC2 can be used as model cell line for the study of LINC01234. CEC2 is the esophageal cancer cell line maintained in our laboratory. KYSE180 and A549 also have shown high expression of LINC01234 as compared to other cell lines as shown in Fig. 2.
qRT-PCR was done for the location of expression of LINC01234 in the cell and results showed that it is expressed both in cytoplasm as well as in nucleus but comparatively high expression was observed in cytoplasm in CEC2 and A549 cell lines as shown in Fig. 3.

![Figure 3. Expression of Linc01234 in Cytoplasm (C) and Nucleus (N) of cell lines.](image)

**Expression of CASC9 in different Cell Lines**

It was found through qRT-PCR that the expression of CASC9 was higher in esophageal cancer cell lines as compared to other cell lines of different cancers (cell lines were used randomly). Kyse450 showed highest expression of CASC9. Most of the cell lines used for comparison of lncRNA expression were esophageal cancer cell lines as shown in Fig. 4.

![Figure 4. Expression of CASC9 in different Cell Lines.](image)

**Discussion**

lncRNAs have gained attention in the last few years due to their role in carcinogenesis. Studies in recent years are providing strong evidence for the association of lncRNAs with growth of tumor, epithelial-mesenchymal transition and metastasis. Unfortunately, the role of lncRNAs in cancer is still at its infancy stage and little knowledge is documented about their roles in esophageal cancer (EC). Different studies have shown their critical oncogenic roles in promoting EC progression and may be used as prognostic biomarkers to predict the survival of
patients. Different Studies about development of different cancers, in the recent years, regarding the dysregulated expression of IncRNAs proved them as important cancer contributors hence can be used as biomarkers and prognostic factors [20].

Upregulated expression of LINC01234 was found in gene chip of malignant cells which were transformed from normal esophageal cells to esophageal cancer cells by HPV18 E6E7 infection showing that this IncRNA may have some important role in carcinogenesis. For further verification, qRT-PCR was used for the measurement of expression of LINC01234. One of the lung cancer cell line (A549) and five esophageal cancer cell lines (CEC2, KYSE180, EC109, EC9706, KYSE450) were used for this purpose. Significantly high expression was found in two esophageal cancer cell lines (CEC2, KYSE180) and one lung cancer cell line (A549). CEC2 showed highest expression and CEC2 is esophageal cancer cell line maintained in our laboratory so it is the perfect model cell line for the study of LINC01234 expression as well as its role in esophageal cancer. To the best of our knowledge, it is the first study showing comparison of expression of LINC01234 in esophageal cancer and lung cancer cell lines. The previous study about LINC01234 documented that three-lncRNA signature can be used for the prediction of survival of patients with esophageal cancer. The esophageal cancer tissues were used for this purpose [14]. Presence of elevated level of LINC01234 provided us one evidence and the three-lncRNA signature study is also another important evidence showing its relation with the EC. Further more, our study gives an initial information about the expression of LINC01234 in different esophageal cancer cell lines and lung cancer cell line. One study also used plasma samples for the detection of lncRNA in EC patients but detection level of LINC01234 was found less than 60% [21].

We used different cell lines for the relative expression of CASC9 and most of them were esophageal cancerous cell lines. Highest relative expression of CASC9 was found in Esophageal cancer cell lines while no significant expression was found in other cancer cell lines. In previous study, expression of CASC9 was found significantly higher in 65% of the ESSC tissue samples as compared to normal adjacent tissue samples [16]. CASC9 was markedly unregulated in ESCC tissues and its high expression was found in TE1 and Kyse150 cell lines in previous study [18]. In this study, many esophageal cancer cell lines are used along with other cancer cell lines. Expression of KYSE 450, KYSE 116 and KYSE 150 was also found significantly higher in the present study by qRT-PCR showing the same results which were previously documented while no significant expression is found in other cancer cell lines.

We used only cell lines for the expression analysis of lncRNAs so this is one of the limitation, further extensive study can be done by using EC tissues and normal esophageal tissues as well as cell lines to evaluate the potency of lncRNAs. Further investigation for the role of the lncRNAs can be done which can make the role of these IncRNAs more clear. So the present study is just an initial but important step and is providing some basic information for further exploration of the role of IncRNAs in cancer.

Summary

In this study, we used two IncRNAs (CASC9, LINC01234) to see their expression in different cell lines mainly focused on the Esophageal cancer cell lines. We found in our model cell line that expression level of LINC01234 was upregulated with the changes in cells from normal to malignant cell transformation so, the changes in the IncRNA level with the changes in cells showing that this IncRNAs may play a critical role in esophageal cancer. CASC9 is also an
important lncRNA because its expression was also found high in Esophageal cancer cell lines as compared to other cell lines.

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**References**


