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Original Article

CHSY1 promotes aggressive phenotypes of hepatocellular carcinoma cells via activation of the hedgehog signaling pathway

Chiung-Hui Liu [a, b], Chyn-Tair Lan [a, b], Jui-Feng Chou [a], To-Jung Tseng [a, b, **], Wen-Chieh Liao [a, b, *]

[a] Department of Anatomy, Faculty of Medicine, Chung Shan Medical University, No.110, Sec.1, Jianguo N. Rd, Taichung, Taiwan
[b] Department of Medical Education, Chung Shan Medical University Hospital, No.110, Sec.1, Jianguo N. Rd, Taichung, Taiwan

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ABSTRACT

Abnormal expression of chondroitin sulfate has been found in many types of cancer, while its biological functions in hepatocellular carcinoma (HCC) progression remain uninvestigated. Here, we report that chondroitin sulfate synthase 1 (CHSY1), the enzyme that mediates the polymerization step of chondroitin sulfate, is a critical mediator of malignant character in HCC that acts via modulating the activity of the hedgehog signaling. CHSY1 was up-regulated frequently in HCC where these events were associated with worse histologic grade and poor survival. Enforced expression of CHSY1 was sufficient to enhance cell growth, migration, invasion, and epithelial-mesenchymal transition, whereas silencing of CHSY1 suppressed these malignant phenotypes. Mechanistic investigations revealed that the increase of cell surface chondroitin sulfate by CHSY1 promoted sonic hedgehog binding and signaling. Inhibiting hedgehog pathway with vismodegib decreased CHSY1-induced migration, invasion, and lung metastasis of HCC cells, establishing the critical role of hedgehog signaling in mediating the effects of CHSY1 expression. Together, our results indicate that CHSY1 overexpression in HCC contributes to the malignant behaviors in cancer cells, we provide novel insights into the significance of chondroitin sulfate in hedgehog signaling and HCC pathogenesis.

Introduction

Hepatocellular carcinoma (HCC) is one of the most prevalent human cancers, and it's being the third-leading cause of cancer death worldwide [1]. The high mortality of this disease mainly attributes to late diagnosis and limited treatment for advanced HCC. Abnormal expression and composition of extracellular matrix (ECM) molecules in tumor microenvironment are hallmarks of cancer [2]. Glycosaminoglycans (GAGs) are unbranched polysaccharide chains which abundant in the ECM of tumor as well as on the surface of cancer cells. GAGs exist as free chains or covalently link to core protein are known as proteoglycans (PG). Although comprehensive genomic and proteomic analyses have identified many key drivers of HCC [3], the complex interactions between cells in tumor and GAG chains are difficult to directly decipher form genomic information. Thus, study roles of GAG in tumor microenvironment may discover new molecular mechanisms underlying HCC.

Accumulated knowledge about GAGs and PG demonstrated that they can regulate cell growth, differentiation, morphogenesis, cell migration, and bacterial/viral infections [4]. Recent studies also indicated that altered structure of GAG is associated with cancer progression and can be taken as biomarkers for disease diagnosis as well as pharmacological targets [5–8]. Chondroitin sulfate (CS) is one of the major type of GAGs. In the past, functions of CS chains were considered only in structure stabilization. Recently, due to many growth factors, proteases, cytokines, chemokines, and adhesion molecules have been found interacting with CS chains, the importance of CS chains in cancer progression has been reevaluated [9–11]. For instance, CS chains in melanoma cells can enhance MMP2 activation, and promote angiogenesis,

Abbreviations: hepatocellular carcinoma, HCC; extracellular matrix, ECM; Glycosaminoglycans, GAGs; proteoglycans, PG; Chondroitin sulfate, CS; sonic hedgehog, SHH; epithelial-mesenchymal transition, EMT.

* Corresponding author. Department of Anatomy, Faculty of Medicine, Chung Shan Medical University, No.110, Sec.1, Jianguo N. Rd, Taichung, Taiwan.
** Corresponding author. Department of Anatomy, Faculty of Medicine, Chung Shan Medical University, No.110, Sec.1, Jianguo N. Rd, Taichung, Taiwan.
E-mail addresses: tjtseng@csmu.edu.tw (T.-J. Tseng), aliceliudog@gmail.com (W.-C. Liao).

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proliferation, as well as cell invasion [12,13]. Highly sulfated CS chains on cell surface could promote metastatic process of lung cancer cells [14] and osteosarcoma cells [15]. Chondroitin sulfate-E was found strongly expressed in ovarian carcinoma and promoted VEGF binding [16].

In human, the biosynthesis of CS chains initiate from *N*-acetylgalactosamine linking to a tetrasaccharide structure by CSGALNACT1 or CSGALNACT2 transferases. Next, the polymerization step is catalyzed by a group of bifunctional enzymes (CHSY1, CHSY2/CHPF, and CHSY3) which have both β1–3 glucuronosyltransferase and β1–4 *N*-acetylgalactosaminyltransferase activities (Fig. 1A). These GlcA-GalNAc repeats can be esterified by sulfate at various positions by a sulfurtransferase family (4-*O*-Sulfotransferase, 6-*O*-Sulfotransferase, and 2-*O*-Sulfotransferase). In addition, C5 epimerase exist for epimerization of glucuronic acid to iduronic acid which consider as dermatan sulfate [17]. Various modifications of CS depend on the expression of these enzymes, making a single CS chains usually composed by different sulfated units, and leading enormous structural diversity and complexity [18].

In HCC, it has been reported that expression of CS chains are increased in a rat hepatocarcinogenesis model [19]. A previous study also indicated that CS chains overexpressed in HCC, and altered sulfation status were associated with poorly histological grade [20]. Importantly, a recent study reported that a distinct modification of "oncofetal CS chains" is highly expressed on many types of cancer, including HCC, which can be used as a marker for cancer diagnosis or target therapy [8]. Although the expression of CS chains in HCC has been investigated in several groups, which enzymes alter the expression of CS chains, and the biological functions of CS chains in HCC progression remain uninvestigated.

Materials and methods

Cell culture

Liver cancer cell lines, HA59T, HA22T, HepG2, and Hepa1-6 were purchased from Bioresource Collection and Research Center in the year 2014 (Hsinchu, Taiwan). HCC36 cells were kindly provided by Prof. Lei Wan (China Medical University). Cells were cultured in DMEM containing 10% FBS in 5% CO_2 at 37 °C.

Reagents and antibodies

Recombinant HGF, TGF-β, and sonic hedgehog (SHH) protein were purchased from ProSpec. Hedgehog antagonist vismodegib (GDC-0449) was purchased from LC Laboratories, and dissolved in DMSO (Sigma).

Full length CHSY1 cDNA clone and antibodies against CHSY1 were purchased from OriGene. Antibodies against E-cadherin, ZO-1, Vimentin, p-Smad2/3, Smad2/3, p-AKT, p-ERK1/2, and ERK1/2 were purchased from Cell Signaling Technology, Inc. Antibodies against total AKT, chondroitin sulfate (CS56), and actin were purchased from GeneTex, Inc. Antibodies against His tag was purchased from Santa Cruz

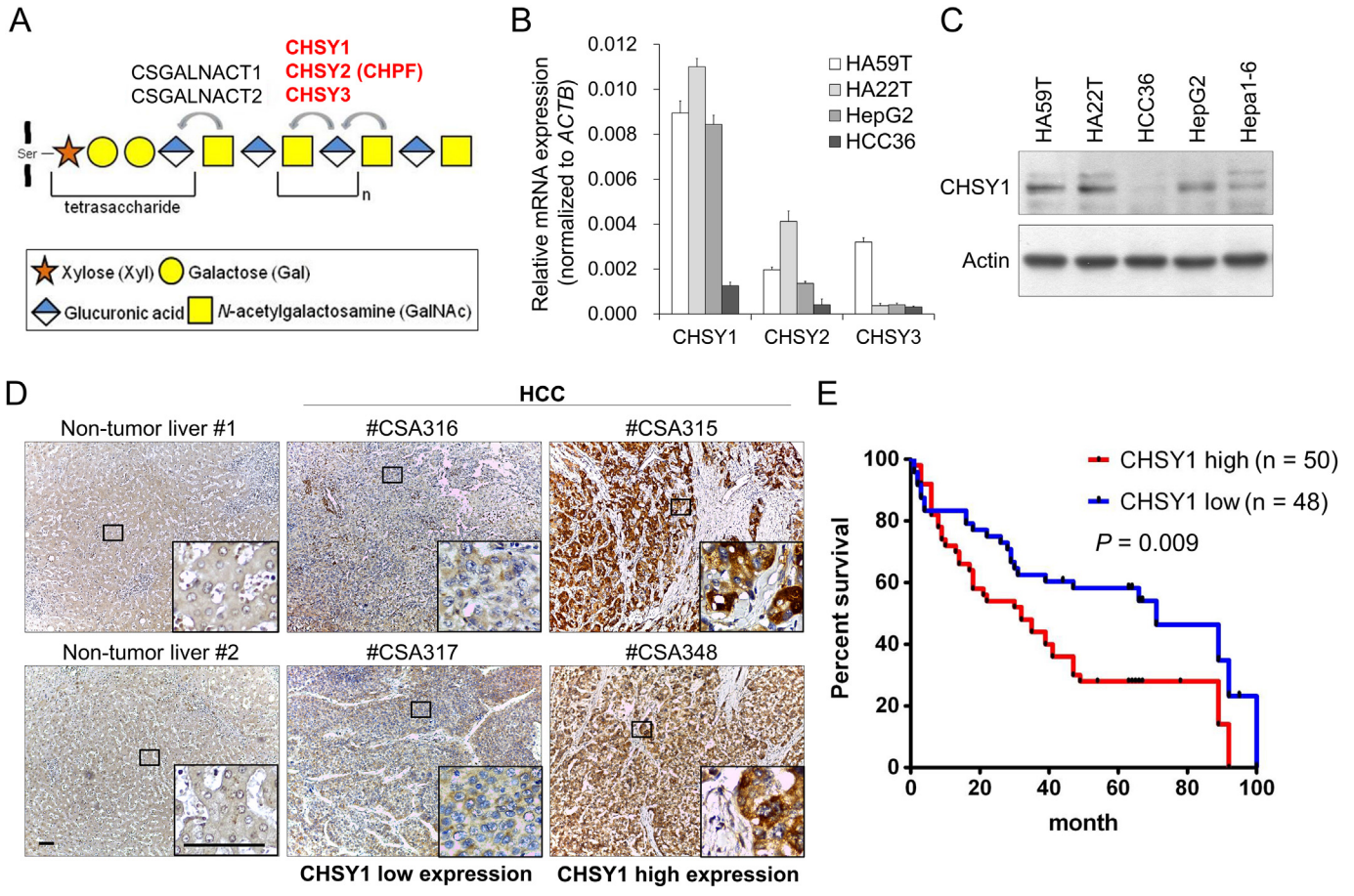

Fig. 1. Expression of CHSY1 in human HCC. (A) Biosynthesis of chondroitin sulfate initiates from *N*-acetylgalactosamine linking to a tetrasaccharide structure by CSGALNACT1 or CSGALNACT2 transferases. Next, the polymerization step is catalyzed by a group of bifunctional enzymes (CHSY1, CHSY2/CHPF, and CHSY3) that have both β1–3 glucuronosyltransferase and β1–4 *N*-acetylgalactosaminyltransferase activities. (B) Expression of *CHSY1*, *CHSY2*, and *CHSY3* mRNA in four human HCC cell lines. The mRNA levels were analyzed by real-time RT-PCR and normalized to *ACTB*. Experiment was done in triplicate and mean ± SD was shown. (C) Expression of CHSY1 in HCC cell lines. The CHSY1 protein expression was analyzed by Western blotting. Actin was used as an internal control. (D) Immunohistochemistry of CHSY1 in non-tumor liver tissue and primary HCC tissue arrays contains 98 cases. All sections were counterstained with hematoxylin. Representative images of CHSY1 low expression cases (meddle) and CHSY1 high expression cases (right) were shown. Amplified images were shown at the bottom right of each image. Scale bars, 150 μm. (E) Kaplan–Meier analysis of overall survival for patients with HCC. The analyses were conducted according to the immunohistochemistry of CHSY1 and the survival information provided by supplier. Log-rank test, $P = 0.009$.