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Urine Lunx mRNA as a Potential Molecular Diagnostic Tool for Lung Cancer

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Abstract: Objective: Exploring the expression of cell-free RNA in urine of normal patients and lung cancer patients, analyze its clinical value in the dignosis of lung cancer. **Methods:** The urine samples were collected from lung cancer patients and healthy people. Extracted the total cf-RNA from all samples. The contents of total cf-RNA in two groups were detected, and the expressions of LUNX mRNA in two groups were measured by quantitative real-time PCR. Receiver-operating characteristics (ROC) curve was established to evaluate the diagnostic value of urine cf-RNA for the differentiation between lung cancer patients and the control group. **Results:** Compare to the normal group (50.50±4.527ng), the urinary cf-RNA content in patients (133.8±8.615ng) was significantly increased, $p<0.05$. The expression of urinary lunx mRNA level from patients was raised, $p<0.05$. ROC curve analysis showed that, in the diagnosis, AUC value of lunx mRNA was 0.83(95% CI, 0.747-0.906), $p<0.05$. **Conclusion:** The expression of urine cf-RNA and Lunx mRNA was elevated, and can be used as a potential molecular diagnostic tool for lung cancer.

Keywords: Urine, Lung Cancer, cf-RNA, Diagnosis

1. Introduction

Lung cancer is a prevalent malignant tumor of high incidence and mortality. It is one of the most dangerous diseases to human health and life. Due to the lack of convenient and efficient cancer screening methods and insufficient cognition of early diagnosis and treatment, the incidence and mortality rate of lung cancer in China are the highest among all cancers [1]. Therefore, it is very important to find convenient and specific biomarkers for early lung cancer [2]. In recent years, with the rise of precision medicine and liquid biopsy, the advantages of non-invasive detection in disease prevention and prognosis monitoring have been gradually recognized [3]. Urine detection is a truly non-invasive detection method. A research by Zhao shows that free RNA in urine is relatively stable, which means that free RNA in urine can be used as a potential biomarker for early discovering diseases[4].

2. Experimental material and method

2.1 Clinical urine samples

Urine samples from 58 lung cancer patients were collected in this study. All the patients were diagnosed in the Affiliated Hospital of Qingdao University. There are 36 males and 22 females, aged 35-76 with a median age of 56. And 35 cases in the control group, including 21 males and 14 females, aged 32-65 with a median age of 52.

2.2 Total urinary RNA extraction

All of the urine samples from patients and control groups were centrifuged at 5000r/min for 40 min at 4°C. Then collected the supernatant fluid and filtered it through a positively charged nylon membrane to absorb the nucleic acid onto the nylon membrane. Then the membrances were drying in a 56°C drying oven for 5 minutes. The nylon membrane was cut into small pieces and collected into 1.5ml Ep tube without RNA enzyme [5]. Put the tubes at -80°C. Total RNA in urine was adsorbed onto the membrances now. Then extracted them by using TRIzol reagent (Takara, China). The content of RNA was detected by a nanodrop, and the difference of total RNA between patients and controls should be analyzed.

2.3 Reverse transcription and real-time qPCR

The total RNA was transcribed into cDNA after the content detection (TransScript One-step gDNA Removal and cNA Synthesis SuperMix, China). Then amplified by fluorescent quantitative PCR (TransStart Tip Green qPCR SuperMix, China). The conditions were as follows: 94°C for 30s, 94°Cfor 5S, 60°C for 30s, 40 cycles. All samples were calculated as exponential of $2^{-\Delta\Delta Ct}$. The primer sequence are listed in table 1.

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Table 1. Primer sequence

gene symbol	forward primer	reverse primer
GAPDH	5'-AGAAGGCTGGGGCTCATTTG-3'	5'-AGGGGCCATCCACAGTCTTC-3'
Lunx	5'-AAGTCTGTTGAGGCTGGCTG-3'	5'-GCCAAGTCCAT CAAGCAGAG-3'

2.4 ROC curve analysis

ROC curve was established to evaluate the diagnostic value of urine cf-RNA for the differentiation between lung cancer patients and control group.

2.5 Statistical analysis

The Statistical analyses were performed with Prism 6.0 and MedCal 17 statistical software. All values were depicted as mean (±) standard deviation (SD) and are considered significant if $p < 0.05$.

3. Results

3.1 Urinary total RNA

The average amount of urinary total RNA from patients was 133.8±8.615 ng whereas it was 50.50±4.527 ng from the normal group. The total RNA amount of patients was increased ($P < 0.05$, Fig. 1).

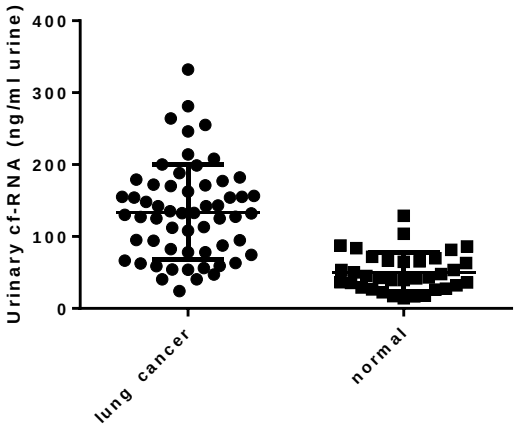

Figure 1. Urinary total RNA from lung cancer patients and normal people

3.2 qPCR results

Urinary lunx mRNA was amplified by qPCR. The expression level of urinary lunx mRNA from patients was increased, $p<0.05$.

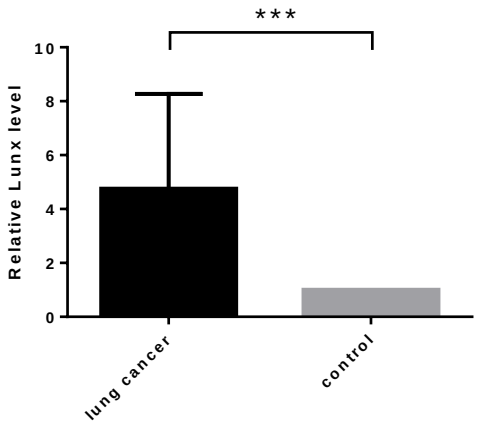

Figure 2. Expression levels of Lunx mRNA in urine samples of lung cancer and control group.
We measured the expression levels of Lunx mRNA from 58 patients and 35 normal people by qPCR, RNA levels were normalized to GAPDH。***：P＜0.001

3.3 The diagnostic value of urinary Lunx mRNA

To assess whether urinary Lunx mRNA expression can be used as a diagnostic tool for lung cancer, we use ROC curves for analysis (Fig. 4). The area under Lunx mRNA curve (AUC) was 0.838 (95% CI: 0.747-0.906), the Jordan index was 0.628, with a sensitivity of 91.38 and specificity of 71.43.

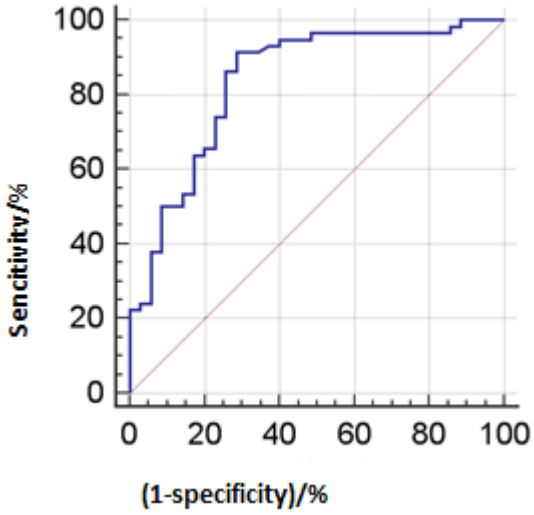

Figure 3. Receiver operating characteristic (ROC) curve analysis by Lunx mRNA

4. Discussion

Lunx mRNA, which encodes human lung-specific protein x (LUNX), is located at 20p11.1-q12. The gene is specifically expressed in lung tissue while other tissues did not. Recent years, there were reports of lunx mRNA expression in the blood too[6].

In this study, we analyzed the expression of the cf-RNA in urine, and found that the urinary total cf-RNA content and the urinary lunx mRNA expression level in the patients were both significantly increased. The ROC curve analysis showed that lunx mRNA had good sensitivity and sensitivity in the diagnosis of lung cancer and could be used as a potential diagnostic biomarker.

Lung cancer usually takes a long time from the onset of disease, clinical symptoms to diagnosis. Therefore, if the disease can be detected as early as possible and then treated, it can effectively improve the survival rate of lung cancer. At present, the study of liquid life is more focused on the free nucleic acid in the blood [7;8]. Because of the inherent stability regulation mechanism of the body, the early changes in the blood are easy to be removed, which is not conducive to detection [9]. There is no such mechanism in urine. Early changes are more sensitive and easy to capture [10]. Urine detection as a routine physical examination items, sampling is convenient and truly non-invasive testing, if the urine can be found in high-efficiency, sensitive biomarkers, it will be able to effectively detect early disease, thus early treatment, improve the survival rate. However, the content of free RNA in urine is low, and further research is needed to extract high quality RNA efficiently and concisely, as well as to screen molecular markers with high sensitivity and specificity.

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