LIM and SH3 Protein 1 Stands for a Bio-Marker for Cholangiocarcinoma Detection

Ning Wang  
the PLA Rocket Force Characteristic Medical Center

Yanni Li  
the PLA Rocket Force Characteristic Medical Center

Yanfang Zheng  
the PLA Rocket Force Characteristic Medical Center

Huoming Chen  
the PLA Rocket Force Characteristic Medical Center

Xiaolong Wen  
University of Science and Technology Beijing

Zhaoxia Li (isdfke@163.com)  
the PLA Rocket Force Characteristic Medical Center  
https://orcid.org/0000-0002-9860-5977

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Abstract

**Background:** *LIM and SH3 protein 1 (LASP-1)* has been demonstrated to be overexpression in several types of cancers. The aim of this study was to verify the serum level of *LASP-1* and investigate its diagnostic value in cholangiocarcinoma (CCA) patients.

**Methods:** Quantitative real-time polymerase chain reaction (qRT-PCR) was performed to detect the expression level of *LASP-1* in CCA patients and healthy controls. The correlation of *LASP-1* expression with clinicopathological characteristic of CCA patients was analyzed via Chi-square test. Receiver operating characteristic (ROC) curve was built to evaluate the diagnostic value of serum *LASP-1* in CCA.

**Results:** Serum levels of *LASP-1* were upregulated in CCA compared with healthy controls (*P*<0.01). And the serum level and tissue level of *LASP-1* mRNA exhibited significant correlation (R=0.454, *P*=0.000). Serum expression of *LASP-1* was closely associated with lymph node metastasis (*P*=0.018) and TNM stage (*P*=0.021). ROC curve analysis revealed that serum *LASP-1* was of great value in differentiating CCA patients from healthy individuals. The area under the ROC curve (AUC) value was 0.879 corresponding with a sensitivity of 81.9% and a specificity of 79.6%.

**Conclusions:** Serum *LASP-1* might be an useful diagnostic biomarker for CCA.

Background

Cholangiocarcinoma (CCA) is the bile duct cancer, one of the highly aggressive malignant cancer, arising from ductular epithelium of biliary tree [1]. This cancer contains two major types including extrahepatic CCA (eCCA) and intrahepatic CCA (iCCA) according to anatomical location [2]. Previous studies have found several risk factors for CCA, such as primary sclerosing cholangitis (PSC), intrahepatic biliary stones, cirrhosis, viral hepatitis C and B, as well as CEA or CA19-9 [3-7]. But the diagnosis of CCA is still difficult due to its location, size and demoplastic characteristics [8]. Surgery is perhaps the main treatment method for cholangiocarcinoma, and despite improvements in surgical techniques, the survival of patients with this neoplasm was still poor. At present, there is no effective tool or specific biomarker that can detect the early stage or monitor status of CCA. Hence, there is a crucial need to explore novel reliable CCA diagnostic biomarker for improving the prediction and therapy efficacy.

The *LIM and SH3 protein 1 (LASP-1)* gene, also called metastatic lymph node gene 50 protein (*MLN50*), was initially identified from a cDNA library of metastatic axillary lymph nodes (MLN) from human breast cancer and the gene was mapped to human chromosome 17q21 [9-11]. *LASP-1* has been demonstrated to play an important role in cancer development and progression and might function as a tumor oncogene in several types of cancers [12-14], such as ovarian cancer [12], breast cancer [13] and colorectal cancer [14]. Furthermore, Zhang et al. have analyzed the effect of *LASP-1* on biology and function of human CCA cell lines HCCC-9810 and RBE. The results showed that *LASP-1* was overexpressed in CCA tissues and was involved in the metastasis and growth of CCA [15]. However, the clinical significance of *LASP-1* in the diagnosis of CCA was few reported.
Quantitative real-time polymerase chain reaction (QRT-PCR) is a sensitive, faster and affordable detection method for gene expression in different biological systems [16]. Currently, qRT-PCR technique is widely used to explore cancer biomarkers in human tissue and fluid samples. For example, Koshkin et al. reported that the expression patterns of 10 mature microRNA (7, 10a, 17, 20a, 21, 23a, 26a, 137, 222) exhibited significant different between glioma specimens and non-cancerous tissues through qRT-PCR analysis, suggesting their potential as diagnostic biomarker for brain tumors [17]. The qRT-PCR analysis carried out by Sun et al. demonstrated that serum levels of miR-770 levels showed significant difference between lung cancer patients and healthy controls, which could be employed as an indicator for early diagnosis and prognosis evaluation of the cancer [18]. QRT-PCR is an easy and reliable method to explore tumor-related biomarkers.

In the present study, we investigated the expression level of LASP-1 in CCA serum samples and tissue specimens using qRT-PCR. In addition, we also estimated the diagnostic value of serum LASP-1 in CCA patients.

Methods

Patients and specimens

In total, 127 CCA patients and 113 healthy controls were contained during their hospitalization and physical examination in the PLA Rocket Force Characteristic Medical Center. The patients were newly confirmed with CCA through pathological examinations. None of them had received surgical treatments, chemotherapy, or radiotherapy before sample collection. The healthy individuals exhibited normal range of all laboratory examinations, and had no history of malignant diseases. We collected 5mL blood from each participator for serum isolation. Blood was collected during physical examination and the serum was separated from whole blood by centrifugation for 10 min at 3000 rpm within 2 h after obtained, and then stored at -80°C until use. In addition, 96 CCA patients in case group accepted surgical treatment in our study. The CCA tissues and corresponding normal tissues were collected from these patients.

The study protocol was approved by the Ethics Committee of the PLA Rocket Force Characteristic Medical Center, and all patients provided written informed consents prior to sampling. All specimens were made anonymous according to the ethical and legal standards.

RNA extraction and quantitative real-time PCR (qRT-PCR)

Total RNA from serum and tissue samples was extracted using TRIzol reagent (Invitrogen) and reverse transcribed to first-strand cDNA using the SuperScript™ II Reverse Transcriptase Kit (Invitrogen) according to the manufacturer's protocol. Quantitative real-time PCR (qRT-PCR) was performed with an Applied Biosystems 7900HT system (Applied Biosystems) using SYBR Premix Ex Taq (Takara). GAPDH was used as endogenous control. The sequences of the primers used for LASP-1 amplification were 5'-TGTCTCCTGACTGGTTGCGT-3', and 5'-TGATCTGGTCCTGGGTCTTC-3'. Primers for GAPDH were: 5'-
ATCAAGAAGGTGGTGAAGCAG-3' and 5'-TACTCCTTGGAGGCCATGTG-3'. The 2^ΔΔCt method was used to determine relative quantitation of LASP-1 expression. Each sample was performed in triplicate.

**Statistical analysis**

The SPSS version 21.0 and GraphPad Prism 5 softwares were used to conduct all statistical analyses. The Student's t-test was used to determine the statistical significance between tumor and healthy control groups. The association between LASP-1 expression and clinicopathological parameters was evaluated using χ^2 test. The receiver operating characteristic (ROC) curves was generated to distinguish CCA patients from healthy individuals and the diagnostic potential of serum LASP-1 evaluated by calculating the area under ROC curve (AUC). *P* value less than 0.05 was considered statistically significant.

**Results**

**Baseline characteristics of the study population**

In our study, 127 CCA patients including 73 males and 54 females were employed as case group. The control group included 66 men and 47 women. The mean age of case group was 53.78±18.75 years, while the average age of control group was 55.16±17.12 years. The case and control groups did not show significant differences in age and gender distributions (*P*=0.552 and 0.105, respectively). The body mass index (BMI) value of CCA patients was 22.56±2.15 kg/m^2, and the data in control group was 21.78±1.56 kg/m^2, without obvious difference (*P*=0.754) (**Table 1**).

**Expression level of LASP-1 was increased in CCA**

The relative mRNA expression level of LASP-1 in serum specimens collected from 127 CCA patients and 113 healthy controls were analyzed by qRT-PCR. The results showed that expression levels of LASP-1 in CCA patients were significantly higher than in healthy controls (*P*<0.01) (**Figure 1A**). Furthermore, we collected CCA tissues from 96 patients. The relative expression of LASP-1 mRNA was also detected in CCA tissues and adjacent normal tissues. QRT-PCR assay suggested that compared to non-cancerous tissues, CCA tissues exhibited significant up-regulation of LASP-1 mRNA (**Figure 1B**).

In addition, the relationship between serum and tissue levels of LASP-1 was also estimated in present study. Pearson's correlation analysis indicated that the serum level and tissue level of LASP-1 mRNA exhibited significant correlation (R=0.454, *P*=0.000) (**Figure 2**).

**Relationship between LASP-1 expression and clinicopathological features of CCA patients**

To investigate whether LASP-1 was involved in the development of CCA, the correlation between LASP-1 expression and clinicopathological characteristics was analyzed. The included CCA patients were divided into high expression group (n=76) and low expression group (n=51), according to their median serum levels of LASP-1 mRNA (the cut-off was 1.99). As shown in **Table 2**, the expression of LASP-1 was significantly correlated with lymph node metastasis (*P*=0.018) and TNM stage (*P*=0.021). However, there
were no significant correlations between serum LASP-1 expression and age, gender, tumor size, or differentiation (all, \(P>0.05\)).

**The diagnostic value of LASP-1 in CCA**

To assess the feasibility of serum LASP-1 as a diagnostic tool for the detection of CCA, ROC curve analysis was performed. As shown in Figure 3, LASP-1 was proved to have a relatively high accuracy in differentiating CCA patients from healthy individuals yielded AUC of 0.897 (95% CI: 0.837-0.921) with a sensitivity of 81.9% and a specificity of 79.6% at the optimal cutoff point 1.77.

**Discussion**

CCA is a type of malignant tumor and patients with CCA mostly appear in late clinical presentation that resulted in high mortality with poor prognosis [19]. Owing to highly chemo-resistant characteristic of CCA, the only curative treatment is surgical resection, however, which may not be suitable for all cases. The incidence and prevalence of CCA vary markedly worldwide and it is still a refractory disease with a low 5-year survival rate [20, 21]. The current major problems are lack of effective biological indicators for monitoring tumor occurrence. Thus, it is critical to find effective indicator markers for early detection of CCA.

Nowadays, more and more specific biological markers were studied which play important roles in the detection and treatment of patients with different cancer types, including CCA [22-28]. Kawin Leelawat et al. measured the level of CEA, CA19-9, MMP-7 and MMP-9 in the serum of cholangiocarcinoma and benign biliary tract diseases patients and found only the serum MMP-7 appears to be a valuable diagnostic marker in the discrimination of CCA from benign biliary tract disease [25]. Zhang et al. pointed that the biology of calretinin and CK5/6 expression in CCA is unclear but calretinin and CK5/6 immunohistochemical stains may be useful for diagnosing a CCA [26]. Yu et al. determined the expression of PKM2 in and its impact on biology and clinical outcome of human hilar cholangiocarcinoma (HC), and pointed that PKM2 is an independent prognostic factor and potential therapeutic target for human HC [27]. In recent years, serum tumor markers have been the objects of extensive investigation to assist CCA diagnosis because of the easy obtaining samples and relatively low cost. However, until now no marker was adequate specificity for CCA diagnosis.

**LASP-1** is a specific focal adhesion protein that has been demonstrated to play an important role in cancer development and progression [13, 29-31]. For instance, Frietsch et al. analyzed the prognostic significance of LASP-1 in breast cancer patients and provided evidence that nuclear LASP-1-positivity may serve as a negative prognostic indicator for long-term survival of breast cancer patients [13]. The LASP-1 was found overexpressed in gastric cancer tissues and cell lines, and associated with poor prognosis, which plays an important role in growth and metastasis of gastric cancer [29]. Wang et al. determined the expression of LASP-1 in primary HCC and the results showed that the high cytosolic LASP-1 expression was associated with poor overall survival in HCC patients [30]. Yang et al. examined LASP-1 expression in ccRCC tissues and in two cell lines and the results indicated that LASP-1 might serve as a
prognostic biomarker for ccRCC patients [31]. Based on the previous reports, we studied the clinical diagnostic value of \textit{LASP-1} in CCA.

In this present study, we examined the expression level of \textit{LASP-1} in serum and tissues of the CCA patients. The results revealed that serum level of \textit{LASP-1} in CCA patients was increased compared with healthy control. The high expression of \textit{LASP-1} was tightly correlated with positive lymph node metastasis and advanced TNM stage, which indicated that \textit{LASP-1} as an oncogene participated in the development and progression of CCA. The study carried out by Zhang et al. reported that the knockdown of \textit{LASP-1} could obviously promote cell apoptosis, inhibit cell proliferation, migration and invasion in vitro and tumorigenesis in vivo. The up-regulation of \textit{LASP-1} in CCA showed close association with malignant clinical characteristics, and poor prognosis [15]. However, the molecular mechanism of \textit{LASP-1} in development of CCA was poorly known. Further investigations are still required.

ROC curve analysis demonstrated that serum \textit{LASP-1} be a valuable indicator for CCA detection. \textit{LASP-1} is a specific focal adhesion protein at cell surface, and its over-production in tumor cells or tissues may lead to alterations in peripheral blood concentration. In our study, we also found that the expression patterns of \textit{LASP-1} in serum and tissue samples exhibited high consistency. Compared to tissue specimens, the blood was easily obtained via non-invasive methods, having repeatability. Thus, serum \textit{LASP-1} might be a potential non-invasive biomarker for early diagnosis of CCA.

\section*{Conclusions}

In conclusion, \textit{LASP-1} expression is increased in CCA patients, and its elevated expression is positively correlated with lymph node metastasis and TNM stage. Serum \textit{LASP-1} may be a promising diagnostic marker for CCA patients.

\section*{List Of Abbreviations}

\textit{LIM and SH3 protein 1 (LASP-1)}

cholangiocarcinoma (CCA)

Quantitative real-time polymerase chain reaction (qRT-PCR)

Receiver operating characteristic (ROC)

area under the ROC curve (AUC)

extrahepatic CCA (eCCA)

intrahepatic CCA (iCCA)

primary sclerosing cholangitis (PSC)
Declarations

Ethics approval and consent to participate

This study was supported by the Ethics Committee of the PLA Rocket Force Characteristic Medical Center and also has been carried out in accordance with the World Medical Association Declaration of Helsinki.

The subjects had been informed the objective. Certainly, written consents were signed by every subject in this study.

Consent for publication

We obtaining permission from participants to publish their data.

Availability of data and materials

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

Competing interests

The authors declare that they have no competing interests.

Funding

Not applicable.

Authors’ contributions

N.W. design of the work; Y.L. the acquisition, analysis, Y.Z. interpretation of data; H.C. the creation of new software used in the work; X.W., Z.L. have drafted the work or substantively revised it. All authors read and approved the final manuscript.

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References

1. Blechacz B, Gores GJ: Cholangiocarcinoma: advances in pathogenesis, diagnosis, and treatment. Hepatology 2008, 48(1):308-321.
2. Everhart JE, Ruhl CE: Burden of digestive diseases in the United States Part III: Liver, biliary tract, and pancreas. Gastroenterology 2009, 136(4):1134-1144.
3. Cai Y, Cheng N, Ye H, Li F, Song P, Tang W: The current management of cholangiocarcinoma: A comparison of current guidelines. Bioscience trends 2016, 10(2):92-102.
4. Razumilava N, Gores GJ: Classification, diagnosis, and management of cholangiocarcinoma. *Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association* 2013, 11(1):13-21 e11; quiz e13-14.

5. Shaib YH, El-Serag HB, Nooka AK, Thomas M, Brown TD, Patt YZ, Hassan MM: Risk factors for intrahepatic and extrahepatic cholangiocarcinoma: a hospital-based case-control study. *The American journal of gastroenterology* 2007, 102(5):1016-1021.

6. Palmer WC, Patel T: Are common factors involved in the pathogenesis of primary liver cancers? A meta-analysis of risk factors for intrahepatic cholangiocarcinoma. *Journal of hepatology* 2012, 57(1):69-76.

7. Boyd S, Mustonen H, Tenca A, Jokelainen K, Arola J, Farkkila MA: Surveillance of primary sclerosing cholangitis with ERC and brush cytology: risk factors for cholangiocarcinoma. *Scandinavian journal of gastroenterology* 2017, 52(2):242-249.

8. Gatto M, Bragazzi MC, Semeraro R, Napoli C, Gentile R, Torrice A, Gaudio E, Alvaro D: Cholangiocarcinoma: update and future perspectives. *Digestive and liver disease : official journal of the Italian Society of Gastroenterology and the Italian Association for the Study of the Liver* 2010, 42(4):253-260.

9. Tyson GL, El-Serag HB: Risk factors for cholangiocarcinoma. *Hepatology* 2011, 54(1):173-184.

10. Tomasetto C, Moog-Lutz C, Regnier CH, Schreiber V, Basset P, Rio MC: Lasp-1 (MLN 50) defines a new LIM protein subfamily characterized by the association of LIM and SH3 domains. *FEBS letters* 1995, 373(3):245-249.

11. Tomasetto C, Regnier C, Moog-Lutz C, Mattei MG, Chenard MP, Lidereau R, Basset P, Rio MC: Identification of four novel human genes amplified and overexpressed in breast carcinoma and localized to the q11-q21.3 region of chromosome 17. *Genomics* 1995, 28(3):367-376.

12. Grunewald TG, Kammerer U, Winkler C, Schindler D, Sickmann A, Honig A, Butt E: Overexpression of LASP-1 mediates migration and proliferation of human ovarian cancer cells and influences zyxin localisation. *British journal of cancer* 2007, 96(2):296-305.

13. Frietsch JJ, Grunewald TG, Jasper S, Kammerer U, Herterich S, Kapp M, Honig A, Butt E: Nuclear localisation of LASP-1 correlates with poor long-term survival in female breast cancer. *British journal of cancer* 2010, 102(11):1645-1653.

14. Zhao L, Wang H, Liu C, Liu Y, Wang X, Wang S, Sun X, Li J, Deng Y, Jiang Y et al: Promotion of colorectal cancer growth and metastasis by the LIM and SH3 domain protein 1. *Gut* 2010, 59(9):1226-1235.

15. Zhang H, Li Z, Chu B, Zhang F, Zhang Y, Ke F, Chen Y, Xu Y, Liu S, Zhao S et al: Upregulated LASP-1 correlates with a malignant phenotype and its potential therapeutic role in human cholangiocarcinoma. *Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine* 2016, 37(6):8305-8315.

16. Gadkar V, Fillion M: New Developments in Quantitative Real-time Polymerase Chain Reaction Technology. *Current issues in molecular biology* 2014, 16:1-6.
17. Koshkin FA, Chistyakov DA, Nikitin AG, Konovalov AN, Potapov AA, Usachov DY, Pitskhelauri DI, Kobyakov GL, Shishkina LV, Chekhonin VP: **Profile of microRNA expression in brain tumors of different malignancy.** *Bulletin of experimental biology and medicine* 2014, **157**(6):794-797.

18. Sun B, Liu HF, Ding Y, Li Z: **Evaluating the diagnostic and prognostic value of serum miR-770 in non-small cell lung cancer.** *European review for medical and pharmacological sciences* 2018, **22**(10):3061-3066.

19. Shaib Y, El-Serag HB: **The epidemiology of cholangiocarcinoma.** *Seminars in liver disease* 2004, **24**(2):115-125.

20. Nakagohri T, Kinoshita T, Konishi M, Takahashi S, Gotohda N: **Surgical outcome and prognostic factors in intrahepatic cholangiocarcinoma.** *World journal of surgery* 2008, **32**(12):2675-2680.

21. Sapisochin G, Fernandez de Sevilla E, Echeverri J, Charco R: **Liver transplantation for cholangiocarcinoma: Current status and new insights.** *World journal of hepatology* 2015, **7**(22):2396-2403.

22. Seeree P, Pearngam P, Kumkate S, Janvilisri T: **An Omics Perspective on Molecular Biomarkers for Diagnosis, Prognosis, and Therapeutics of Cholangiocarcinoma.** *International journal of genomics* 2015, **2015**:179528.

23. Davis RL, Liang C, Sue CM: **A comparison of current serum biomarkers as diagnostic indicators of mitochondrial diseases.** *Neurology* 2016, **86**(21):2010-2015.

24. Ma Y, Zhou W, He S, Xu W, Xiao J: **Tyrosine kinase inhibitor sunitinib therapy is effective in the treatment of bone metastasis from cancer of unknown primary: Identification of clinical and immunohistochemical biomarkers predicting survival.** *International journal of cancer* 2016, **139**(6):1423-1430.

25. Leelawat K, Sakchinabut S, Narong S, Wannapraset J: **Detection of serum MMP-7 and MMP-9 in cholangiocarcinoma patients: evaluation of diagnostic accuracy.** *BMC gastroenterology* 2009, **9**:30.

26. Zhang L, Frank R, Furth EE, Ziober AF, LiVolsi VA, Zhang PJ: **Expression and diagnostic values of calretinin and CK5/6 in cholangiocarcinoma.** *Experimental hematology & oncology* 2014, **3**:12.

27. Yu G, Yu W, Jin G, Xu D, Chen Y, Xia T, Yu A, Fang W, Zhang X, Li Z et al. **PKM2 regulates neural invasion of and predicts poor prognosis for human hilar cholangiocarcinoma.** *Molecular cancer* 2015, **14**:193.

28. Uhm KO, Lee JO, Lee YM, Lee ES, Kim HS, Park SH: **Aberrant DNA methylation of integrin alpha4: a potential novel role for metastasis of cholangiocarcinoma.** *Journal of cancer research and clinical oncology* 2010, **136**(2):187-194.

29. Zheng J, Yu S, Qiao Y, Zhang H, Liang S, Wang H, Liu Y, Zhou F, Jiang J, Lu S: **LASP-1 promotes tumor proliferation and metastasis and is an independent unfavorable prognostic factor in gastric cancer.** *Journal of cancer research and clinical oncology* 2014, **140**(11):1891-1899.

30. Wang H, Li W, Jin X, Cui S, Zhao L: **LIM and SH3 protein 1, a promoter of cell proliferation and migration, is a novel independent prognostic indicator in hepatocellular carcinoma.** *Eur J Cancer* 2013, **49**(4):974-983.
31. Yang F, Zhou X, Du S, Zhao Y, Ren W, Deng Q, Wang F, Yuan J: LIM and SH3 domain protein 1 (LASP-1) overexpression was associated with aggressive phenotype and poor prognosis in clear cell renal cell cancer. PloS one 2014, 9(6):e100557.

Tables

Table 1. Baseline characteristics of the CCA patients and healthy controls

| Parameters             | CCA group (n=127, %) | Healthy controls (n=113, %) | P values |
|------------------------|----------------------|----------------------------|----------|
| Age (years)            |                      |                            | 0.304    |
| <50                    | 68 (53.54)           | 53 (46.90)                 |          |
| ≥50                    | 59 (46.46)           | 60 (53.10)                 |          |
| Average age (years)    | 53.78±18.75          | 55.16±17.12                | 0.552    |
| Gender                 |                      |                            | 0.105    |
| male                   | 73 (57.48)           | 66 (58.41)                 |          |
| female                 | 54 (42.52)           | 47 (41.59)                 |          |
| BMI values (kg/m$^2$)  | 22.56±2.15           | 21.78±1.56                 | 0.754    |

Notes: CCA: Cholangiocarcinoma; BMI: Body mass index.

Table 2. Relationship between LASP-1 expression level and clinicopathologic parameters of CCA patients
| Parameters                        | Cases (n=127) | LASP-1 expression | $\chi^2$ | $P$  |
|----------------------------------|---------------|-------------------|----------|------|
|                                  |               | Low   | High  |       |      |
| **Age**                          |               |        |       | 1.796| 0.180|
| <50                              | 68            | 31    | 37    |      |      |
| ≥50                              | 59            | 20    | 39    |      |      |
| **Gender**                       |               |        |       | 0.967| 0.326|
| Male                             | 73            | 32    | 41    |      |      |
| Female                           | 54            | 19    | 35    |      |      |
| **Tumor size**                   |               |        |       | 0.381| 0.537|
| <5cm                             | 73            | 31    | 42    |      |      |
| ≥5cm                             | 54            | 20    | 34    |      |      |
| **Lymph node metastasis**        |               |        |       | 5.595| 0.018|
| negative                         | 71            | 35    | 36    |      |      |
| positive                         | 56            | 16    | 40    |      |      |
| **Differentiation**              |               |        |       | 3.697| 0.055|
| poor                             | 69            | 33    | 36    |      |      |
| moderate+well                    | 58            | 18    | 40    |      |      |
| **TNM stage**                    |               |        |       | 5.320| 0.021|
| I-II                             | 74            | 36    | 38    |      |      |
| III-IV                           | 53            | 15    | 38    |      |      |

**Figures**
Figure 1

The relative LASP-1 expression levels in CCA patients. A: The expression of LASP-1 in CCA serum was significantly higher than that in healthy control individuals. B: Compared to adjacent non-cancerous tissues, CCA tissues showed obviously increased expression of LASP-1 (**, P<0.01).
Figure 2

The interaction between serum and tissue expression of LASP-1 in CCA patients. The serum levels of LASP-1 was significantly correlated with its expression in CCA tissues (R=0.454, P=0.000).
The diagnostic significance of LASP-1 was analyzed via establishing ROC curve. ROC curve analysis results showed serum LASP-1 expression to discriminate CCA patients from healthy controls.