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# Treatment-damaged hepatocellular carcinoma promotes activities of hepatic stellate cells and fibrosis through GDF15

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ABSTRACT

The aim of this study was to investigate whether treatment-damaged hepatocellular carcinoma (HCC) would accelerate liver cirrhosis through promoting the activities of hepatic stellate cells (HSCs). HCC cells were exposed to chemotherapeutic agent or hypoxia to mimic the transarterial chemoembolization (TACE)-like treatment. Growth differentiation factor 15 (GDF15) expression was increased in cisplatin- or hypoxia-treated HCC cells. Treatment-induced GDF15 increase in HCC cells was mediated by p38MAPK, JNK, ERK1/2 activation. GDF15 from treatment-damaged HCC cells enhanced the proliferation and collagen synthesis of HSCs through ERK1/2- and Smad3-dependent pathways. Metformin significantly reduced the GDF15 production from treatment-damaged HCC cells by targeting JNK. The use of metformin could attenuate the *in vivo* fibrotic activities of HSCs promoted by treatment-damaged HCC cells and inhibit GDF15 expression. In conclusion, treatment-damaged HCC accelerates fibrosis by promoting the activities of HSCs *via* GDF15 secretion, which could be reversed by metformin. This provides a potential therapeutic target for alleviating TACE-aggravated liver cirrhosis.

## 1. Introduction

Hepatocellular carcinoma (HCC) is one of the highly prevalent cancers in the world [1]. Most of patients are diagnosed at the intermediate-advanced stage ineligible for curative therapies. In those cases, transarterial chemoembolization (TACE) is the mainstay of non-surgical treatments. Two randomized controlled trials have shown that TACE provides survival advantages for patients with unresectable HCC [2,3]. TACE exerts its antitumor effect by damage to tumor through intra-arterial chemotherapy with embolization-induced ischemia. However, TACE is a non-curative procedure with the low complete response rate [4]. Patients are required to undergo repeated TACE courses of treating the residual viable tumor and tumor recurrence. Because HCC often occurs in the underlying fibrotic or cirrhotic liver, repeated TACE in the cirrhotic liver will further deteriorate liver function [5–9]. The benefits of TACE derived from tumor control may be offset by the worsening hepatic dysfunction. Therefore, how to avoid or alleviate the exacerbation of liver damage during TACE could reduce liver-related morbidity and ultimately improve patients' outcomes.

The degree of cirrhosis directly influences liver function and survival of patients. The long-term effects of TACE procedure on liver tissue and preserved liver function have been reported [6–10]. The significant atrophy of nontumorous liver caused by TACE has been observed, suggesting that TACE can aggravate liver cirrhosis [10]. Hepatic stellate cells (HSCs) are major fibrogenic cells in the liver [11]. In an animal study, TACE induced hepatocyte damage and fibrosis progression *via* HSCs activation, thus leading to the compromised liver function [12]. Since TACE chemotherapeutic and embolic agents are almost delivered to tumor, whether TACE treatment-damage to HCC would accelerate liver fibrosis remains unknown.

GDF15, a transforming growth factor (TGF)-β superfamily member, is a hallmark of stress response to various injuries (e.g. chemotherapy, hypoxia) [13,14]. Similar to TGF-β, GDF15 promotes the proliferation and expression of extracellular matrix in NIH3T3 fibroblasts [15]. TACE, a treatment of integrating chemotherapy and embolization-induced hypoxia to damage liver tumors, induces stress response in HCC

*Abbreviations:* TACE, transarterial chemoembolization; HCC, hepatocellular carcinoma; GDF15, growth-differentiation factor 15; HSCs, hepatic stellate cells; TGF, transforming growth factor; JNK, c-Jun N-terminal kinase; pHSCs, primary hepatic stellate cells; DMEM, Dulbecco's modified Eagle's medium; MAPK, mitogen activated protein kinases; ERK, extracellular regulated protein kinases; BMP7, bone morphogenetic protein-7; α-SMA, alpha-smooth muscle actin; FBS, fetal bovine serum; BSA, bovine serum albumin

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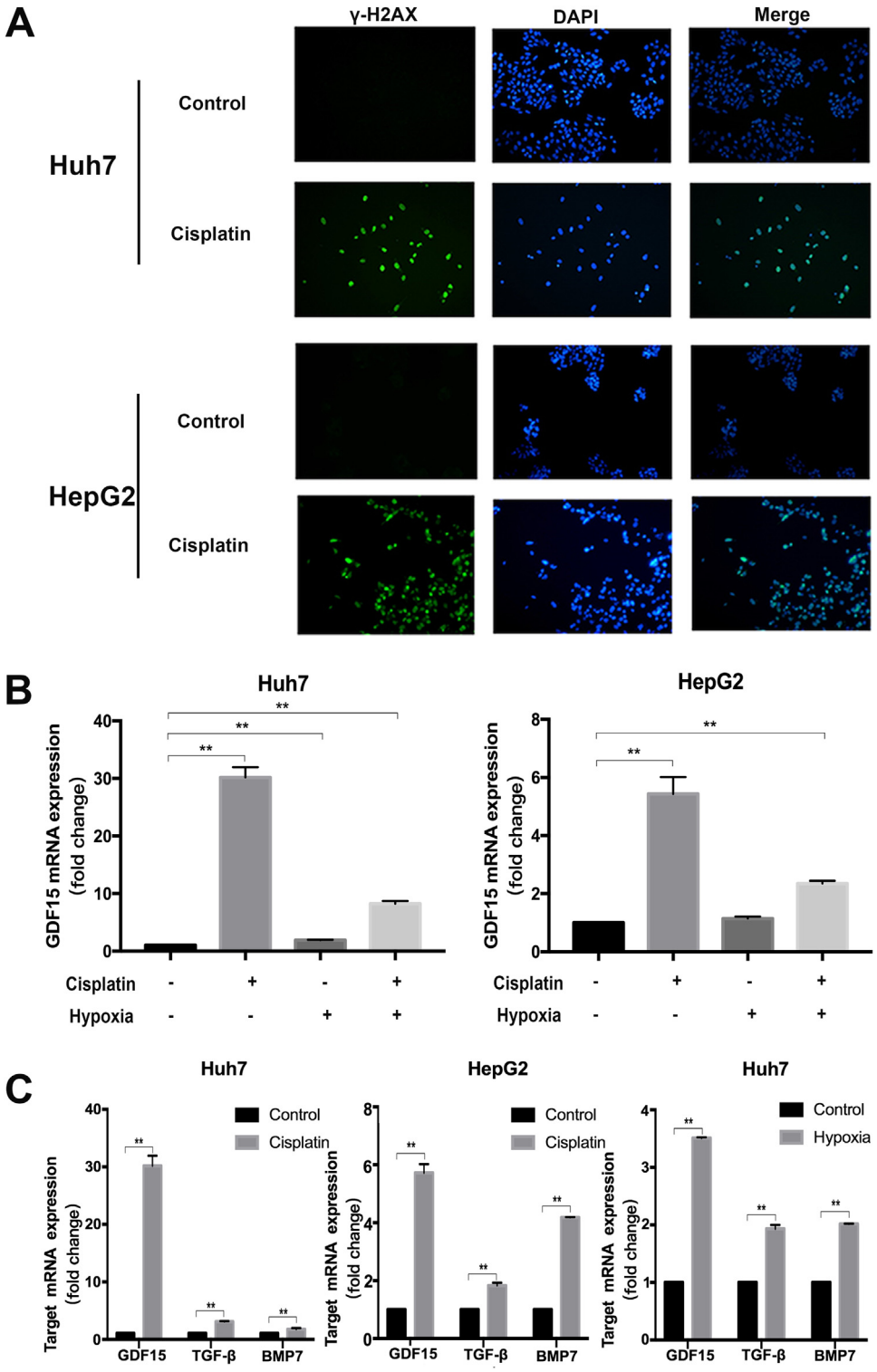


**Fig. 1. GDF15 upregulation in chemotherapy- or hypoxia-treated HCC cells. (A)** DNA damage foci in cisplatin-treated HCC cells (Huh7, HepG2). DNA damage was detected using the antibody recognizing γ-H2AX (green signals) and nuclei were counterstained with DIPA (blue). **(B)** GDF15 mRNA expression in cisplatin- or hypoxia-treated Huh7 and HepG2 cells. **(C)** The mRNA expression of GDF15, TGF-β and BMP7 in cisplatin or hypoxia-treated HCC cells. $^{*}P < 0.05$, $^{**}P < 0.01$.

tissue. This prompted us to hypothesize whether GDF15 derived from TACE treatment-damage to HCC would promote fibrotic activities by targeting HSCs. The present study investigated this hypothesis using in vitro and in vivo experiments with HCC cell lines under "TACE-like" conditions.

Herein, we demonstrated that (i) GDF15 was increased in chemotherapy- and hypoxia-treated HCC cells *via* P38MAPK, ERK1/2, JNK activation; (ii) GDF15 enhanced the proliferation and collagen production of HSCs through ERK1/2 and Smad3 pathways; (iii) metformin attenuated treatment-damaged-HCC-cells-promoted fibrosis *via* suppressing GDF15 expression.

From a perspective view of the interaction between treatment-damaged HCC cells and HSCs, this study provides a novel insight into the mechanism of treatment-accelerated liver cirrhosis in HCC patients