Research Paper

Expression and Clinical Significance of Long Non-Coding Ribonucleic Acid LOC554202 and H19 in Serum of Cervical Cancer

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To explore the serum expression of long non-coding ribonucleic acid H19 and LOC554202 in cervical cancer as well as the clinical significance is the main objective. Serum samples were collected from 52 preoperative cervical cancer patients, at the same time, the serum samples were also collected from 39 age-matched women admitted due to gynecological benign diseases with cervical cancer screening negative were selected as the control group. Among them, 10 cervical cancer patients were used for preoperative and postoperative comparisons. The serum long non-coding ribonucleic acid H19 and LOC554202 levels in the above specimens were detected through real-time quantitative reverse transcription-polymerase chain reaction and the cervical cancer patients before and after surgery were compared. Meanwhile, the relationships of serum long non-coding ribonucleic acid H19 and LOC554202 levels in cervical cancer patients with their clinical pathological parameters were also analyzed and the receiver operating characteristic curve was adopted to evaluate the diagnostic efficiency of serum long non-coding ribonucleic acid H19 and LOC554202 levels. The serum long non-coding ribonucleic acid H19 and LOC554202 levels in cervical cancer patients were apparently elevated compared with those in control group, those after surgery were markedly declined relative to those before surgery, and the difference was of statistical significance ($p<0.05$). The serum long non-coding ribonucleic acid H19 and LOC554202 levels in cervical cancer patients were not correlated with age, pathological type, clinical classification ($p<0.05$). The area under the receiver operating characteristic curve, sensitivity and specificity of serum long non-coding ribonucleic acid H19 in independently diagnosing cervical cancer were 0.661, 30.8 % and 94.9 %, respectively, while those of serum long non-coding ribonucleic acid LOC554202 in independently diagnosing cervical cancer were 0.707, 69.2 % and 66.7 %, respectively. Serum long non-coding ribonucleic acid H19 and LOC554202 can serve as the potential circulatory markers for the clinical diagnosis and postoperative monitoring of cervical cancer.

Key words: Cervical cancer, long non-coding ribonucleic acid, H19, LOC554202, reverse transcription-polymerase chain reaction

Long Non-Coding Ribonucleic Acids (lncRNAs) are a class of RNA molecules with the length of over 200 nucleotides, which are expressed in regulatory genes such as epigenetic, transcriptional and post-transcriptional genes in the manner of RNA, thus becoming a research hotspot in tumor[1,2]. Recent studies indicate that lncRNA H19[3,4] and LOC554202[5,6] are highly expressed in cancer tissues, which are promising to become the biomarkers in cervical cancer diagnosis. At present, few studies have investigated the expression of lncRNA H19 and LOC554202 in the peripheral blood of cervical cancer. This study aimed to detect the expression of lncRNA in cervical cancer through real-time quantitative Reverse Transcription-Polymerase Chain Reaction (qRT-PCR), compare the serum lncRNA H19 and LOC554202 levels in cervical cancer patients before and after surgery, and further analyze the relationships of serum lncRNA H19 and LOC554202 levels in cervical cancer patients with clinical pathological parameters, as well as the

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diagnostic values of these two in cervical cancer, so as to provide theoretical foundation for using serum lncRNA H19 and LOC554202 as the circulatory diagnostic markers of cervical cancer.

MATERIALS AND METHODS

Sample collection:

52 serum samples of inpatient cervical cancer patients were collected from January to October 2021 at our hospital as the case group, with the age ranging from 23-66 y and the median age of 48 y. The cervical cancer stage was classified according to the World Health Organization (WHO) criteria, as shown below, stage I (n=28), stage II (n=21), stage III (n=2) and stage IV (n=1). All the selected cases were pathologically confirmed and were naive to treatment before surgery. At the same time, 10 matched patients were selected and the serum specimens at 5-7 d after surgery were retained. In addition, the epidemiological data (including age, tumor and family history) and biochemical detection indexes (Human Papillomavirus (HPV) infection and subtype) of each patient were collected. Moreover, 39 patients with benign cervical disease but cervical cancer screening negative during the hospitalization period were selected at the same period. These patients did not receive any clinical treatment prior to blood collection and were aged 25-65 y, with the median age of 47 y. All sample collection in this study was approved by the hospital ethics committee upon patient informed consent.

Major reagents and equipment:

Serum RNA extraction kit (Qiagen 217184, Qiagen), PrimeScript™ RT reagent kit (RR037A), SYBR® Premix Ex Taq™ II (RR820A, Takara Bio), PCR primers (Shanghai, Sangon Biotech), fluorescence quantitative PCR machine (LightCycler 480 II, Roche, Switzerland) and multifunctional microplate reader (Synergy™ H1, BioTek, United States of America (USA)).

RNA extraction and qRT-PCR:

Total RNA was extracted from serum according to the serum RNA extraction kit instruction and the RNA concentration and purity were detected using the multifunctional microplate reader. The extracted RNA was reversely transcribed using PrimeScript™ RT reagent kit and SYBR® Premix Ex Taq™ II for qRT-PCR, with Glyceraldehyde 3-Phosphate Dehydrogenase (GAPDH) as the internal reference. H19 upstream primer: 5'-TGATGACGGGTGGAGGGGCTA-3', H19 downstream primer: 5'-TGATGTCGCCCTGTCTGCACG-3', LOC554202 upstream primer: 5'-GGCGGATGCAAGTTAATAAAAC-3', LOC554202 downstream primer: 5'-TACGCCTGAGTGTTCACGAG-3', GAPDH upstream primer: 5'-GCACCGTCAAGGCTGAGAAC-3', GAPDH downstream primer: 5'-TGGTGAAGACGCCAGTGGA-3'. The relative expression quantities of serum lncRNA H19 and LOC554202 were calculated according to the $2^{-\Delta\Delta Ct}$ method, among which ΔΔCt=cervical cancer (Ct target gene-Ct internal reference gene)-control group (Ct target gene-Ct internal reference gene).

Evaluation indexes:

Differences in serum lncRNA H19 and LOC554202 levels between cervical cancer patients and control group were compared. The serum lncRNA H19 and LOC554202 levels in cervical cancer patients after surgery were compared with those before surgery. The relationships of serum lncRNA H19 and LOC554202 levels in cervical cancer patients with their clinicopathological parameters (age, pathological type, clinical classification and Cancer Antigen 125 (CA125) level) were analyzed. The diagnostic efficiency of the Receiver Operating Characteristic (ROC) curve for serum lncRNA H19 and LOC554202 levels in cervical cancer was evaluated.

Statistical analysis:

The Statistical Package for the Social Sciences (SPSS) 21.0 software was used to process and analyze the data. The measurement data were expressed as ($\bar{x}\pm s$), comparison between two groups was carried out using the Mann-Whitney U test and the ratio was compared through chi-square test. The ROC curve was plotted based on the lncRNA H19 and LOC554202 levels, while the Youden index (sensitivity+specificity-1) was used to determine the threshold. A two-sided difference of $p<0.05$ was deemed as statistically significant.

RESULTS AND DISCUSSION

Serum expression levels of lncRNA H19 and LOC554202 in cervical cancer patients was explained in detail. The ΔCt value of serum lncRNA H19 in cervical cancer was 1.71±0.90, while that in control group was 2.27±0.85 (fig. 1A). The serum H19 expression level in cervical cancer calculated upon the $2^{-\Delta\Delta Ct}$ method was 2.01±1.30, which was markedly elevated compared

with that in control group (1.32±0.70) (fig. 1B) and the difference was statistically significant (p<0.01). The ΔCt value of serum LOC554202 in cervical cancer patients was 2.26±1.26, while that in control group was 3.36±1.45 (fig. 1C) and the serum LOC554202 expression level in cervical cancer group (2.76±1.87) was evidently elevated compared with that (1.51±1.24) in control group (fig. 1D), and the difference was statistically significant (p<0.01).

Comparisons of serum lncRNA H19 and LOC554202 levels in cervical cancer patients before and after surgery were shown below. 10 serum samples from cervical cancer patients collected at 5-7 d after surgery were collected to compare the serum lncRNA H19 and LOC554202 levels with those before surgery. When collecting the preoperative specimens, all patients have not received any kind of treatment. Our results suggested that, the serum H19 and LOC554202 expression levels in cervical cancer patients after surgery were notably reduced compared with those before surgery and the differences were of statistical significance (p<0.05, fig. 2).

The relationships of serum lncRNA H19 and LOC554202 levels in cervical cancer patients with the clinical data of patients were further analyzed. The results suggested that, the serum lncRNA H19 and LOC554202 levels in cervical cancer patients were not correlated with patient age, clinical classification and CA125 (p>0.05, Table 1).

Diagnostic value of serum lncRNA H19 and LOC554202 levels in cervical cancer were shown here. The ROC curves were plotted based on the serum lncRNA H19 and LOC554202 levels in cervical cancer patients and control group, while the Youden index (sensitivity+specificity-1) was used to determine the threshold (Table 2). The results indicated that, the Area Under the Curve (AUC) of serum lncRNA H19 was 0.661 (95 % Confidence Interval (CI): 0.549-0.773, p=0.009, fig. 3A). The AUC of serum lncRNA LOC554202 was 0.707 (95 % CI: 0.601-0.813, p=0.001, fig. 3B).

Cervical cancer is one of the most common malignant tumors in the world, which ranks the fourth place in terms of the cancer mortality worldwide[7,8]. Cervical cancer is a disease induced by multi-step and multi-factor, but the pathogenesis has not been completely illustrated at present. Therefore, it is of great clinical prospect to search for the novel targets for the early diagnosis, treatment and prognosis judgement of cervical cancer. As the non-coding genes, lncRNAs have no protein-encoding potential due to the lack of open reading frame; as a result, they are initially regarded as the "dark substance" during the transcription process and receive little attention[1,9]. However, a large number of recent studies suggest that[10-12], lncRNAs participate in various regulatory processes in the body, such as growth development, cell proliferation, differentiation and apoptosis; besides, they are closely correlated with multiple tumors, neurological disorders, infectious diseases, as well as cardiovascular and cerebrovascular diseases.

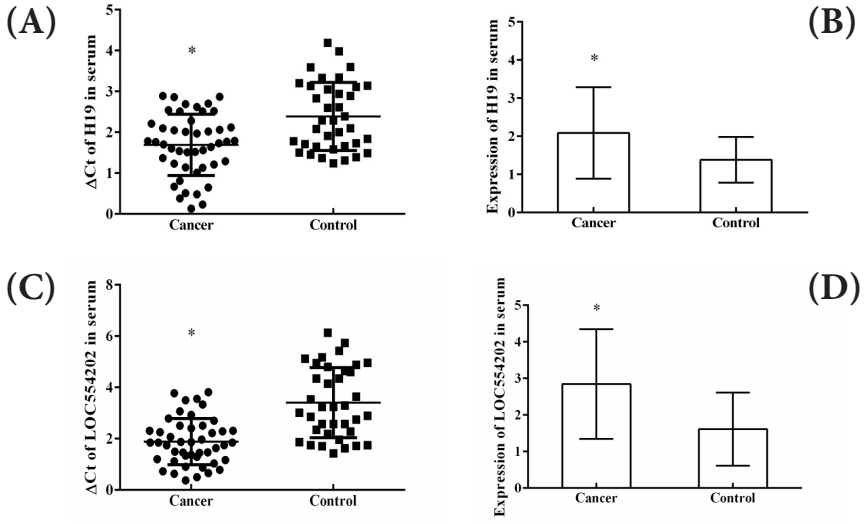

Fig. 1: Expression of lncRNA H19 and LOC554202 in serum of patients with cervical cancer, (A) ΔCt of lncRNA H19 in serum of patients with cervical cancer; (B) The relative expression of lncRNA H19 in serum of patients with cervical cancer; (C) ΔCt of lncRNA LOC554202 in the serum of patients with cervical cancer and (D) The relative expression of lncRNA LOC554202 in serum of patients with cervical cancer. Cancer groups: Cervical cancer group; control groups: Control group and *: The difference was statistically significant, p<0.05

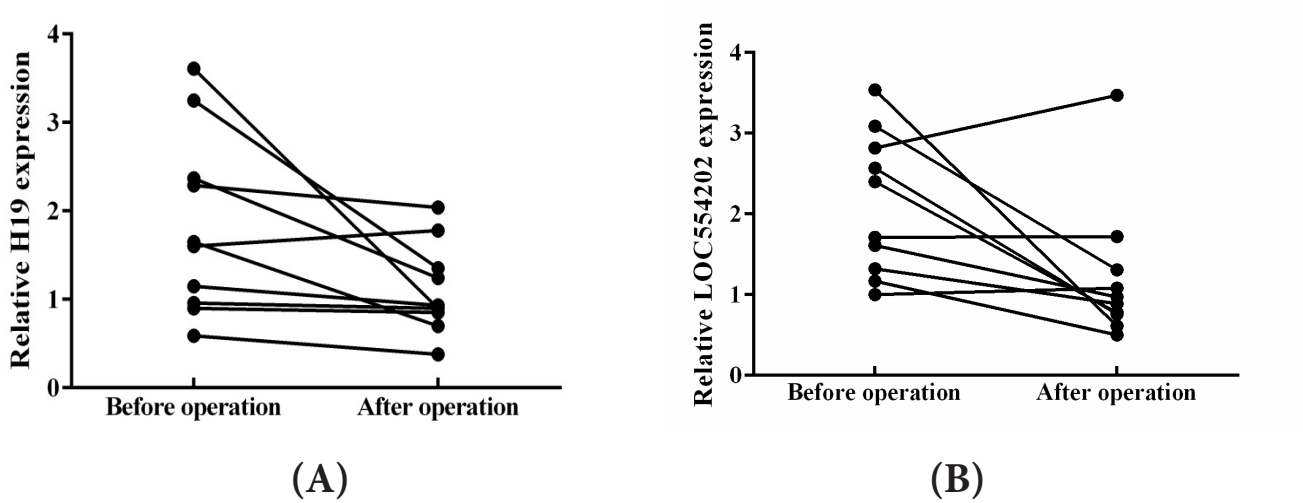

Fig. 2: Expression of lncRNA (A) H19 and (B) LOC554202 in patients with cervical cancer before and after operation

TABLE 1: CORRELATION OF SERUM lncRNA H19 AND LOC554202 WITH CLINICOPATHOLOGICAL PARAMETERS IN PATIENTS WITH CERVICAL CANCER ($\bar{x}\pm s$)

Pathological parameters	n	H19 expression level	p	LOC554202 expression level	p
Age (y)			0.212		0.870
≥48	31	2.19±1.27		2.58±1.69	
<48	21	1.73±1.32		2.66±2.10	
Clinical stages			0.927		0.696
I-II	49	2.01±1.28		2.79±1.84	
III-IV	3	1.93±1.90		2.35±2.67	
CA125 (U/ml)			0.504		0.894
≥35	31	2.11±1.34		2.73±1.73	
<35	21	1.86±1.25		2.80±2.10	

TABLE 2: EFFICACY ANALYSIS OF SERUM lncRNA H19 AND LOC554202 IN THE DIAGNOSIS OF CERVICAL CANCER

Index	AUC	p	95 % CI	Sensitivity (%)	Specificity (%)	Cut-off value
Serum lncRNA H19	0.661	0.009	0.549-0.773	30.8	94.9	2.334
Serum lncRNA LOC554202	0.707	0.001	0.601-0.813	69.2	66.7	1.705

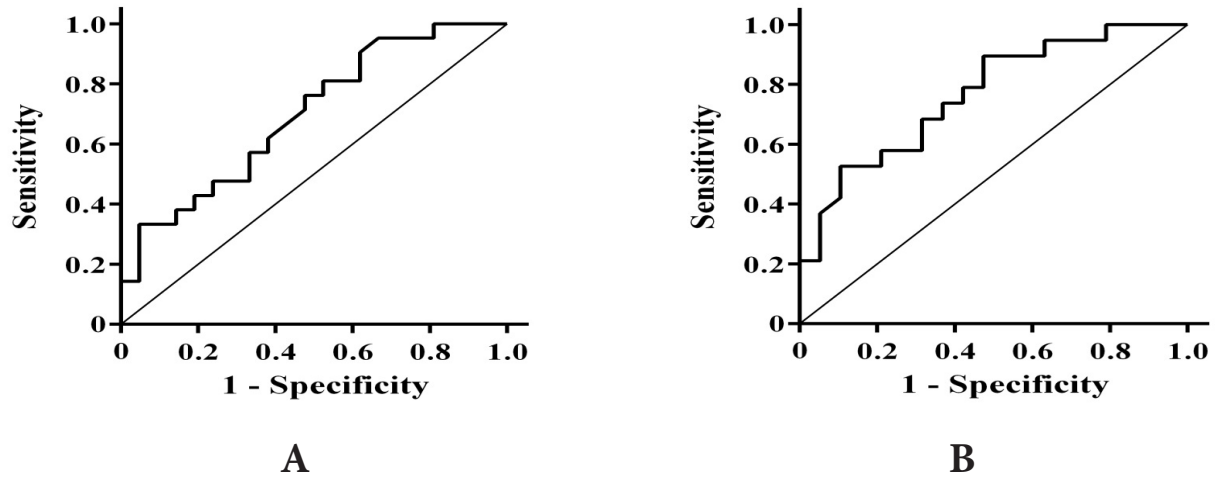

Fig. 3: ROC of serum lncRNA (A) H19 and (B) LOC554202 in patients with cervical cancer

H19 is the first lncRNA discovered to be related to tumor, which has dual functions of tumor-promoting and tumor-suppressing effect[13-15]. Besides, it is highly expressed at embryonic period and down-regulated after birth. Existing studies suggest that, the lncRNA H19 expression level in cervical cancer tissues is apparently higher than that in normal cervical tissues[16]. LOC554202 is discovered as the first lncRNA with transaction, some scholars discover that the expression level of LOC554202 in cervical cancer tissues is notably higher than that in corresponding para-carcinoma tissues ($p<0.001$) and the expression level is related to the clinical classification and lymph node metastasis of patients[5]. These results indicate that lncRNA H19 and LOC554202 are promising to become the biomarkers for diagnosing cervical cancer.

Existing studies discover that, some lncRNAs can stably exist in the serum (plasma) of multiple malignant tumors and that external environmental changes like temperature and pH have little influence on their stability[17]. A study has analyzed and compared the serum lncRNA Plasmacytoma Variant Translocation 1 (PVT1) expression levels among 88 cervical cancer patients, 64 precancerous lesion of cervical cancer and 111 healthy controls and discovers that serum lncRNA PVT1 is highly expressed in cervical cancer tissues, which is related to tumor size, clinical classification and lymph node metastasis[18]. Such results reveal that serum lncRNA PVT1 may serve as a novel non-invasive biomarker for the early diagnosis of cervical cancer. This finding indicates that serum lncRNAs can be used as the novel circulating diagnostic markers for cervical cancer.

So far, research on the circulatory lncRNA markers of cervical cancer remains at the starting stage. However, it is relatively convenient and minimally-invasive to detect the peripheral blood biomarkers. Therefore, searching for the new cervical cancer circulatory diagnostic markers is of great significance to the clinical diagnosis and treatment of cervical cancer. In this experiment, the serum levels of lncRNA H19 and LOC554202 in cervical cancer, which had not been reported in research, were selected for research. Our results suggested that, the serum levels of lncRNA H19 and LOC554202 in cervical cancer patients were markedly higher than those in control group, while those in cervical cancer patients after surgery were apparently reduced relative to those before surgery. These research results suggested that the serum levels of lncRNA H19 and LOC554202 were promising to become the potential circulatory biomarkers for the clinical diagnosis and postoperative monitoring of cervical cancer. The correlations of serum levels of lncRNA H19 and LOC554202 in cervical cancer patients with clinicopathological parameters were analyzed and the results indicated that the serum levels of lncRNA H19 and LOC554202 were not correlated with patient age, pathological type, stage and CA125. Such result might be related to the limited total sample size and small sample size in some groups, as well as great inter-group bias. Some studies suggest that lncRNAs are differentially expressed in multiple malignant tumors, which can serve as the novel circulatory diagnostic markers[14]. Therefore, this study had plotted the ROC curve based on the serum levels of lncRNA H19 and LOC554202 to evaluate the diagnostic efficiencies of these two for cervical cancer. The results revealed that the AUC of serum lncRNA H19 was 0.661 (95 % CI: 0.549-0.773, $p=0.009$), while that of serum lncRNA LOC554202 was 0.707 (95 % CI: 0.601-0.813, $p=0.001$) and these two had favorable diagnostic efficiencies for cervical cancer. Nonetheless, this was a single-center small sample size study, which was associated with limited clinical guiding significance. Consequently, multi-center combined experimental studies with large sample size should be carried out, so as to provide theoretical foundation for using serum lncRNA H19 and LOC554202 as the circulatory diagnostic markers for cervical cancer.

Conflict of interests:

The authors declared no conflict of interest.

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This article was originally published in a special issue, "Modern Applications in Biomedical Research and Pharmaceutical Sciences" Indian J Pharm Sci 2022:84(3) Spl Issue "247-252"