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ORIGINAL ARTICLE

Caveolin-1 promotes tumor growth and metastasis via autophagy inhibition in hepatocellular carcinoma

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Summary

Background: Caveolin-1 is a member of the caveolae family of membrane proteins. Although some researchers have investigated the function of Caveolin-1 in hepatocellular carcinoma, the mechanism of Caveolin-1 action and its prognostic value in hepatocellular carcinoma remain unclear.

Methods: Caveolin-1 expression was measured in hepatocellular carcinoma cell lines and tissues using quantitative reverse transcription-polymerase chain reaction, western blot, and immunofluorescence assays. In in vitro experiments, Caveolin-1 was depleted using a short hairpin RNA lentiviral vector, and tumor cell behavior was analyzed. The effect of Caveolin-1 on hepatocellular carcinoma cell autophagy was investigated. Prognostic value of Caveolin-1 was analyzed by immunohistochemistry in two cohorts that included a total of 721 hepatocellular carcinoma patients.

Results: We found that Caveolin-1 was overexpressed in highly metastatic hepatocellular carcinoma cell lines and tumor tissues. Moreover, Caveolin-1 promoted hepatocellular carcinoma cell proliferation, migration, and angiogenesis and inhibited autophagy. Finally, Caveolin-1 expression in hepatocellular carcinoma tissues was inversely correlated with patient overall survival and time to recurrence.

Abbreviations: Cav-1, Caveolin-1; HCC, Hepatocellular carcinoma; qRT-PCR, Quantitative reverse transcription-polymerase chain reaction; OS, Overall survival; TTR, Time to recurrence; EMT, Epithelial-to-mesenchymal transition; TMA, Tissue microarray; AFP, α-fetoprotein; γ-GT, γ-glutamyl transferase; TNM, Tumor-nodes-metastasis; HR, Hazard ratio; CI, Confidential interval.

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Conclusion: Our data obtained from cell lines suggest an oncogenic role for Caveolin-1 in hepatocellular carcinoma, Caveolin-1 contributed to hepatocellular carcinoma cell autophagy deficiency. Furthermore, Caveolin-1 may function as a novel prognostic indicator for hepatocellular carcinoma patients after curative resection, and combination of targeted therapy aimed at Caveolin-1 and autophagy modulation may represent an effective way to treat hepatocellular carcinoma.

Introduction

Liver cancer is the fifth most frequently diagnosed cancer worldwide but is the second most common cause of cancer-related deaths [1]. Among the types of liver cancer, hepatocellular carcinoma (HCC) accounts for 70% to 85% of the total liver cancer burden [2]. HCC recurrence and metastasis are significant factors influencing the 5-year overall survival (OS) and time to recurrence (TTR) rates. Thus, identifying biomarkers that predict HCC metastasis is highly warranted.

Caveolae are invaginations of the plasma membrane that play a pivotal role in cholesterol transport, endocytosis, potocytosis, and signal transduction. The caveolae family are predominantly formed by three proteins: Caveolin-1 (Cav-1), Cav-2, and Cav-3 [3,4]. The oncogenic role of Cav-1 in cancer has been widely investigated in multiple studies. For example, Cav-1 functions as a crucial regulator of the epithelial-to-mesenchymal transition (EMT) in gastric cancer [5]. Cav-1 also contributes to prostate cancer progression by interacting with LRP6, which leads to IGF-IR/IR activation, Akt-mTORC1 activation, and aerobic glycolysis [6]. The Cavin-1/Caveolin-1 and FoxM1-Cav-1 pathways were also shown to play a critical role in pancreatic cancer progression [7]. In contrast, a tumor suppressor role for Cav-1 in cancer has also been reported [8,9].

In HCC, the role of Cav-1 remains controversial. Cav-1 has been shown to promote hepatocarcinoma cell adhesion by upregulating integrin $\alpha 2$, 6-sialylation [10], and Cav-1 also contributes to HCC tumorigenesis by promoting metastasis [11]. In contrast, Du et al. reported that Cav-1 contributes to DLC1-mediated tumor suppression via a RhoGAP-independent mechanism [12]. Cav-1 has been suggested to play a causative role in invasive and poor-prognosis HCCs [13]. Another study found that Cav-1 may play a tumor suppressor role via its modulation of eNOS [14]. Although previous studies have examined Cav-1 protein expression using immunohistochemistry in archived HCC tissues, the case numbers were limited and lacked validation. Thus, further investigation into the mechanism of Cav-1 function in HCC is warranted. Our current study represents the largest analysis of Cav-1 protein expression in HCC; we investigated the mechanisms of Cav-1 function in HCC progression and also evaluated the expression of Cav-1 in a series of metastatic HCC cell lines. Our results provide evidence of an oncogenic role for Cav-1 in HCC cells in vitro, and our findings also reveal the prognostic value of Cav-1 through the use of tissue microarrays (TMAs) generated from HCC samples.

Materials and methods

Human HCC tissues and cell lines

Thirty-one sets of fresh matched tumor and peritumoral tissue samples were randomly collected from HCC patients who underwent curative resection between 2009 and 2010 at the Liver Cancer Institute, Zhongshan Hospital, Fudan University, Shanghai, China. All patients provided informed consent, and the samples were also used for quantitative reverse transcription-polymerase chain reaction (qRT-PCR) analysis. This study was approved by the Institutional Review Board of the Liver Cancer Institute. The human HCC cell lines MHCC97L, MHCC97H, and HCCLM3 were established at our institute and exhibit the same genetic background but different lung metastatic potentials [15,16]. Normal hepatocyte cells (L02) and the HCC cell lines Hep3B, HepG2, SMMC-7721, and Huh7 were purchased from the Institute of Biochemistry and Cell Biology, Chinese Academy of Science. All cell lines were routinely maintained and cultured.

RNA isolation and qRT-PCR

Total RNA was isolated from each cell line using the RNeasy Mini Kit (Qiagen, Valencia, CA, Germany) according to the manufacturer's instructions. Equal amounts of RNA were reverse transcribed into cDNA using the SuperScript III System (Invitrogen, Carlsbad, CA). Cav-1 mRNA expression in the HCC cell lines and tumor tissues isolated from 31 HCC patients was measured by qRT-PCR using an ABI7900 HT instrument (Applied Biosystems, Foster, Foster City, CA). qRT-PCR was performed using the SYBR PrimeScript RT-PCR Kit (Takara Bio, Shiga, Japan) according to the manufacturer's instructions. β-Actin was used as an internal control. The mRNA levels were calculated based on the Ct values, which were corrected for β-actin expression according to the following equation: $2^{-\Delta\Delta Ct}$ (ΔCt = Ct [Cav-1] - Ct [β-actin]). All experiments were performed in triplicate. The primers used for all genes were as follows: Cav-1-F: 5′-agaaccagaagggacacacagt-3′, Cav-1-R: 5′-agatggaatagacacggctgat-3′; P62-F: atcggaggatccgagtgt, P62-R: tggctgtgagctgctctt; Atg5-F: tgggccatcaatcggaaactc, Atg5-R: tgcagccacaggacgaaacag; Beclin-1-F: agctgccgttatactgttctg, Beclin-1-R: actgcctcctgtgtcttcaatctt; β-actin-F: 5′-caactgggacgacatggagaaaat-3′, β-actin-R: 5′-ccagaggcgtacagggatagcac-3′.