

Expression Level of lncRNA CYTOR in Iranian Cervical Cancer Patients

Ali Rajabi¹, Narges Dastmalchi¹, Neda Shokri¹, Samaneh Tayefeh-Gholami¹,
Seyyed Mohammad Yaghoubi², Reza Safaralizadeh*¹

Abstract

Background: A critical role has been known for lncRNAs in the initiation and development of cancers. Therefore, lncRNAs have been reported as the possible biomarkers in relation to the diagnosis and therapy of malignancies. This project examined the change in CYTOR lncRNA expression in human cervical cancer samples as compared with adjacent healthy ones.

Methods: We provided one hundred fifteen pairs of tumorous and adjacent healthy tissue specimens of cervical cancer patients. RNAs were isolated from tissue specimens and cDNAs were synthesized. We considered quantitative Real-time PCR (qRT-PCR) to examine the expression levels of CYTOR lncRNA. In addition, the biomarker activity of CYTOR and the associations between the lncRNA and clinicopathological characteristics were evaluated.

Results: The significant increased expression of CYTOR was obtained in cancerous samples as compared with non-cancerous ones ($P < 0.0001$). A significant correlation was indicated between CYTOR expression and the squamous subtype of cervical cancer ($p = 0.046$). The receiver operating characteristic (ROC) curve-related AUC (area under the curve), specificity, and sensitivity were calculated 0.88, 81.74%, and 80%, respectively, which may introduce CYTOR as a potential biomarker.

Conclusions: CYTOR may be an effective oncogene and biomarker in cervical cancer cases given its increased expression in human cervical cancer tissues.

Keywords: Biomarker, Cervical cancer, CYTOR, qRT-PCR, lncRNA.

Introduction

Cervical carcinoma was introduced as the third noticeable cause of malignancy-associated mortality among women worldwide (1). Despite the great development of diagnostic and therapeutic methods, such as screening, and chemotherapy for cervical carcinoma, significant long-term survival was not detected in the cases (2, 3). The decreased prognostic values of cervical carcinoma cases resulted in the detection of the carcinoma in the last stages. The aggressiveness of cervical carcinoma cells to other tissues is the most effective factor that contributes to the progression of cervical carcinoma (4, 5). Therefore, there is a vital need to assess the

molecular mechanisms of cervical carcinoma initiation and development and introduce more effective therapeutic approaches for cervical carcinoma cases (6).

Recognizing the effective biomarkers with diagnostic and prognostic values is essential for cervical cancer (7, 8). Long intergenic non-coding RNA (lincRNA) groups include more than 200 nucleotide RNAs which do not encode proteins (9). LincRNAs have been found to contribute to different cellular and molecular activities (10, 11), such as effective regulation of cellular processes, including splicing, and gene expression (11, 12). Numerous investigations showed that

1: Department of Animal Biology, Faculty of Natural Sciences, University of Tabriz, Tabriz, Iran.

2: Department of Biology, Faculty of Natural Sciences, University of Tabriz, Tabriz, Iran.

*Corresponding author: Reza Safaralizadeh; Tel: +98 9125140527; E-mail: Safaralizadeh@tabrizu.ac.ir.

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lincRNAs are frequently deregulated and play vital roles in different malignancies (13-15). LincRNAs may be effective biomarkers in relation to diagnosis and therapy of different cancers, such as cervical cancer (15-17). LincRNAs have been indicated within various signaling pathways essential for malignancies, and control numerous oncogenes and tumor suppressors in different malignancies, such as cervical cancer (18-21).

Several studies examined the involvement of lincRNAs in Iranian cervical carcinoma cases. Naghashi et al. evaluated the dysregulation of Cervical Cancer High-Expressed lncRNA 1 (CCHE1) in 50 cervical carcinoma cases. They showed that CCHE1 was noticeably overexpressed in Iranian cervical carcinoma cases (22). Jannat Alipoor et al. assessed 40 Iranian cervical carcinoma specimens and demonstrated the significant increased expression of MIAT, a well-characterized disease-related lncRNA, in cervical cancerous tissues. They indicated that downregulation of MIAT reduced cell viability and resulted in G1 phase accumulation in cervical carcinoma cells by controlling Cyclin D1 and Proliferating cell nuclear antigen (PCNA) (PCNA) (23).

lncRNA cytoskeleton regulator RNA (CYTOR), Gene ID: 112597, lncRNA was synthesized from the intronic position of E3 ubiquitin-protein transferase RMND5A protein coding sequence (24). It has been reported that CYTOR may participate in the progression of some cancers via controlling numerous biological pathways, including proliferation and apoptosis (25-28). The overexpression and amplification of the CYTOR gene on chromosome 2 were commonly detected in some cancers. Recent projects have also demonstrated the upregulation of CYTOR in cervical cancer (29). However, the clinical functions and biomarker roles of CYTOR in cervical cancer have not been clearly identified.

In the present study, we examined the hypothesis that increased CYTOR expression correlates with cervical carcinoma progression. Considering that the lncRNAs are considered as

biomarkers with crucial contribution to the diagnosis, prognosis, and therapy of cervical carcinoma, was aimed in this study to estimating the expression levels, and clinical importance of lncRNA CYTOR, as well as detection of biomarker in malignant tissue samples of individuals with cervical cancer.

Materials and Methods

Sampling

In this project, 115 pairs of tumorous and adjacent non-tumorous cervix tissues from women with mean age of 51 ± 10.16 attended to the international hospital of Tabriz, Iran were obtained. The vials containing tissue samples were stored in liquid nitrogen until RNA extraction. A pathologist confirmed the pathological features of the samples. All of the patients signed informed consent voluntarily. The patients' pathological features are described in Table 1.

RNA extraction

TRIZOL-mediated total RNA extraction was performed on both tumoral and normal cervix tissue samples using the guidelines of GeneAll Company (Korea). The quality of the RNAs was estimated by calculation the ratio of absorbance at 260 and 280 nm (260/280) by using Spectrophotometer (TermoFisher). The RNAs were kept at -196°C until conversion to cDNA.

Converting the RNA to cDNA

The Takara kit for converting the RNAs to cDNAs in accordance with the instructions (TaKaRa, Japan). After examining the quality of the extracted RNAs, the treatment with DNase I was performed for removing the DNAs. Each reaction comprised $0.5\ \mu\text{l}$ DNase, $0.5\ \mu\text{l}$ DNase I 10x buffer, and $4\ \mu\text{l}$ RNA. The reaction vials were then kept at 37°C for 30 min. After that, DNase I was deactivated by adding EDTA to the reaction vials followed by incubation at 65°C for 10 min. After adding $3.5\ \mu\text{l}$ Master Mix, the vials were kept at 37°C and 85°C , respectively for 60 min.

qRT-PCR

The primers used were displayed as follows:

CYTOR forward: 5' -
AGAATGAAGGCTGAGGTGTG-3', and
CYTOR reverse: 5'-
CAGCGACCATCCAGTCATTTA-3' and for
 β -actin forward: 5'-
AGAGCTACGAGCTGCCTGAC-3' and β -
actin reverse: 5'-
AGCACTGTGTTGGCGTACAG-3'

The qRT-PCR assay was carried out by Amplicon SYBR Green Mix (Denmark) on Roche light Cyclor qRT-PCR system (USA). Final volume (15 μ l) in each vial contained Master Mix (7 μ l), 0.5 μ l of each forward and reverse primer (5 pmol), and ddH₂O (6.3 μ l), and cDNA (1 μ l). The qRT-PCR program was as follows: Step 1: 94 °C for 15 min, Step 2: 40 cycles of 94 °C for 30 sec, 61 °C for 30 sec, and 72 °C for 30 sec, and final step: 72 °C for 4 min. Each reaction was repeated in a triplicate manner. β -actin was considered for normalization of the CYTOR expression.

Statistical analyses

The values of $2^{-\Delta C_t}$ were estimated to evaluate the CYTOR expression in tissue specimens using qRT-PCR-determined Cts. The data assessment was then carried out using Student's t-test in GraphPad Prism 7 program. The Mann-Whitney test in SPSS and ANOVA test in GraphPad Prism 7 were used to estimate the correlation between the CYTOR and clinicopathological characteristics. In addition, to examine the biomarker activity of CYTOR in cancerous cases, the ROC curve test was used to estimate the sensitivity (%), specificity (%), and cutoff via GraphPad Prism 7. In the analyses, the *p* values less than 0.05 were considered as statistically significant.

Results

The results showed an increased level of CYTOR (Fig. 1) in cervical cancer tissue specimens as compared with adjacent non-tumorous ones ($P < 0.0001$).

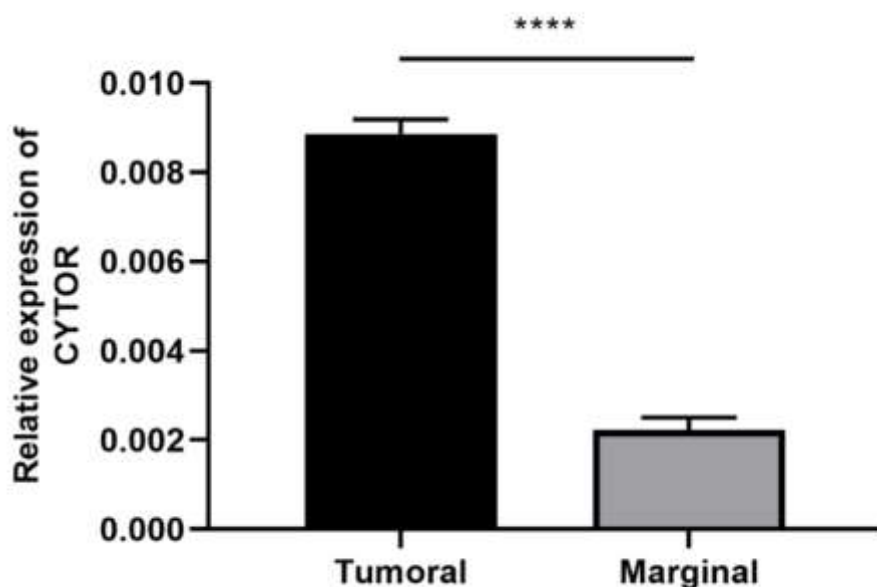


Fig. 1. The overexpression of CYTOR in cervical cancer tumor samples as compared with adjacent non-tumorous samples ($P < 0.0001$).

The correlation of increased expression of CYTOR with clinicopathological characteristics, including age, tumor size, differentiation, stage, histology, depth of cervical invasion, herpes virus (HPV) infection, and lymph node involvement in

cervical cancer individuals were calculated. The upregulation of CYTOR expression was significantly correlated with the squamous subtype in comparison with the adenocarcinoma subtype of cervical cancer samples ($P = 0.046$) (Table 1).

Table 1. Correlation between CYTOR expression levels and clinicopathological characteristics of cervical carcinoma cases.

Variable	Number of patients	Expression (mean ± SD) ×10 ⁻³ (Tumor sample)	Expression (mean ± SD) ×10 ⁻³ (Marginal sample)	P value	Significance
Age (year)					
≤ 50	57	7.1±2.1	3.7±1.3	0.091	NS
>50	58	6.4±2	3.5±1.3		
Histology					
Squamous	83	7±2.2	3.5±1.2	0.046	Sig
Adenocarcinoma	32	6.1±1.8	4±1.8		
Lymph node involvement					
Yes	86	6.8±2.2	3.6±1.4	0.08	NS
No	29	6.6±2	3.6±1.2		
Stage					
I	49	6.9±2.1	3.6±1.3	0.09	NS
II	38	6.9±2.1	3.6±1.3		
III	21	6.2±2	3.7±1.3		
IV	7	6.2 ±2	3.7±1.3		
Size (cm)					
≤ 5	58	6.9±2.1	3.7±1.3	0.4	NS
> 5	57	6.6±2.1	3.5±1.3		
Differentiation					
Poor	67	6.6±2.1	3.6±1.2	0.66	NS
Moderate	12	6.5±1.8	4.1±1.5		
Well	36	7±2.2	3.5±1.5		
Depth of cervical invasion					
≥2/3	87	6.5±2	3.5±1.3	0.072	NS
<2/3	28	7.4±2.3	4±1.3		
HPV					
Positive	71	6.7±2.1	3.5±1.3	0.78	NS
Negative	44	6.7±2.2	3.7±1.3		

NS; non-significant. Sig; significant.

NS: non-significant, Sig: significant.

A receiver operating characteristic curve (ROC curve) analyses demonstrated that the CYTOR is having a great potential to be

examined as a new biomarker for better diagnosis of patients with cervical cancer (Fig. 2, Table 2).

Table 2. The ROC curve-associated statistical values for CYTOR biomarker activity in cervical carcinoma cases. The ROC curve data demonstrates the CYTOR with great potential to be a new biomarker for cervical cancer.

ROC curve data	Values
	CYTOR
The area under the ROC curve (AUC)	0.88
Sensitivity (%)	78.26
Specificity (%)	93.91
Cutoff score	<0.004975
Standard error	0.022
95% confidence interval	0.8393 to 0.9272
p value	<0.0001
The sum of marginal specimens	115
The sum of tumor specimens	115

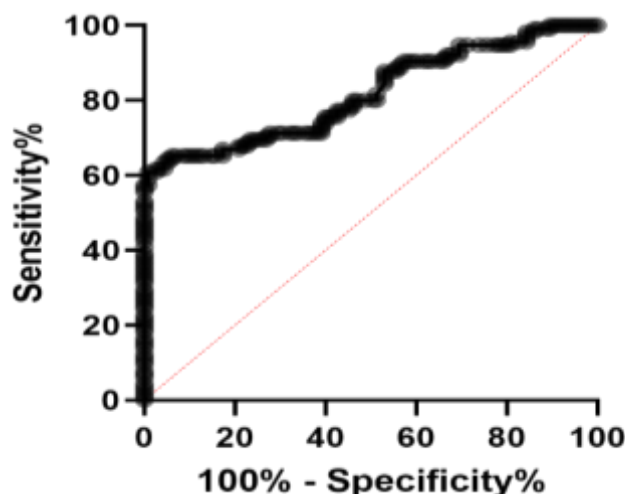


Fig. 2. The CYTOR-related ROC curve; (AUC=0.88, specificity=81.74%, sensitivity=80%, the cutoff score= <0.004975 , and 95%CI=0.9399 to 0.9844).

Discussion

The acquired information from the qRT-PCR confirmed that the increased expression of CYTOR lncRNA in cervical carcinoma tissues as compared with healthy cervix specimens. This may verify the oncogenic role of CYTOR in the patients. The results revealed the significant relationship between CYTOR upregulation and squamous subtype in cervical cancer samples. These observations show that CYTOR upregulation may result in developed cervical carcinoma.

The expression of CYTOR in Iranian cancer patients has not completely been assessed in previous studies. Moradi et al. revealed the noticeable upregulation of the CYTOR expression and indicated CYTOR as a novel possible biomarker for breast carcinoma diagnosis (30). Several projects have shown the activity of CYTOR during tumorigenic processes and the induction of malignant tumors (31, 32). A project evaluating colorectal carcinoma indicated that the CYTOR with an oncogenic activity may stimulate development of colorectal carcinoma invasiveness both *in vitro* and *in vivo* (26). Liu et al. showed an essential involvement of CYTOR in promoting tamoxifen insensitivity of breast carcinoma cells (27). In addition, it has been reported that CYTOR upregulation was considerably correlated with the aggressiveness, lymph node invasion, and higher stages of gastric carcinoma (28).

The analyses of results showed a noticeable biomarker activity for CYTOR in cervical carcinoma, therefore CYTOR might be an excellent factor in diagnosis and prognosis of cervical carcinoma cases. However, evaluation of numerous cervical carcinoma cases would be more valuable. Moreover, it would be valuable to show the molecular targets of CYTOR in malignancies, such as different subtypes of cervical carcinoma. The limitation of this investigation is the lack of experiments on the essential molecular contribution of CYTOR in cervical carcinoma. Therefore, the next phase in indicating the activity of CYTOR in cervical carcinoma is estimating the molecular functions of this lncRNA and its impacts on cell-related processes, including apoptosis, autophagy, migration etc.

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Conflicts of Interest

None.

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