Up Regulation of LINC00473 Promotes Cervical Cancer Progression and Predicts Poor Prognosis

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Research

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Abstract

Objective Long noncoding RNAs imbalance exert momentous effect in cancer. LINC00473 can accelerate the extensive tumors proceeding. Nevertheless, LINC00473 effects in cervical cancer has less reported. This study focused on estimate the LINC00473 effects in the developing process of cervical cancer and its unknown molecular biological regulation mechanism.

Methods qRT-PCR was applied for not only measuring LINC00473 expression but also c-myc, cyclinD1 and β -catenin mRNA expression. Both cell count and transwell methods were utilized for determining cell proliferation and invasion. Western blot was employed to estimate c-myc, cyclinD1 and β -catenin protein expression.

Results LINC00473 high-expressed was showed in organizations and cells. The LINC00473 gene knockout muffled the cells development, while LINC00473 overexpressed occurred opposite trend. Besides, its knockout dramatically inhibited c-myc, cyclinD1 and β -catenin expression. β -catenin overexpression could rescue restrain effect of LINC00473 low-expression on cells progress.

Conclusion The LINC00473 high-expression may promote cervical cells progress through adjusting Wnt/ β -catenin pathway.

1. Foreword

Cervical cancer, as serious gynecological malignancy, with has high metastasis and recurrence. it is a wonder that cervical cancer presents a younger trend ^[1]. About 85% of cervical cancer patients occur in developing countries ^[2]. HPV infection is considered to be major elements of cervical cancer. Yet, the pathogenesis of it is still blurry. Hence, in-depth understanding of the prevention and clinical treatment of cervical cancer is of great sense to raise the survival rate of patients.

Multiple evidences showed that numerous noncoding RNAs are preternaturally expressed in assorted cancers ^[3-9]. LncRNAs have become candidates for cancer biomarkers and therapeutic targets ^[8-12]. LncRNAs, as precursors of small RNAs with more than 200 nucleotides, regulate epigenetic traits and cell differentiation ^[13-19]. Many LncRNAs exert the role of vital anti-cancer genes or pro-cancer genes effects in terms of different types of tumor cells, including the cells in cervical cancer ^[20-26]. LINC00473 promotes the pancreatic cancer and prostate cancer progress ^[27-29]. Its abnormal expression is an important factor in tumor development. Nevertheless, there is no report about LINC00473 effects in cervical cancer.

Wnt signaling pathway regulates cell development through adjusting membrane protein receptor ^[30]. Some people have observed that wnt pathway is positively correlated with tumor development ^[31, 33]. Our crew studied LINC00473 may accelerate cervical cells progress through adjusting wnt pathway.

2. Methods

2.1 Antibodies and materials

Trizol was supplied by Jiangsu enmoasai Biotechnology Co., Ltd. Reverse transcriptase kit was supplied by Shanghai Future Industrial Co., Ltd. Rabbit monoclonal antibodies c-myc (1:1000), cyclin D1 (1:500), β -catenin (1:1500) and β -actin (1:1000) were supplied by Wuhan youliante Biotechnology Co., Ltd.

2.2 Patients

Cervical cancer 50 patients were confirmed in my hospital from October 2017 to September 2019. Tissue were kept at -80 °C for further experiments. All participants received written informed consent.

2.3 Cell culture

Five cells were supplied by Baienwei Biotechnology Co., Ltd. Substrate 1640 with 10% FBS (Shanghai chuanqiu Biotechnology Co., Ltd) as well as 1% penicillin-streptomycin (Shaanxi maiyuan Biotechnology Co., Ltd) from Roswell Park Memorial Institute (RPMI) was adopted to cultivate all cellular systems mentioned above. All the cells grew under 37 °C in cultivation box with 5% CO₂ for humidifying.

2.4 qRT-PCR Analysis

Our members applied Trizol to distill total RNA from organizations and cells. 5cDNAg was taken to be reversely transcribed to cDNA under the guide of qRT-PCR kits. The 2- $\Delta\Delta$ Ct approach was adopted to calculate fold changes. PCR primers are as follows: LINC00473: F: AGA GAG GTC TGA GTC CGA AGT; R: CAA AAG GCG AGT GTG TGG. C-Myc: F: GAT TCT CTG CTC TCC TCG AC; R: TCC AGA CTC TGA CCT TTT GC. Cyclin D1: F: CCG AGA AGC TGT GCA TCT AC AC; R: CCG AGT TGG AAC CAC GAC GC. β -catenin: F: AAC AGG GTC TGG GAC ATT AG TC; R: CGA AAG CCA ATC AAA CAC AA AC. β -actin: F: TAC AAC TCC TTG CAG CTC C; R: ATC TTC ATG AGG TAG TCA GTC. The experiments were repeated three times.

2.5 Cell counting experiments

Those cells on the exponential phase and their suspension were placed in environment of 37 °C and 5% CO₂. Our team added trypan blue into cell upper fluid. The living and dead cells were counted by counting plate.

2.6 Cell invasion assay

Our crew added Matt Riegel gel (100 μ l) into serum-free culture medium prepared with ice. The whole system was added Transwell tiny chamber under 37 °C, conserving for 30 min. 100 μ l cellular suspension and 200 μ l no-serum culture fluid were added to upper strata. Transwell sublayer and complete culture medium with 500 μ l 0.05% fetal bovine serum were incubated together for two days with 5% CO₂. Our crew fixed and dried cells that migrated under membrane surfaces as well as dyed cells with crystal violet. Cellular numbers were counted under the microscope.

2.7 Statistical analysis

Statistical analyses were accomplished making use of GraphPad Prism 7.0. data was expressed mean $\pm$ standard deviation and was analyzed by t-test between different groups. $P < 0.05$, statistically significant.

3. Outcomes

3.1 LINC00473 over-expression affected poor TNM staging and lymph node metastasis

Our crew detected LINC00473 expression in organizations and cells. The results showed that more LINC00473 expressed not only in organizations but also in cells (Fig. 1A-1B). See Table 1 for more details. Besides, LINC00473 high-expression was associated with TNM staging difference and lymph shift ($P < 0.05$).

Table 1
LINC00473 expression and the clinical peculiarity relevance.

Kind	LINC00473 expression			<i>P</i>
	Patients	High(%)	Low(%)	
Age				0.568
≤ 60	22	10	12	
> 60	28	15	13	
Gender				0.564
Male	30	16	14	
Female	20	9	11	
Differentiation				0.215
Well	5	3	2	
Moderate	30	12	18	
Poor	15	10	5	
Lymphatic metastasis				0.005*
Yes	26	18	8	
No	24	7	17	
Clinical period				0.004*
I-II	20	5	15	
III-IV	30	20	10	
Note: * represented statistically significant.				

3.2 LINC00473 high-expression implied bad prognosis

Our crew discovered that the holistic exist time of LINC00473 more-expression was shorter than LINC00473 less-expression ($P < 0.05$). See Fig. 2A for more details. Later, Our crew found that the LINC00473 high-expression could be used as an auxiliary factor to predict the prognosis of cervical cancer (Fig. 2B). Bioinformatics further confirmed the relationship between LINC00473 expression and tumor phase and lymph shift (Fig. 2C-2E).

3.3 LINC00473 down-regulation controlled cells progress

Figure 3A discovered that siRNA transfection effectively reduced LINC00473 expression. Cell counting methods detected that C-33A and ME-180 cells grew slowly in the situation of LINC00473 was down-regulated (Fig. 3B). Besides, LINC00473 low-expression lessened cellular invasion ability (Fig. 3C).

3.4 LINC00473 over-regulation accelerated cells progress

Figure 4A analysis discovered that LINC00473 over-expression substantially raised LINC00473 expression. On the contrary, LINC00473 over-expression facilitated cellular proliferation and invasion ability (Fig. 4B-4D).

3.5 LINC00473 positive regulation wnt/ β -catenin pathway

Figure 5A-5D displayed that LINC00473 low-expression prominently down-regulated β -catenin, cyclinD1 as well as c-myc mRNA/protein levels while its overexpression reversed the trend.

3.6 β -catenin repair reversed siLINC00473 effects

Our crew removed LINC00473 and supplemented the β -catenin expression in C-33A cells (Fig. 6A). Surprisingly, supplemented β -catenin reversed the effects. Later, LINC00473 over-expression saved cell progress induced through LINC00473 gene removed (Fig. 6B-6D).

4. Discussion

Cervical carcinoma was still regarded as the most common malignant cancers bothering women. Because of its poor prognosis, it is momentous to talk over cervical cancer mechanism. Some specific miRNAs can be used as molecular markers for the diagnosis of cervical cancer^[34–36]. MALAT1 less-expression in CaSki cells substantially weakened cell movement ability^[37]. The research showed that the XLOC_010588 over-expression restrains the proliferation of HeLa as well as SiHa^[38–40]. It was concluded that lncRNAs expression is tissue-specific and individual specific attributing to abundant target genes^[41–44]. Extensive lncRNAs effects in cervical cancer is blurry.

We discovered more LINC00473 in organizations and cells. Besides, LINC00473 high-expression had a bearing on lymph transfer. Bioinformatics and ROC analysis further confirmed the above results. LINC00473 low-expression halted NSCLC cellular migrating ability as well as viability.

Mutations of wnt pathway components boost tumor proceeding when they lead to excessive activation or imbalance^[37]. Some lncRNAs inhibit tumor by regulating wnt related proteins^[38]. LINC00473, β -catenin, cyclinD1 as well as c-myc mRNA and protein expression levels lessen confirmed this hypothesis while LINC00473 over-expression reversed this effect. In addition, LINC00473 knockdown markedly lessened the β -catenin mRNA/protein level. Surprisingly, β -catenin repair reversed the effect of LINC00473, indicating LINC00473 effects was rely on wnt/ β -catenin pathway.

5. Conclusion

we reported that more LINC00473 expressed in organizations and cells. The LINC00473 low-expression could control cervical cells proceeding. It also lessened β -catenin, cyclinD1 as well as c-myc mRNA/protein expression. Besides, β -catenin overexpression could relieve the inhibition triggered by LINC00473 knockdown. LINC00473 raised the movement ability of cervical cells through adjusting wnt/ β -catenin pathway, implying that LINC00473 treated as a cervical cancer fresh target.

6. Abbreviation

Not applicable.

7. Declarations

Funding

Not applicable

Ethics statement

The experiment was often approved by the Ethics Committee of the First Affiliated Hospital of Nanhua University No. 336, and all patients participating in this study provided written informed consent in accordance with the "Helsinki Declaration".

Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article

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Authors' contributions

Xi Zhou conceived and designed the study. Xia Zhao and Yan Ma analyzed the data. Zhouyi Wu contributed to literature review. Xi Zhou and Heng Li wrote the manuscript. Xi Zhou and Heng Li reviewed and edited the manuscript. All authors read and approved the final manuscript.

Acknowledgments

Not applicable.

8. References

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Figures

Figure 1

LINC00473 was highly expressed in cervical cancer. (A) qRT-PCR was utilized for determining LINC00473 expression in organizations. (B) Our crew determined LINC00473 expression in cells. * P < 0.05.

Figure 2

LINC00473 high-expression implied bad prognosis in cervical cancer. (A) LINC00473 overall survival curve. (B) LINC00473 data in survival and death of cervical cancer. (C) LINC00473 expression level during different T-scores. (D) LINC00473 expression level in different N stages. (E) LINC00473 expression in different TNM stages.

Figure 3

LINC00473 low-expression restrained movement ability of cervical cancer cells. (A) qRT-PCR was utilized for determining LINC00473 expression by transfected siNC or siLINC00473. (B) Proliferation curve of transfected siNC or siLINC00473. (C-D) Invasiveness of transfected siNC or siLINC00473. * $P < 0.05$.

Figure 4

LINC00473 high-expression raised movement ability of cervical cancer cells. (A) qRT-PCR was utilized for determining LINC00473 expression by transfected EV or pCDNA3-LINC00473. (B) Proliferation curve of transfected EV or pCDNA3-LINC00473. (C-D) Invasiveness of transfected EV or pCDNA3-LINC00473. * $P < 0.05$.

Figure 5

LINC00473 positive regulation wnt/β-catenin pathway. (A-D) qRT-PCR and WB were adopted to detect β-catenin, cyclinD1 as well as c-myc mRNA/protein levels by transfected siNC or siLINC00473 and EV or pCDNA3 LINC00473. * $P < 0.05$.

Figure 6

The repair of β -catenin abolished the LINC00473 effect gene knockdown. (A) qRT-PCR was applied for measuring β -catenin expression. (B-D) Cells proceeding was detected through proliferation curve and transwell. * $P < 0.05$