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LIM and SH3 protein 1, a promoter of cell proliferation and migration, is a novel independent prognostic indicator in hepatocellular carcinoma

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Abstract LIM and SH3 protein 1 (LASP-1) plays a significant role in the formation of several malignant tumours. However, the biological and clinical significances of LASP-1 in hepatocellular carcinoma (HCC) remain largely unknown. Using immunohistochemistry, we analysed LASP-1 expression in 144 clinicopathologically characterised HCC cases. Using gene and transfection and RNA interference, we investigated the effects of LASP-1 overexpression and depletion on tumour cellular behaviour *in vitro*. LASP-1 expression was detected in not only cytoplasm and but also nucleus of HCC and liver cells. The positive rates of both cytosolic and nuclear LASP-1 expression in HCC were higher than adjacent non-tumourous tissues. Statistical analysis showed that heterogeneous LASP-1 expression is associated with hepatitis B surface antigen (HBsAg) and serum alpha-fetoprotein (AFP) level of HCC patients. A significant trend was identified between cytosolic LASP-1 overexpression in HCC and worsening clinical prognosis. Multivariate survival analysis showed that cytosolic LASP-1 expression was recognised as an independent prognostic factor of patient's survival. *In vitro* study showed LASP-1 promoted cell proliferation and migration, and resulted in aggressive phenotypes of cancer cells. LASP-1 is a valuable marker of HCC progression. High cytosolic LASP-1 expression is associated with poor overall survival in HCC patients.

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1. Introduction

Hepatocellular carcinoma (HCC) ranks as the fifth most common malignant disorder and the third leading cause of cancer-related deaths worldwide.[1] Although it is especially prevalent in certain areas of Asia and Africa, an increasing incidence in western countries, including the United States, has recently been observed.[2] The prognosis of HCC remains poor despite advances in surgical or locoregional therapies. The search for an effective and efficient therapy for HCC is still ongoing.[3] With the increasing understanding of the tumour biology of HCC, more and more molecular markers with high sensitivity and specificity for HCC have been found and could be helpful for early diagnosis and the development of future targeted HCC therapeutics.

LIM and SH3 protein 1 (LASP-1) was initially identify from a cDNA library of metastatic axillary lymph nodes of breast cancer patients, and the gene was mapped to human chromosome 17q21.[4,5] Human LASP-1 gene encodes a protein of 261 amino acids containing an N-terminal LIM domain followed by two actin binding domains in the core of the LASP-1 protein mediating an interaction between LASP-1 and the actin cytoskeleton at the site of cell membrane extensions, but not along the actin stress fibres.[6–9] The SH3 domain at the C-terminus is involved in protein–protein interactions through binding to praline-rich sequences, specifically with zyxin, pallidin, lipoma preferred partner (LPP) and vasodilator-stimulated phosphoprotein (VASP).[7,10] The exact functions of LASP-1 are still not well known, it is localised on multiple sites of dynamic actin assembly such as focal contacts, focal adhesions, lamellipodia membrane ruffles and pseudopodia.[4,8,11–13]

In our previous study, we observed that LASP-1 was upregulated in colorectal cancer (CRC), especially in those with metastasis, implying its relationship with poor clinical outcome. LASP-1 stimulated cancer cell growth and migration *in vitro*, and promoted aggressive phenotypes of CRC cells *in vivo* by regulating the expression of various key molecules.[14] Similarly, LASP-1 expression has been reported to be increased in many malignant tumours, such as breast cancer,[15] ovarian cancer[16] bladder cancer[17] and medulloblastoma.[13] According our current knowledge, however, the biological function and clinical significance of LASP-1 in development and progression of HCC have remained largely unknown. Recent studies have demonstrated LASP-1 protein as both a novel p53 transcriptional target[18] and one of uPA downstream proteins,[19] suggesting the potential roles of LASP-1 in HCC.

In the present study, we determined the expression of LASP-1 in primary HCC using Western blotting and immunohistochemistry and investigated the relationship between its expression and clinicopathological parameters. We also evaluated the potential prognostic value of LASP-1 in the postresectional survival of HCC patients. We then performed gene transfection-mediated overexpression and RNA interference (RNAi)-mediated gene silencing to investigate the effect of LASP-1 on the biological behaviour of HCC cells and discussed the possible mechanisms involved in genesis and metastasis of HCC.

2. Materials and methods

2.1. Tumour tissue sample

All cases of tumour tissue were provided by the Tumor Tissue Bank of Nanfang Hospital. A total of 152 patients were involved in the study. Fresh frozen tumour samples from eight patients of them and matched adjacent non-tumours tissues were selected for Western blot analysis. Formalin-fixed tumour tissues from other 144 patients including 97 matched adjacent non-tumour tissues were used for immunohistochemical analysis. In each case, a diagnosis of primary HCC had been made, and the patients had undergone elective surgery for HCC in Nanfang Hospital during 2001–2002. The Tumor Tissue Bank of Nanfang Hospital possesses a comprehensive set of clinicopathological data, including age, gender, size of primary tumour, tumour differentiation, tumour metastasis, clinical stage, serum alpha-fetoprotein (AFP), hepatitis B surface antigen (HBsAg) and liver cirrhosis. Complete follow-up, ranging from 0 to 83 months, was available for all patients and the median survival was 34 months. At the time of censoring the data, there had been 88 (61.1%) deaths in the patient group. The pathological diagnosis was performed at the Department of Pathology of Nanfang Hospital, Southern Medical University. The tumour tissues were fixed in 4% formaldehyde, embedded in paraffin, sectioned and stained with haematoxylin and eosin (HE). Permission for this study was obtained from the Ethics Committee of Southern Medical University.

2.2. Cell line

Human liver immortal cell line L02 was obtained from the Committee of Type Culture Collection of Chinese Academy of Sciences (Shanghai, China). HCC cell lines Huh-7 (RCB 1366) was obtained from the RIKEN cell bank (Ibaraki, Japan). Hep3B (HB-8064), HepG2 (HB-8065) and SK-Hep1 (HTB-52) were obtained from American Type Culture Collection (ATCC). MHCC-97L, MHCC-97H, HCC-LM3 and HCC-LM6, kindly provided by Liver Cancer Institute of Zhongshan Hospital, Fudan University (Shanghai, China). All the cells were maintained in RPMI1640 (Hyclone, Logan, UT) or Dulbecco's modified Eagle's