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# Curcumin raises lipid content by Wnt pathway in hepatic stellate cell

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ABSTRACT

*Background:* Activation of hepatic stellate cells (HSCs) is a pivotal event in liver fibrosis, which is characterized by dramatic disappearance of lipid droplets. However, the underlying molecular mechanisms are largely unknown. We aimed to explore the role of Wnt/β-catenin pathway in HSC lipogenesis and to examine the effects of curcumin in this molecular context.

*Methods:* Primary rat HSCs were cultured *in vitro* for experiments. The Wnt activator WAY-262611 and β-catenin activator lithium chloride (LiCl) were used to activate the pathway at distinct levels in HSCs. Cell proliferation, fibrogenic markers, intracellular lipids and triglyceride, and adipogenic transcription factors were examined in HSCs.

*Results:* Both WAY-262611 and LiCl promoted proliferation and upregulated the expression of α-smooth muscle actin and α1(I) procollagen, but they decreased the contents of intracellular lipids and triglyceride in HSCs. Analyses of adipogenic transcription pattern showed that the two compounds reduced the expression of peroxisome proliferator–activated receptor γ, CCAAT/enhancer binding protein α, retinoid X receptor-α, and retinoic acid receptor-β, four key transcription regulators of HSC adipogenic phenotype. Curcumin also reduced the expression of Frizzled and β-catenin, upregulated the expression of adipogenic transcription factors, and restored lipid content in HSCs. However, both WAY-262611 and LiCl abrogated curcumin restoration of lipogenesis and inhibition of fibrogenic marker expression in HSCs.

*Conclusions:* Wnt/β-catenin pathway was a profibrogenic signaling and inhibited lipogenesis by suppressing adipogenic transcription pattern in HSCs. Blockade of this pathway was associated with curcumin stimulation of HSC lipogenesis. We revealed a novel mechanism underlying curcumin restoration of lipid droplets during HSC activation.



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## 1. Introduction

Liver fibrosis is a reversible wound-healing response following liver injury. Hepatic stellate cells (HSCs) play a pivotal role in liver wound healing through fibrogenic regulation. HSCs normally maintain a quiescent or lipocyte phenotype characterized by storage of lipid droplets in the cytoplasm. As a consequence of liver injuries, HSCs display a myofibroblastic phenotype in which they proliferate more rapidly and lose their lipid droplets accompanied by excessive production of extracellular matrix that entails changes in hepatic architecture [1]. Notably, disappearance of lipid droplets is considered one of the hallmarks of HSC activation [2]. It is recognized that transcriptional regulation is required for the adipogenic phenotype of HSCs. The major transcription factors involved in HSC adipocyte differentiation include peroxisome proliferator–activated receptor γ (PPARγ), CCAAT/enhancer binding protein α (C/EBPα), retinoid X receptor-α (RXRα), and retinoic acid receptor-β (RARβ) [3]. Expression of these transcription factors is abundant in quiescent HSCs but is lost on activation [3]. However, little is known about how the repressed lipogenesis affects HSC activation and differentiation.

The role of the Wnt signaling in liver biology has come to the forefront over the last few years, and this pathway has turned out to be among the central players in maintaining liver health [4]. β-Catenin is the chief downstream effector of the canonical Wnt signaling [5]. Dickkopf-1 (Dkk1) is identified as an endogenous inhibitor of Wnt pathway, which disrupts the formation of heterodimeric receptor complex consisting of Frizzled and the low density lipoprotein receptor-related protein 5/6 (LRP5/6) [5]. Recent evidence revealed that activation of Wnt/β-catenin signaling inhibited the differentiation and lipogenesis in 3T3-L1 adipocytes [6]. Another study demonstrated that Wnt signaling was critically involved in *de novo* lipogenesis and adipogenesis in nonalcoholic fatty liver disease [7]. These emerging data give the possibility that the Wnt/β-catenin signaling could regulate the adipogenic phenotype of HSCs. We and others previously demonstrated that the natural compound curcumin could be a therapeutic remedy for liver fibrosis [8–10]. Interestingly, it was reported that curcumin could accumulate intracellular lipids in activated HSCs [11]. However, the underlying mechanisms remain to be elucidated. To this end, the present study aimed to investigate the role of Wnt/β-catenin pathway in regulation of lipogenesis in HSCs and to examine the effects of curcumin in this molecular context.

## 2. Materials and methods

### 2.1. Reagents and antibodies

The following compounds were used in this study: WAY-262611 (EMD Millipore, Billerica, MA), lithium chloride (LiCl), and curcumin (Sigma, St Louis, MO). WAY-262611 and curcumin were dissolved in dimethyl sulfoxide, and LiCl was dissolved in phosphate-buffered saline for experiments. The following primary antibodies were used in this study: α-smooth muscle actin (α-SMA) and α1(I)procollagen (Epitomics, San Francisco, CA); PPARγ, C/EBPα, RXRα, RARβ, Frizzled, β-catenin, and β-actin (Cell Signaling Technology, Danvers, MA).

### 2.2. Cell culture

Primary rat HSCs were obtained from Jiangyin CHI Scientific, Inc (Wuxi, China). HSCs were cultured in Dulbecco-modified eagle medium (DMEM; Invitrogen, Grand Island, NY) with 10% fetal bovine serum (FBS; Wisent Biotechnology Co, Ltd, Nanjing, China), 1% antibiotics, and grown in a 5% $CO_2$ humidified atmosphere at 37°C. Cell morphology was assessed with an inverted microscope with a Leica QWin System (Leica, Solms, Germany).

### 2.3. Cell proliferation assay

HSCs were seeded in 96-well plates and cultured in DMEM with 10% FBS for 24 h, and then were treated with vehicles, WAY-262611, or LiCl at indicated concentrations for 24 h. Then, the medium was replaced with 100-μL phosphate-buffered saline containing 0.5 mg/mL 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide incubating at 37°C for 4 h. Next, the crystals were dissolved with 200-μL dimethyl sulfoxide. The spectrophotometric absorbance at 490 nm was measured by a SPECTRAmax microplate spectrophotometer (Molecular Devices, Sunnyvale, CA). Results were from three independent experiments, and each experiment had six replicates.

### 2.4. Oil red O staining

HSCs were seeded in 6-well plates and cultured in DMEM with 10% FBS for 24 h, and then were treated with vehicles, WAY-262611, LiCl, and/or curcumin at indicated concentrations for 24 h. HSCs were stained with oil red O reagents (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) to visualize the lipids with a light microscope (×200 amplification). Lipids in HSCs were colored dark red by oil red O. Images were taken in a blinded fashion at random fields. Results were from triplicate experiments.

### 2.5. Intracellular triglyceride determination

HSCs were seeded in 6-well plates and cultured in DMEM with 10% FBS for 24 h, and then were treated with vehicles, WAY-262611, or LiCl at indicated concentrations for 24 h. Levels of intracellular triglyceride (TG) were colorimetrically determined using assay kits (Nanjing Jiancheng Bioengineering Institute) following the protocol provided by the manufacturer. The intracellular TG levels were expressed as fold changes of the control group. Results were from triplicate experiments.

### 2.6. Western blot analyses

Whole cell protein extracts were prepared from cultured cells or liver tissues. The protein levels were determined using a bicinchoninic acid (BCA) assay kit (Thermo Scientific Pierce, Rockford, IL). Proteins (50 μg per well) were separated by sodium dodecyl sulfate (SDS)-polyacrylamide gel, transferred to a poly(vinylidene fluoride) (PVDF) membrane (Millipore,