Journal of Clinical and Basic Research (JCBR): 2018: Vol. 2: N.4.P: 48-53 Original Article

Overexpression of Long Noncoding RNA POU3F3 in Esophageal Squamous Cell Carcinoma is Associated with TNM Stage and Family History

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ABSTRACT

Background and objectives: Esophageal cancer (EC) is the second most common gastrointestinal cancer, and esophageal squamous cell carcinoma (ESCC) is the dominant type of EC in Iran. One of the most important challenges in cancer management is the early diagnosis. As tumor suppressors or oncogenes, long non-coding RNAs (lncRNAs) play a vital role in tumor initiation, progression and metastasis. Recent studies have reported that assessing expression of lncRNAs might have prognostic or diagnostic potential for ESCC. In this study, we evaluated expression of lnc-POU3F3 in ESCC and its relationship with some clinical features of the disease.

Methods: Blood samples from 32 cancer patients (18 males and 14 females) and 32 healthy individuals were collected from the Biobank of Sayyad Shirazi Hospital and the Danesh Medical Diagnostic Laboratory in Gorgan (Golestan Province, Iran), respectively. The subjects were matched in terms of age and gender. Following total RNA extraction and cDNA synthesis, quantitative real-time PCR was performed using RealQ Plus 2x Master Mix Green (Ampliqon, Denmark) in the ABI Applied Biosystems 7300 device.

Results: The lnc-POU3F3 was significantly overexpressed in samples from ESCC patients compared to controls. The increased lnc-POU3F3 expression was significantly correlated with family history (P=0.03) and TNM stage (P=0.02).

Conclusions: Our findings suggest that lnc-POU3F3 may be used as a diagnostic biomarker for ESCC. However, further studies with a larger sample size are required to confirm this finding.

KEYWORDS: Esophageal cancer, ESCC, lncRNA, lnc-POU3F3

Received: 2018/12/12 **Revised: 2019/01/03** **Published: 2019/01/22**

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[DOI: 10.29252/jcbr.2.4.48]

INTRODUCTION

Cancer is a genetic disorder characterized by uncontrolled growth, proliferation and spread of abnormal cells (1, 2). Esophageal cancer (EC) is the eighth most common cancer and the sixth most frequent cause of cancer death worldwide (3, 4). In Iran, an estimated 35,000 deaths from cancer occur every year, 5800 of which are related to EC. The highest rates of EC have been found in the Golestan and the North Khorasan Provinces of Iran (5, 6). The main types of EC include esophageal squamous cell carcinoma (ESCC) which predominates in Iran and esophageal adenocarcinoma (EA). Despite recent advances in the treatment of ESCC, it is difficult to diagnose early stages of the disease. Hence, identification of effective biomarkers is essential for the early diagnosis and treatment of the disease (7, 8).

According to recent extensive genomic transcription studies, about 2% of the human genome codes for messenger RNA (mRNA) that translates into protein. However, a very large part of the genome transcribes into non-coding RNAs (ncRNAs) that function at RNA level. Long ncRNAs (lncRNAs) are longer than 200 nucleotides and do not break up into smaller RNA molecules (9, 10). A number of investigations have recently confirmed the aberrant expression of lncRNAs in EC. For instance, Lv et al. revealed increased level of lncRNA HOTAIR in ESCC, which was associated with tumor progression and poor prognosis (11). In a study by Hu et al., lncRNA MALAT1 was found to be an oncogene in ESCC and its high expression was associated with clinical stage, primary tumor size and metastasis of the lymph nodes. This study also demonstrated that this lncRNA could regulate ESCC growth by redirecting the ATM-CHK2 pathway (12). In a group of 62 patients with ESCC, significant overexpression of TUG1 in ESCC tissues compared to adjacent normal tissues was associated with a family history. Besides, silencing TUG1 has been shown to inhibit proliferation, migration and cell cycle progression in ESCC cells by using small interfering RNA (13).

Lnc-POU3F3 is a 747-nucleotide transcript containing four exons, which is located on the reverse strand of chromosome 2q12.1. It is transcribed from upstream of the *POU3F3* gene, a member of the class III POU family of transcription factors (14-16). Li et al. and Tong et al. reported increased expression of lncRNA POU3F3 in ESCC (14, 15). However, these results were only reported from China, and therefore cannot be generalized to other areas. In the present study, we evaluate expression pattern of lnc-POU3F3 in total blood samples of patients with ESCC and healthy controls. In addition, we examined the relationship between lnc-POU3F3 expression and clinical features of ESCC.

MATERIALS AND METHODS

Blood samples from 32 cancer patients (18 males and 14 females) and 32 healthy individuals were collected from the Biobank of Sayyad Shirazi Hospital and the Danesh Medical Diagnostic Laboratory in Gorgan (Golestan Province, Iran), respectively. All samples were transferred to the laboratory under appropriate temperature and maintained at -80 °C until RNA extraction. The subjects were matched in terms of age and gender. Mean age of patients and controls was 59±14 and 60±13 years, respectively.

Extraction of total RNA from the blood samples was performed using Trizol reagent (Invitrogen, CA, USA). The extracted total RNA was reverse transcribed in a final volume of 20 µL using random primers and cDNA synthesis kit (Takara, Japan) according to the manufacturer's instructions. Subsequently, 2 µL of cDNA were used for quantitative real-time PCR (qRT-PCR) using RealQ Plus 2x Master Mix Green (Ampliqon, Denmark) in the ABI Applied Biosystems 7300 device based on the manufacturer's protocol: 95 °C for 15 minutes, 40 cycles of 95 °C for 15 seconds, 59 °C for 45 seconds and 72 °C for 40 seconds. Expression of the

[DOI: 10.29252/jcbr.2.4.48]

target gene was normalized to that of GAPDH, which was used as internal control. Relative quantitative value was expressed using the 2-ΔΔCt method. Sequences of the primers used in this study are as follows: POU3F3-F: 5'-AAT CAC TGC AAT TGA AGG AAA AA-3' and POU3F3-R: 5'-CCT TGT TTT CCA ACC CTT AGA CT-3', GAPDH-F: 5'-GAA GGT GAA GGT CGG AGT-3', and GAPDH-R: 5'-GAA GAT GGT GAT GGG ATT TC-3'.

Mean values and standard deviation of data were calculated using Microsoft Excel 2013. Statistical analysis was performed using SPSS 16.0 software. Differences between the groups were analyzed with t-test. A P-value of <0.05 was considered statistically significant.

RESULTS

LncRNA POU3F3 was expressed in both cancer and control samples. We observed that the lncRNA was significantly overexpressed in blood samples of ESCC patients compared to that of the control samples (P=0.01) (Figure1).

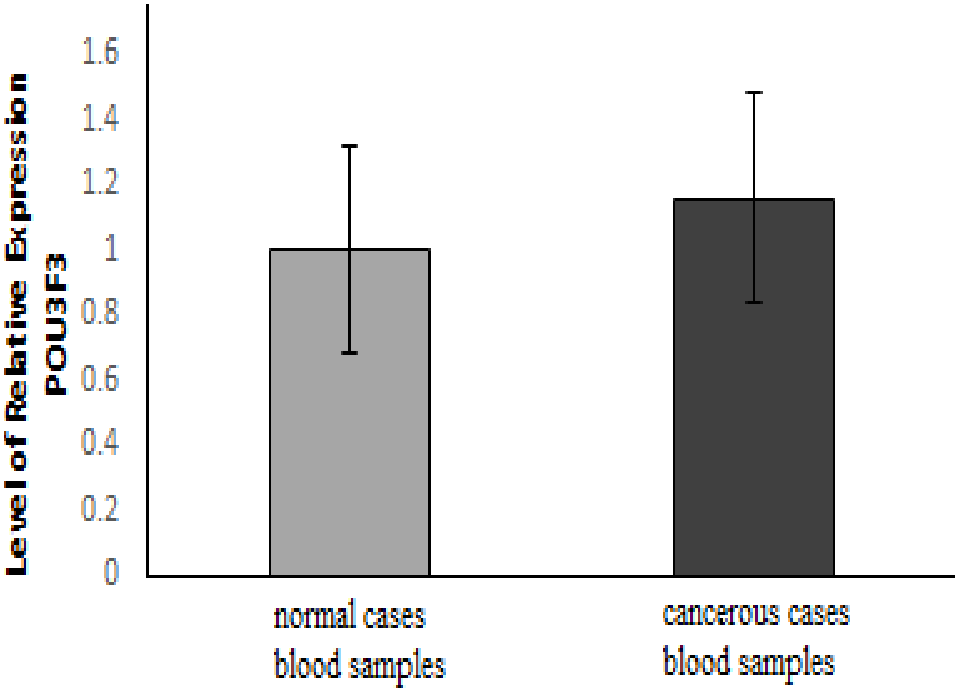

Figure 1. Relative expression level of POU3F3 in samples from ESCC patients and healthy controls

[DOI: 10.29252/jcbr.2.4.48]

The overexpression of lncRNA POU3F3 was significantly associated with stage III ESCC (P=0.03) and family history (P=0.02). However, no association was observed between the lncRNA POU3F3 expression and other clinicopathologic factors in ESCC patients, including age, sex and metastasis (Table 1).

Table 1. **Association between clinicopathological factors and lnc-POU3F3 expression in ESCC patients**

Characteristic	Number	Percent	Fold change	P-value
Age (year)				
<50	10	31.25	1.142365	0.880
≥50	22	68.75	1.161682	
Gender				
Male	18	56.25	1.198829	0.405
Female	14	43.75	1.101504	
Family history				
Positive	9	28.13	1.260205	0.024
Negative	23	71.87	1.121597	
Smoking				
Positive	12	37.5	1.19999	0.587
Negative	20	62.5	1.133804	
Metastasis				
M_0	16	50	1.180167	0.223
M_1	6	18.75	0.952754	
Stage				
T1,T2	10	31.25	1.007284	0.032
T3	22	68.75	1.217071	

DISCUSSION

We evaluated the expression of lncRNA POU3F3 in blood samples from ESCC patients and healthy controls using qRT-PCR. The expression of lncRNA POU3F3 in ESCC blood samples was significantly higher than in control samples. Li et al. also reported the significant overexpression of lncRNA POU3F3 in ESCC tissues compared to adjacent normal tissues (15). Moreover, Tong et al. demonstrated an increase in the expression of lncRNA POU3F3 in plasma of ESCC patients compared to that of healthy controls (14). In addition to ESCC, the significant overexpression of this lncRNA has been detected in gastric, colorectal and glioma cancers (16-18).

In the present study, we investigated the relationship between the expression of lncRNA POU3F3 and clinical features of ESCC including age, sex, TNM stage, smoking and family history. Our findings showed that the expression of this lncRNA was significantly associated with TNM stage and family history. Contrary to this finding, Tong et al. found no significant correlation between lncRNA POU3F3 expression and clinical features of ESCC (14). This observation suggests that lncRNA POU3F3 may have an oncogenic role in the development of ESCC.

Changes in the lncRNA POU3F3 expression in ESCC patients have been mainly reported from China, and therefore cannot be generalized to other regions. Therefore, we assessed the expression level of this lncRNA in the Golestan Province, which is an ESCC hotspot in Iran. Investigating the expression of this lncRNA in other countries and regions could be beneficial.

CONCLUSION

Our results showed significant correlation of lncRNA POU3F3 overexpression with family history and TNM stage in ESCC patients. This may indicate that the lncRNA POU3F3 may play an important role in the development of ESCC.

[DOI: 10.29252/jcbr.2.4.48]

ACKNOWLEDGEMENTS

We would like to thank Masood Bazori for his insightful comments and helpful suggestions.

DECLARATIONS

Funding

This study received financial support from the Gonbad Kavous University, Iran (thesis code: 203226).

Ethics approvals and consent to participate

Written informed consent was obtained from all participants.

Conflict of interest

The authors declare that there is no conflict of interest.

REFERENCES

1. Otto T, Sicinski P. Cell cycle proteins as promising targets in cancer therapy. Nature. 2017;17(2):93-115.

2. Malumbres M, Barbacid M. Cell cycle, CDKs and cancer: a changing paradigm. Nature Reviews Cancer. 2009;9(3):153–166. [DOI:10.1038/nrc2602]

3. Hao Y, Wu W, Shi F, Dalmolin RJS, Yan M, Tian F, et al. Prediction of long noncoding RNA functions with co-expression network in esophageal squamous cell carcinoma. BMC Cancer. 2015;15(168):1-10. [DOI:10.1186/s12885-015-1179-z]

4. Wang YL, Bai Y, Yao WJ, Guo L, Wang ZM. Expression of long non coding RNA ZEB1-AS1 in esophageal squamous cell carcinoma and its correlation with tumor progression and patient survival. Int J Clin Exp Pathol. 2015;8(9):11871-11876.

5. Sadjadi A, Nouraie M, Mohagheghi M, MousaviJarrahi A, Malekzadeh R, Donald M. Cancer occurrence in Iran in 2002, an international perspective. Asian Pac J Cancer Prev. 2005;6(3):359-63.

6. Yarhusseini A, Sharifzadeh L, Delpisheh A, Veisani Y, Sayehmiri F, Sayehmir K. Survival Rate of Esophageal Carcinoma in Iran – A Systematic Review and Meta-analysis. Iran J Cancer Prev. 2014;7(2):61-5.

7. Li JY, Ma X, Zhang CB. Overexpression of long non-coding RNA UCA1 predicts a poor prognosis in patients with esophageal squamous cell carcinoma. Int J Clin Exp Pathol. 2014;7(11):7938-7944.

8. Gibb EA, Brown CJ Lam WL. The functional role of long non-coding RNA in [DOI:10.1186/1476-4598-10-38].

9. Huttenhofer A, Schattner P, Polacek N. Non-coding RNAs: hope or hype?. Trends in Genetics. 2005;21(5):289-297. [DOI:10.1016/j.tig.2005.03.007].

10. Inamura K. Major Tumor Suppressor and Oncogenic Non-Coding RNAs: Clinical Relevance in Lung Cancer. Cells. 2017;6(2):12-27. [DOI:10.3390/cells6020012].

11. Lv XB, Lian GY, Wang HR, Song E, Yao H, Wang MH. Long noncoding RNA HOTAIR is a prognostic marker for esophageal squamous cell carcinoma progression and survival. Plos One. 2013;8(5):e63516. [DOI:10.1371/journal.pone.0063516]

12. Hu L, Wu Y, Tan D, Meng H, Wang K, Bai Y, et al. Up-regulation of long noncoding RNA MALAT1 contributes to proliferation and metastasis in esophageal squamous cell carcinoma. J. Experimental & Clinical Cancer Research. 2015;34(7):1-12. [DOI:10.1186/s13046-015-0123-z]

13. Xu Y, Wang J, Qiu M, Xu L, Li M, Jiang F, et al. Upregulation of the long noncoding RNA TUG1 promotes proliferation and migration of esophageal squamous cell carcinoma. J. Tumor Biol. 2015;36(3):1643–1651. [DOI:10.1007/s13277-014-2763-6]

14. Tong YS, Wang XW, Zhou XL, Liu ZH, Yang TX, Shi WH, et al. Identification of the long non-coding RNA POU3F3 in plasma as a novel biomarker for diagnosis of esophageal squamous cell carcinoma. J. Molecular Cancer 2015;14(3):1-13. [DOI:10.1186/1476-4598-14-3]

15. Li W, Zheng J, Deng J, You Y, Wu H, Li N, et al. Increased levels of the long intergenic non-

[DOI: 10.29252/jcbr.2.4.48]

protein coding RNA POU3F3 promote DNA methylation in esophageal squamous cell carcinoma cells. Gastroenterology. 2014;146(7):1714-1726. [DOI:10.1053/j.gastro.2014.03.002]

16. Shan TD, Xu JH, Yu T, Li JY, Zhao LN, Ouyang H, et al. Knockdown of linc-POU3F3 suppresses the proliferation, apoptosis, and migration resistance of colorectal cancer. Oncotarget. 2015;7(1):961-975.

17. Guo H, Wu L, Yang Q, Ye M, Zhu X. Functional linc-POU3F3 is overexpressed and contributes to tumorigenesis in glioma. J Gene. 2015;554(1):114-119. [DOI:10.1016/j.gene.2014.10.038]

18. Xiong G, Yang L, Chen Y, Fan Z. Linc-POU3F3 promotes cell proliferation in gastric cancer via increasing T-reg distribution. Am J Transl Res. 2015;7(11):2262-2269.

[DOI: 10.29252/jcbr.2.4.48]