A Novel Peripheral Whole Blood hsa_circ_0005430 is a Potential Biomarker for Lung Adenocarcinoma

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Research Article

Abstract

Background: This study was aimed to determine the circular RNA 5430(hsa_circ_0005430) in peripheral whole blood（PWB）from lung adenocarcinoma (LA) patients is a novel biomarker for screening LA.

Methods: The expression levels of hsa_circ_0005430 in PWB from 20 LA patients and 10 healthy controls were performed using real-time quantitative reverse transcription-polymerase chain reaction(RT-qPCR). Serum CEA, CA199 and CA50 were detected by chemiluminescent immunoassay. Spearman' correlation test was used to evaluate the correlation of hsa_circ_0005430 and clinical variables. The receiver operating characteristic (ROC) curve was established to evaluate its diagnostic value.

Results: PWB hsa_circ_0005430 was significantly elevated in LA patients, comparing with healthy controls. There was no significant difference in hsa_circ_0005430 level among different pathological factors of LA. In these LA patients, we found a significant correlation between PWB hsa_circ_0005430 and disease activity, including carcinoembryonic antigen(CEA), carbohydrate antigen 199(CA199) and carbohydrate antigen 50 (CA50). ROC analysis showed that PWB hsa_circ_0005430 had an area under the curve(AUC) of 0.75 (95% *CI* 0.567-0.928, *P* < 0.001) to discriminate LA patients from controls with sensitivity of 0.900 and specificity of 0.65. The highest Youden index was 0.55 and the corresponding optimal cut-off value was 0.14.

Conclusions: This study revealed that PWB hsa_circ_0005430 in LA may serve as a potential biomarker for LA patients diagnosis.

Introduction

Lung cancer is a leading malignant tumor worldwide, which can be divided into small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). NSCLC is for 80%, including lung adenocarcinoma (LA) and lung squamous cell carcinoma (LSCC). The 5-year survival rate of NSCLC is less than 18%. The 10-year survival rate of early stage is about 90% with the best cure, but approximately 85% of patients with lung cancer are diagnosed at a terminal stage [1, 2]. Early detection of lung cancer is very important for improving overall survival. Therefore, it is necessary to seek a novel biomarker for lung cancer to assist the timely diagnosis and treatment.

Recent studies have shown that circRNAs play an important role in the occurrence and development of cancer and could be used as therapeutic and prognostic factors for lung cancer [3−6]. Some studies have provided evidence that circRNAs are differentially expressed(DE) in tumors tissue of LA and play an important role in carcinogenesis because of participating in cancer related pathways [7−9]. Our team's previous study showed that the expression of hsa_circ_0005430 in lung adenocarcinoma PWB was significantly up-regulated [10]. However, its value in the diagnosis of lung adenocarcinoma needs further study. This study was designed to determine whether hsa_circ_0005430 in the PWB of LA patients could be used as a novel biomarker for the diagnosis of LA.

Methods

Clinical specimens

20 LA samples were obtained from the Department of Respiratory, Ningbo Li Huili Hospital. 10 controls were obtained from the Department of Medical Examination Center, Ningbo Li Huili Hospital. Clinical information of LA patients is summarized in Table 1. All patients provided informed consent prior to sample collection and the ethics committee of Ningbo Li Huili Hospital approved the study.

Serum Sample collections and processing

Serum was collected with 5 ml fasting blood and was separated by centrifugation at 3500 r/min(2410 x g) for 10 min, completed within 4 hr. PWB samples were immediately snap-frozen in liquid nitrogen after resection and then stored at −80°C until RNA extraction.

Reagents and equipment

CEA, CA199 and CA50 levels were measured by a DXI 800 immunoassay analyzer (Beckman, USA). Serum levels of CA50 were detected by a Maglumi4000P immunoassay analyzer (Snibe, China). All regents use instrument matching reagents.

Quantitative real-time reverse transcription PCR

Total RNA was extracted from PWB samples with a Trizol reagent (Invitrogen, Carlsbad, CA, USA), and then reverse transcribed to get cDNA. CircRNAs expression was detected through RT-qPCR using the SYBR Green PCR kit. The PCR primers used in this study were listed in Table 3. RT-qPCR reactions were carried out with 1 μl cDNA, 5 μl SYBR Green Supermix, 0.5 μl reverse primer, 0.5 μl forward primer, and 3 μl sterilized distilled water. Pre-denaturation maintained at 95°C for 3min, denaturation at 95°C for 10 sec, annealing at 58°C for 30 sec and extension at 79°C for 4 sec. The circle was repeated 39 times. The relative expression of hsa_circ_0005430 was calculated by the $2^{-\Delta\Delta Ct}$ method.

Statistical analysis

Results were all expressed as mean ± standard deviation. The man Whitney *U-test* or student t-test was used to assess significant differences between the two groups. Using the ROC curve, we analyze the specificity, sensitivity and AUC using the 95% *CI*. Spearman's correlation coefficient for the correlation between variables. $P<0.05$ was considered statistically significant.

Results

Expression levels of hsa_circ_0005430

In order to verify the accuracy of our predictions, we measured the expression levels of hsa_circ_0005430 in 20 clinical samples of LA and 10 controls by RT-qPCR. The expression levels of hsa_circ_0005430 in LA PWB were significantly higher than those in the controls(Fig. 1). And there was no significant difference in hsa_circ_0005430 level among different pathological factors of LA(Table 2).

ROC curve analysis of PWB hsa_circ_0005430

We performed ROC curve analysis to assess the potential value of PWB hsa_circ_0005430 for LA diagnosis. ROC curves of PWB hsa_circ_0005430 showed that it had an AUC of 0.75 (95% *CI* 0.567–0.928, *P* < 0.001) to discriminate individuals with LA from controls with sensitivity of 0.65 and specificity of 0.90 (Fig. 2). The highest Youden index was 0.55 and the corresponding optimal cut-off value was 0.14.

Spearman's correlation test of clinical variables and PWB hsa_circ_0005430 in LA patients

To determine whether the expression level of PWB hsa_circ_0005430 in LA patients was a relevant biomarker for the disease activity, we performed Spearman's correlation test to assess the correlation between LA-related clinical features and hsa_circ_0005430 in PWB from LA patients. As shown in Table 3, the level of PWB hsa_circ_0005430 in LA patients correlated with CEA(r = 0.55, P = 0.036), CA199(r = 0.648, P < 0.001) and CA50 (r = 0.479, P = 0.007), which reflect disease activity(Table 4).

Table 4
Correlation coefficients of clinical variables and PWB hsa_circ_0005430 in LA patients

		Hsa_circ_0005430	CEA	CA199	CA50
	Correlation coefficients	1	0.385[a]	0.648[b]	0.479[b]
hsa_circ_0005430	Sig (2-tailed)		0.036	0.000	0.007
	N	20	20	20	20
a, Correlation is significant at the 0.05 level (2-tailed);					
b, Correlation is significant at the 0.01 level (2-tailed);					
PWB, peripheral whole blood;					
LA, Lung Adenocarcinoma.					

Discussion

CircRNAs are more stable in mammalian cells comparing with microRNAs and long noncoding RNAs. These properties give circRNAs the potential of being ideal biomarkers for human diseases [11–16].Therefore, the use of circRNAs as a biological marker of patients with different diseases is very attractive to researchers[17–22]. Our previous studies have shown that the detection of circRNAs has an important value in the diagnosis of lung adenocarcinoma. The expression of hsa_circ_0005430 was significantly up-regulated. In this study, We further confirmed that hsa_circ_0005430 has a high sensitivity (65%) and specificity (90%) for the diagnosis of lung adenocarcinoma, which can be used as a biomarker for the diagnosis of lung adenocarcinoma.

Our study also found that the expression of hsa_circ_0005430 in PWB of LA patients correlated positively with CEA, CA199 and CA50, which reflect the disease activity, indicating that PWB hsa_circ_0005430 is a potential biomarker of LA patients. In addition, in this study, we found that the level of hsa_circ_0005430 in PWB had an AUC of 0.75 to discriminate individuals with LA from controls with high sensitivity and specificity, indicating its high potential as a noninvasive diagnostic biomarker of LA. Lung biopsy is the standard of diagnosis and classification, but it is not suitable for continuous monitoring due to the surgical invasion of complications [23].

However, there are some limitations in our study. First, the sample size was too small to make accurate conclusions.We would increase the number of specimens in the future investigations. Second, we do not analyze the role of hsa_circ_0005430 in LA. Therefore, an experimental functional study is needed to determine the causal relationship between PWB hsa_circ_0005430 and LA. Third, a larger study should be performed to confirm whether hsa_circ_0005430 can serve as novel biomarkers for LA diagnosis treatment.

Conclusions

Our results showed that hsa_circ_0005430 could be used as a novel biomarker for LA with higher accuracy, specificity and sensitivity. More prospective studies are needed to validate these results.

Abbreviations

circRNA, circular RNA; PWB, peripheral whole blood; LA, lung adenocarcinoma; ROC, receiver operating characteristic; CEA, carcinoembryonic antigen; CA199, carbohydrate antigen 199; CA50, carbohydrate antigen 50; NSCLC, non-small cell lung cancer; SCLC, small cell lung cancer; RT-qPCR, real-time quantitative reverse transcription-polymerase chain reaction; AUC, area under the curve; DE, differentially expressed

Declarations

Acknowledgements

None

Authors' contributions

Yinyu Mu contributed to the research design, data collection, data analysis, and manuscript writing. Chunxia Ye and Dongdong Yang contributed to the data collection and manuscript writing. Fuyi Xie contributed to the data analysis and manuscript editing. All authors read and approved the final manuscrip.

Availability of data and materials

Not applicable.

Ethics approval and consent to participate

This study was approved by the ethics committee of Ningbo Li Huili Hospital. Informed consent was obtained from all individual participants included in the study

Competing interests

The authors declare that they have no competing interests.

Consent for publication

Written informed consent for publication was obtained from all participants.

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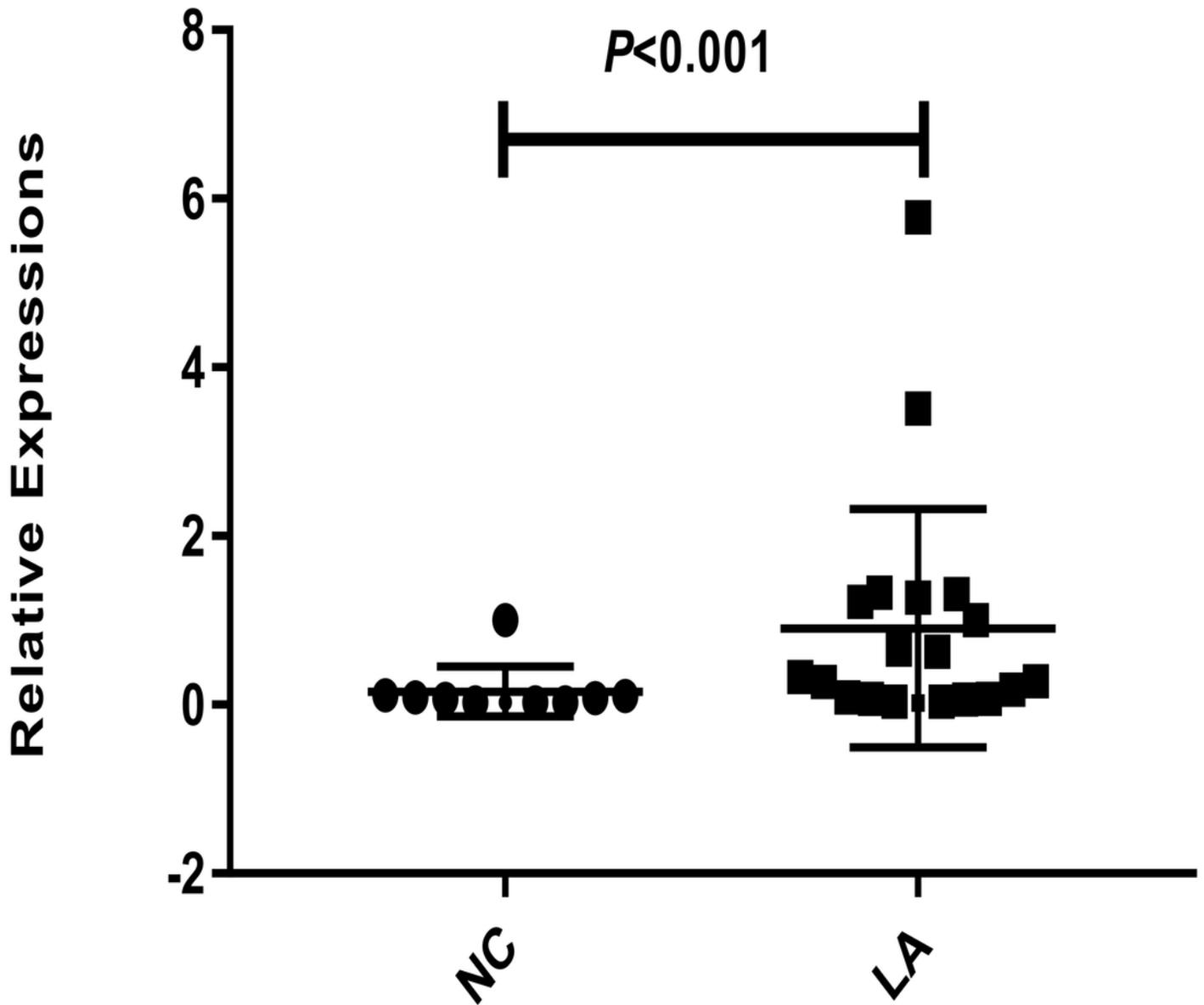
Figures

Figure 1

Relative expression of circRNA5430 in peripheral whole blood samples from the 20 lung adenocarcinoma(LA) and 10 normal controls(NC). RT-qPCR results showed that relative expression of circRNA5430 was significantly higher in LA. circRNA, circular RNA; RT-qPCR, real-time quantitative reverse transcription-polymerase chain reaction.
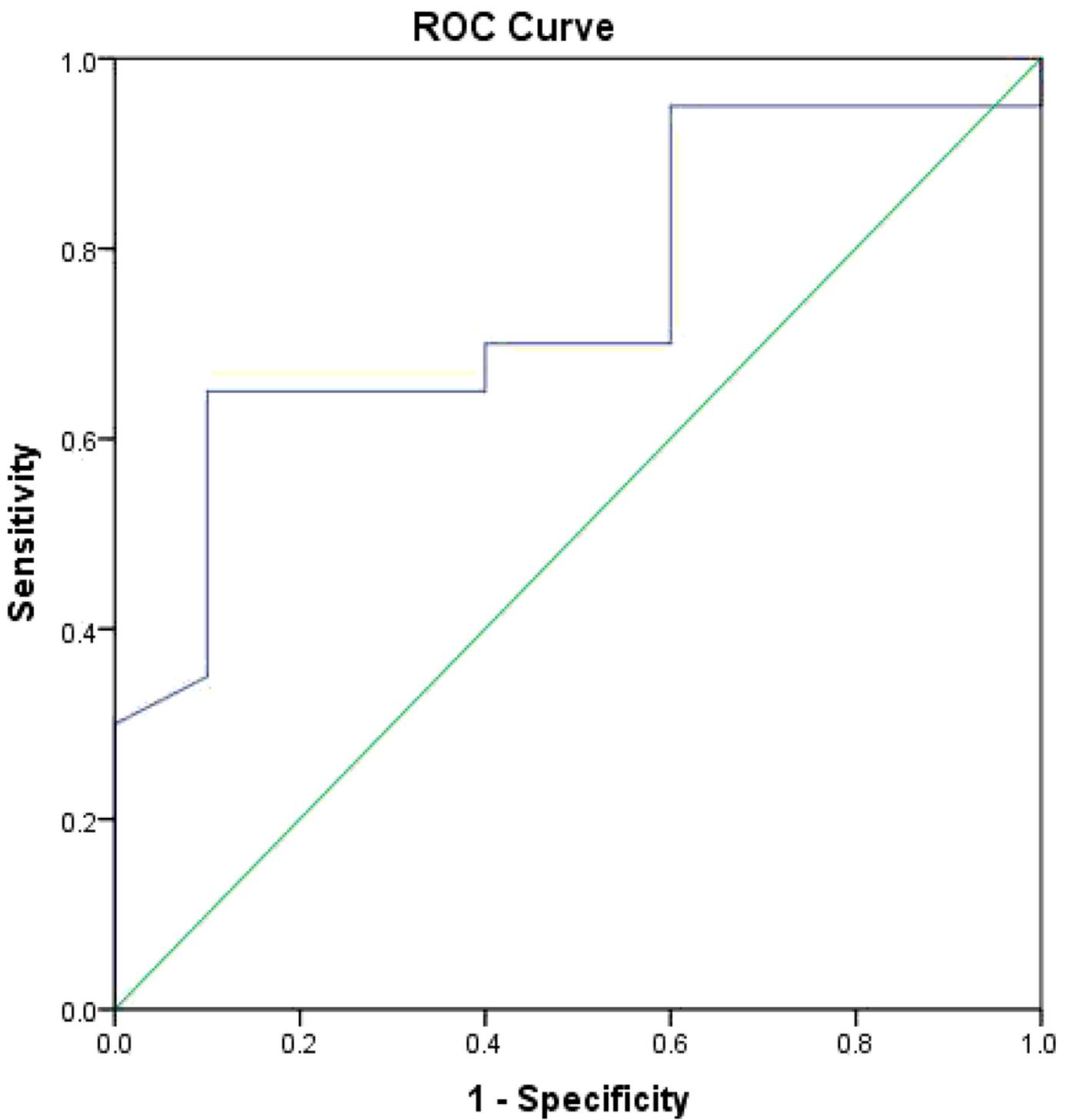
Figure 2

The receiver operating characteristic curve for discriminating patients with LA from healthy controls based on the PWB circRNA5430 expression. LA, lung adenocarcinoma; PWB, peripheral whole blood.