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Stearoyl-CoA desaturase plays an important role in proliferation and chemoresistance in human hepatocellular carcinoma

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ABSTRACT

Background: Hepatocellular carcinoma (HCC) is often diagnosed at an advanced stage, when it is not amenable for aggressive therapies such as surgical resection or liver transplantation. Current therapeutic options achieve clinical responses in only a small percentage of cases. As a consequence, effective approaches for prevention and treatment are greatly needed. Altered lipid metabolism has been recently linked to HCC pathogenesis. The aims of this study were to define the cellular and molecular mechanisms linking stearoyl-CoA desaturase (SCD), the rate-limiting enzyme and an essential regulator of lipid homeostasis in liver cells, to carcinogenesis in HCC.

Material and methods: HCC and normal liver specimens were collected. Human HCC cell lines: HepG2, Hep3B, and PLC/PLF/5 were used for immunoblot, cell viability, proliferation, and apoptosis assays. Small interfering RNAs were used for genetic inhibition, and 10, 12 conjugated linoleic acid was used for pharmacologic SCD inhibition.

Results: SCD was strongly expressed in surgically resected HCC ($n = 64$) and various human HCC cell lines (HepG2, Hep3B, and PLC/PLF/5). The levels of SCD negatively correlated with degree of tumor differentiation ($P < 0.01$). Treatment of these HCC cell lines with a panel of chemotherapeutic drugs resulted in a time-dependent, phosphatidylinositol 3 kinase- and c-Jun N-terminal kinases1/2–mediated upregulation of SCD expression, which paralleled the degree of resistance to drug-induced apoptosis. Specific genetic or pharmacologic SCD suppression resulted in inhibition of cell proliferation ($P < 0.001$) and significantly increased sensitivity to chemotherapy-induced apoptosis.

Conclusions: Our data suggest that increased SCD expression plays an important role in HCC development and resistance to chemotherapy-induced apoptosis, and this is in part mediated by phosphatidylinositol 3 kinase/c-Jun N-terminal kinases activation. Specific targeted interruption of this pathway in HCC could be a desirable approach in designing novel therapeutic strategies.

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1. Introduction

Hepatocellular carcinoma (HCC), the third leading cause of cancer death worldwide, is often diagnosed at an advanced stage, when it is not amenable for aggressive therapies such as surgical resection or liver transplantation [1–3]. There is currently no reliably effective therapy for patients with advanced or metastatic disease, and standard chemotherapeutic agents such as doxorubicin or 5-fluorouracil (5-FU) have relatively low response rates of only 10%–20% with no effect on survival [1–6].

Long-chain free fatty acids (FFA) are biologically active molecules that not only serve as a source of metabolic energy and substrates for cell and organelle membranes but also play a key role in fundamental cell processes such as cell growth and proliferation as well as apoptotic cell death [7,8]. The apoptosis effect appears to be specific for saturated fatty acid (SFA) [9,10]. Altered lipid metabolism, characterized by increased endogenous fatty acid (FA) synthesis, has been linked to HCC pathogenesis and several other human malignancies including breast, prostate, colon, and lung cancers [11–13].

Stearoyl-CoA desaturase (SCD), predominantly located in endoplasmic reticulum, is a key regulator of intracellular FA composition and catalyzes the conversion of SFA into monounsaturated FAs [14,15]. This enzyme facilitates the channeling of both exogenous (uptake from circulation) and endogenous (from *de novo* lipogenesis) FFA into triglyceride storage, phospholipids, and cholesterol ester synthesis [16]. By doing this, SCD may have a dual role in cell survival by protecting against SFA-induced lipotoxicity and at the same time favor cell growth and proliferation [17,18]. However, very little is known regarding the relationship between SCD, HCC survival, and resistance to apoptotic cell death.

We hypothesized that SCD plays an important role in cell growth and proliferation in HCC and confers resistance to chemotherapy when HCC is exposed to conventional chemotherapeutic agents. In this study, we sought to investigate the expression and role of SCD in human HCC and its role in resistance to chemotherapy-induced apoptosis.

2. Materials and methods

Institutional review board approval was obtained as per the institution protocols.

2.1. Cell lines, tissues, and reagents

Human hepatoma cell lines HepG2, Hep3B, and PLC/PRF/5 were purchased from ATCC (Manassas, VA). The cells were grown in Dulbecco's modified Eagle medium supplemented with 10% fetal bovine serum (Sigma, St. Louis, MO), 100 U/mL of penicillin, and 100 mg/L of streptomycin at 37°C in 5% CO_2 in a humidified incubator. The cells were plated at 600,000 cells per 100-mm tissue culture dish and allowed to adhere overnight (18 h) before any treatment began. The cells were incubated with three chemotherapeutic agents: Staurosporine (STS; 1 μmol/L) (Calbiochem, San Diego, CA), 5-FU (10 μg/mL; Sigma), and doxorubicin (1 μg/mL; Sigma) for up to 24 h. Pooled human-liver microsomal protein from normal human hepatocytes (BD Biosciences, Woburn, MA) served as control. Sixty-four paraffin-fixed HCC tissue samples and 10 normal human liver tissue samples, obtained from adjacent to hepatic metastatic tumors, were obtained from the Department of Pathology, Cleveland Clinic. Selective SCD inhibitor 10, 12 conjugated linoleic acid (10, 12 CLA) and 9, 11 CLA were from Matreya (Pleasant Gap, PA). The c-Jun N-terminal kinases (JNK)1/2 inhibitor SP600125 was from Biomol International (Plymouth, PA), the p38 mitogen-activated protein kinases (MAPK) inhibitor SB203580 was from Calbiochem (San Diego, CA), and the phosphatidylinositol 3 kinase (PI3K) inhibitor LY294002 was purchased from Cell signaling Technology (Beverly, MA).

2.2. Immunoblot analysis

For whole cell lysates, cells were homogenized in a lysis buffer containing 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate (SDS), 10 μg/mL of aprotinin, 100 μg/mL of phenylmethylsulfonyl fluoride, 1 mmol/L of sodium orthovanadate, 50 mmol/L of sodium fluoride, 5 μg/mL of pepstein, 5 μg/mL of leupeptin, 2 mmol/L of Pefabloc (4-(2-Aminoethyl) benzenesulfonyl fluoride hydrochloride; Sigma Aldrich), and phosphate-buffered saline (PBS), pH 7.4. Nuclear protein extracts were prepared as previously described [19]. Total or nuclear protein of 20 μg per lane was electrophoretically separated by SDS–8% polyacrylamide gels, transferred to nitrocellulose membrane, and probed with anti–SCD monoclonal antibody (Ab) or anti–sterol regulatory element–binding protein 1 (SREBP-1) monoclonal Ab (Abcam, Cambridge, MA). The blots were developed by an enhanced chemiluminescence system (Immune-Star HRP substrate kit, BioRad Laboratories, Hercules, CA). As a control for sample loading, the blot was stripped and reprobed with β-actin or anti–glyceraldehyde-3-phosphate dehydrogenase (GAPDH) polyclonal Ab (Ambion, Austin, TX). Optical densities of bands in each blot were analyzed using the Image-Pro software.

2.3. Immunohistochemistry

Immunohistochemistry was performed using an anti–SCD monoclonal Ab (Abcam). The samples were incubated with primary Ab 1:100 in the blocking solution for 3 h at room temperature. After washing with PBS, the sections were incubated with the ready-to-use secondary Ab (biotin-labeled affinity-isolated goat anti-rabbit immunoglobulin; DAKO Corporation, Carpinteria, CA) for 30 min at room temperature. After washing in PBS, the samples were incubated with a ready-to-use streptavidin peroxidase conjugate in PBS-containing carrier protein and anti-microbial agents (DAKO corporation) for 30 min at room temperature. After washing with PBS, the samples were stained with 3,3′-diaminobenzidine (vector) for 2–5 min, washed in PBS, counterstained with hematoxylin for 2–3 min, and dehydrated by transferring them through increasing ethanol solutions (30%, 50%, 70%, 80%, 95%, and 100% ethanol). After dehydration, the slices were soaked twice in a xylene bath at room temperature for 5 min, respectively, mounted, and examined. Immunohistochemical results were semiquantitatively evaluated in a blinded fashion using a four-