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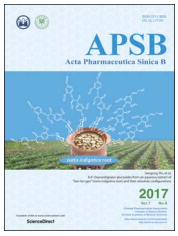

ORIGINAL ARTICLE

Garlic-derived compound *S*-allylmercaptocysteine inhibits hepatocarcinogenesis through targeting LRP6/Wnt pathway

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KEY WORDS

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Abstract Whether and how garlic-derived *S*-allylmercaptocysteine (SAMC) inhibits hepatocellular carcinoma (HCC) is largely unknown. In the current study, the role of low-density lipoprotein receptor (LDLR)-related protein 6 (LRP6) in HCC progression and the anti-HCC mechanism of SAMC was examined in clinical sample, cell model and xenograft/orthotopic mouse models. We demonstrated that SAMC inhibited

Abbreviations: Axin1, axis inhibition protein 1; DKK-1, Dickkopf Wnt signaling pathway inhibitor 1; DVL2, disheveled 2; FADD, Fas-associated protein with death domain; HCC, hepatocellular carcinoma; KD, knock-down; LDH, lactate dehydrogenase; LRP6, low-density lipoprotein receptor (LDLR)-related protein 6; MCL-1, myeloid cell leukemin-1; NAFLD, non-alcoholic fatty liver disease; PCNA, proliferating cell nuclear antigen; SAC, *S*-allylcysteine; SAMC, *S*-allylmercaptocysteine; SPR, surface plasmon resonance; TCF/LEF, T-cell factor/lymphoid enhancing factor; TSA, thermal shift assay; T_m, melting temperature

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LRP6;
Human;
Nude mice

cell proliferation and tumorigenesis, while induced apoptosis of human HCC cells without influencing normal hepatocytes. SAMC directly interacted with Wnt-pathway co-receptor LRP6 on the cell membrane. LRP6 was frequently over-expressed in the tumor tissue of human HCC patients (66.7% of 48 patients) and its over-expression only correlated with the over-expression of β-catenin, but not with age, gender, tumor size, stage and metastasis. Deficiency or over-expression of LRP6 in hepatoma cells could partly mimic or counteract the anti-tumor properties of SAMC, respectively. *In vivo* administration of SAMC significantly suppressed the growth of Huh-7 xenograft/orthotopic HCC tumor without causing undesirable side effects. In addition, stable down-regulation of LRP6 in Huh-7 facilitated the anti-HCC effects of SAMC. In conclusion, LRP6 can be a potential therapeutic target of HCC. SAMC is a promising specific anti-tumor agent for treating HCC subtypes with Wnt activation at the hepatoma cell surface.

1. Introduction

Currently, liver cancer is the sixth most common malignant disease and the second leading cause of cancer death worldwide. There are approximately 50.5% of new liver cancer cases in China in each year, ~75% of which is hepatocytes-derived hepatocellular carcinoma (HCC)[1]. The survival rate after the onset of HCC symptoms is generally less than one year and as to date no effective clinical therapeutic strategy with desirable effects has been developed[2]. Therefore, elucidating the molecular mechanisms on the initiation and progression of HCC is critical for the control of this fatal disease.

The canonical Wnt/β-catenin pathway is aberrantly activated in HCC[3]. Activation of the Wnt pathway is through the binding of Wnt family proteins (*e.g.*, Wnt3a) to the cell surface receptors low-density lipoprotein receptor (LDLR)-related protein 5 (LRP5) and/or LRP6. After that, phosphorylated receptors recruit disheveled homologue proteins, *e.g.*, disheveled 2/3 (DVL2/3), and axis inhibition protein 1 (axin1) to stabilize and promote the nuclear translocation of β-catenin, which acts as a transactivator of T-cell factor/lymphoid enhancing factor (TCF/LEF) transcription factors to regulate the expression of key genes for cell proliferation, differentiation, and tumorigenesis[4]. In the liver, many temporal roles of the Wnt/β-catenin pathway have been identified during its development and maintenance of physiological homeostasis[5]. Emerging evidence suggests that dysregulated signaling of the Wnt/β-catenin pathway lead to hepatic carcinogenesis[6–8]. Recently, LRP6 has been identified as a novel nutritional therapeutic target for several liver diseases, including non-alcoholic fatty liver disease (NAFLD) and hyperlipidemia[9] while another study also found that up-regulation of LRP6 was associated with enhanced hepatic carcinogenesis and cell invasion[10]. Therefore, we hypothesized that LRP6 might be a direct target for nutraceutical agents with anti-HCC properties.

Garlic is used as a medicinal food for its anti-bacterial, immunoregulatory and anti-tumor properties in many countries for more than 2000 years[11]. Epidemiological studies indicate an association between garlic consumption and decreased risk of gastrointestinal tract cancers[11]. *S*-allylmercaptocysteine (SAMC) is a water-soluble active compound derived from aged garlic. We have demonstrated its potent hepato-protective properties and mechanisms in acute liver injury and NAFLD[12,13]. Its anti-tumor effects have been demonstrated in colon cancer[14], prostate cancer[15,16], bladder cancer[17], breast cancer[18], and gastric cell cancer[19]. A very recent study found that SAMC induced apoptosis in human HepG2 cell through targeting the cross-talk between the transforming growth factor-β and the mitogen-activated protein kinase pathways[20]. However, mechanistic data regarding the detailed anti-HCC functions of SAMC, particularly its "immediate receptor" when in contact with the tumor cell, is lacking. Therefore, in the current study, we aimed to investigate the anti-tumor effects and mechanisms of SAMC in human and mouse HCC cell lines and xenograft/orthotopic models, with emphasis in its direct target on the cell membrane.

2. Materials and methods

2.1. Patient samples and analysis

Use of human tissue samples in this project was approved by the Ethical Committee of Shenzhen Third People's Hospital. All patients were given formal notification and written consent on the use of the clinical specimens for research. Forty-eight pairs of HCC tissues and their corresponding non-tumorous liver tissues (1 cm away from the tumor), as well as 6 liver tissues from healthy people, were employed for analyses. The clinicopathological features of all these patients are listed in Supplementary information Table S1.

2.2. Generation of LRP6 rescue and over-expressed constructs

The cloning and generation of a codon-modified shRNA-resistant *LRP6* (*LRP6* rescue) construct was conducted as previously reported[21].

2.3. GST–E-cadherin pull-down assay

The GST–E-cadherin pull-down assay was performed as previously described[22]. Western blotting was performed using an antibody to β-catenin.

2.4. Surface plasmon resonance (SPR) and thermal shift assay (TSA)

Analysis of direct binding between SAMC and LRP6 protein was performed by using SPR and TSA as previously described[23]. Apparent equilibrium dissociation constants (K_d) were then