Biomedicine & Pharmacotherapy 98 (2018) 214–221

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

journal homepage: www.elsevier.com/locate/biopha

STAT3 aggravates TGF-β1-induced hepatic epithelial-to-mesenchymal transition and migration

Bin Wang[a,1], Ting Liu[b,1], Jun-Cheng Wu[b], Sheng-Zheng Luo[b], Rong Chen[b], Lun-Gen Lu[b], Ming-Yi Xu[b,*]

[a] Department of Gastroenterology, Yangpu Hospital, Tong Ji University, Shanghai, 200090, China
[b] Department of Gastroenterology, Shanghai General Hospital, Shanghai Jiao Tong University School of Medicine, 200080, Shanghai, China

ARTICLE INFO

Keywords:
Hepatocellular carcinoma (HCC)
Epithelial-to-mesenchymal transition (EMT)
Signal transducer and activator of transcription 3 (STAT3)
Transforming growth factor-β1 (TGF-β1)
Migration

ABSTRACT

Signal transducer and activator of transcription 3 (STAT3) has been shown to affect epithelial-to-mesenchymal transition (EMT) in cancers. We investigated the underlying molecular mechanisms of STAT3 crosstalk with Snail-Smad3/transforming growth factor (TGF)-β1 signaling pathways during the EMT in hepatocellular carcinoma (HCC). STAT3 and TGF-β1 expressions are examined in liver tissues of HCC patients and rats. The effect of IL-6/ STAT3 crosstalk with Snail-Smad3/TGF-β1 on EMT, carcinogenesis, migration and invasion are tested in vitro and in vivo. Phosphorylation of STAT3 and TGF-β1 proteins are universally high and positively co-expressed in HCC tissues from human and rats. Hepatic lower p-STAT3 proteins are related to earlier tumor stages in HCC patients. AG490 (a JAK2-specific inhibitor) treatment could reduce tumor numbers and sizes depending on suppression of STAT3 signaling in HCC rats. TGF-β1 could induce EMT along with an E-cadherin decrease, while vimentin, Snail, p-Smad2/3, and p-STAT3/STAT3 increase in HepG2. SIS3 (a specific inhibitor of Smad3) could markedly inhibit Snail, Vim and p-STAT3 along with blocking phosphorylation of Smad3, but E-cadherin could be activated in HepG2. IL-6 activates STAT3 signaling and then has cascading consequences for activating Snail-Smad3/TGF-β1 and vimentin as well as migration and invasion in liver cancer cells. In contrast, AG490 has an effect that inhibits phosphorylation of STAT3, lowers Snail-p-Smad3 protein levels, decreases TGF-β1-related PAI-1 promoter activation and then reduces migration or invasion of liver cancer cells. STAT3 functions as a positive regulator to activate TGF-β1-induced EMT and metastasis of HCC. STAT3 and the Snail-Smad3/TGF-β1 signaling pathways synergistically augment EMT and migration in HCC.

1. Introduction

Hepatocellular carcinoma (HCC) patients are often diagnosed at the metastatic stage. The high frequency of metastasis contributes to extremely poor prognosis for these patients. Therefore, identifying the molecular pathogenesis underlying HCC metastasis is critical so that new strategies could be provided for treating HCC. Growing evidence has demonstrated that epithelial-to-mesenchymal transition (EMT) is critical for proliferation, invasion and migration in HCC [1].

Signal transducer and activator of transcription 3 (STAT3) normally acts as a transcription factor for regulating cell proliferation, transformation and motility [2,3]. Inappropriate activation of the IL-6/STAT3 pathway can initiate EMT lead to HCC progression [4,5]. Transforming growth factor-β1 (TGF-β1) is a multifunctional cytokine and governs a sophisticated signaling network that has a dual role in tumor progression [6]. Aberrant TGF-β1 signaling probably has a pro-ontogenetic role in HCC occurrence [7] and is a well-characterized inducer of EMT in HCC.

Intriguingly, some recent studies indicate that there is a correlation between IL-6/STAT3 and TGF-β1 signaling-induced invasion in pancreatic [8] and lung cancer [9]. However, the intricate molecular mechanisms of STAT3, TGF-β1 and EMT in HCC remain unclear. Our study focuses on elucidating how STAT3 regulates TGF-β1-mediated EMT in HCC progression and tries to provide new perspectives for HCC therapies.

* Corresponding author at: Department of Gastroenterology, Shanghai General Hospital, Shanghai Jiao Tong University School of Medicine, No. 100, Haining Road, Shanghai, 200080, China.
E-mail address: xumingyi2014@163.com (M.-Y. Xu).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.biopha.2017.12.035
Received 24 July 2017; Received in revised form 10 December 2017; Accepted 13 December 2017

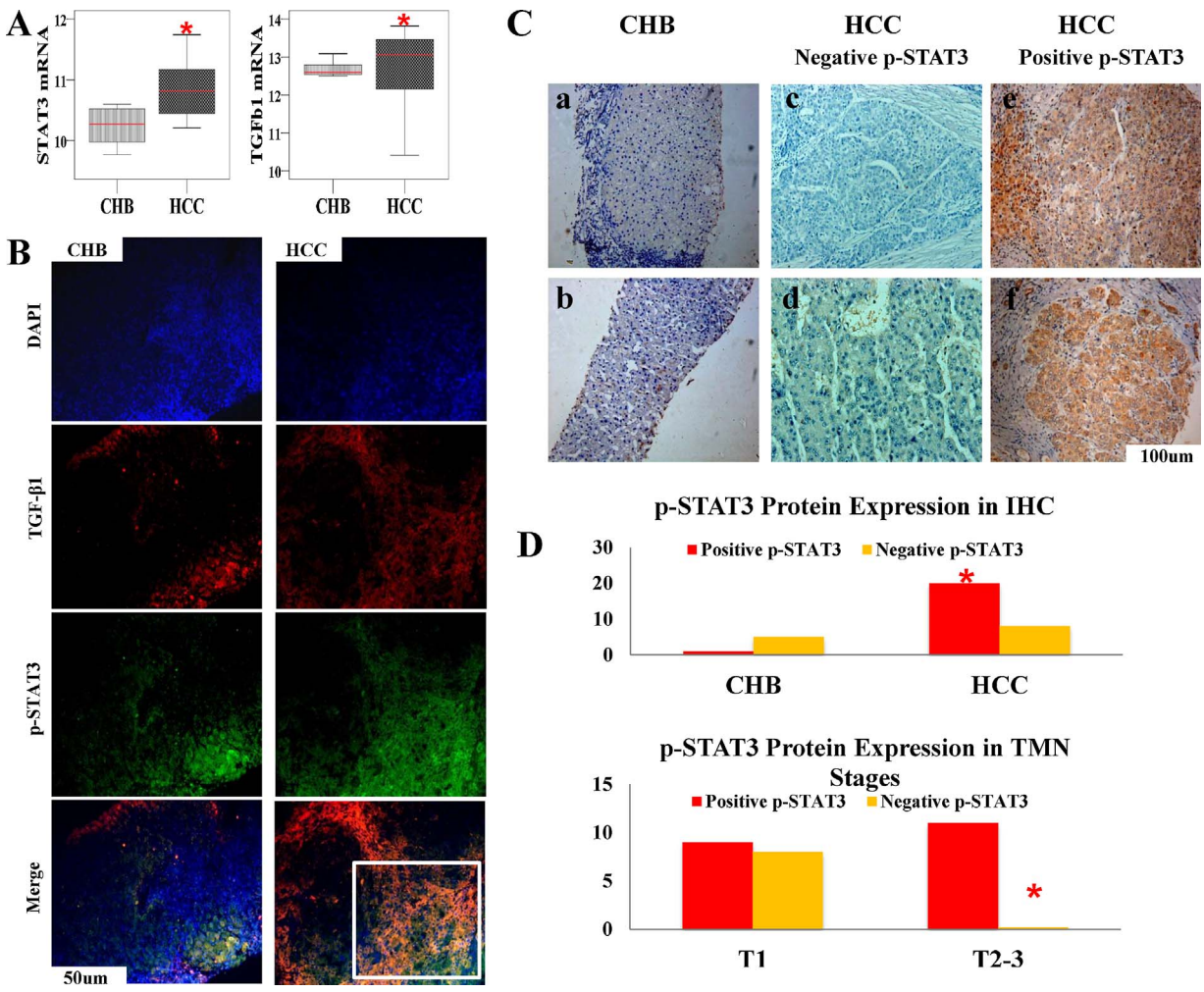

Fig. 1. Hepatic p-STAT3/STAT3 and TGF-β1 expression in HCC patients.
STAT3, TGF-β1 mRNA and protein expression are examined in the liver tissues of 28 HCC and 6 CHB patients. (A) STAT3 and TGF-β1 mRNA are statistically up-regulated in HCC patients compared to CHB patients. (B) IF staining of DAPI (blue), p-STAT3 (green) and TGF-β1 (red) is examined in the liver tissue of a CHB and HCC patient. A small portion of p-STAT3 positive cells in liver tissue also co-expresses TGF-β1 (see white frame). (C) IHC-stained liver tissues are shown in CHB and HCC patients. (D) The percentages of negative or positive p-STAT3 expression in HCC tissues from 2 groups are analyzed. *, means compared to CHB group or compared to T1 group, $p < 0.05$. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

2. Materials and methods

2.1. Patients

Human liver tissues were collected from patients receiving a partial liver resection for HBV-related HCC (HBV-HCC, n = 28) and a percutaneous liver biopsy for chronic hepatitis B (CHB) with fibrosis stages (Scheuer 0–1, n = 6) in our hospital from 01/2011 to 12/2012. PCR was performed on 12 human liver tissues (CHB and HCC group: each group n = 6). Immunohistochemistry (IHC) was performed on 34 human liver tissues (CHB group: n = 6; HCC group: n = 28). All HBV-HCC patients met the following requirements: [1] chronic HBV infection; [2] early HCC with a single tumor nodule and T1N0-1M0 stage according to clinical and histological diagnosing criterion for HCC in China [10]; and [3] para-cancerous tissue showed no atypical hyperplasia. Patients who were co-infected with HIV or HCV, who consumed more than 30 g of alcohol per day, were at risk of other chronic liver disease, received a previous anti-tumor treatment, or who had recurring cancer were excluded. All patients provided written informed consent. The study was approved by the Ethics Committee of our hospital.

2.2. Experimental rat models

Male Wistar rats were used in this study and separated into 3 groups (Control; HCC and HCC + AG490 group: each group n = 10). An HCC rat model was induced by giving animals 0.05 g/L diethylnitrosamine (DENA) daily in their water. HCC + AG490 rats were intraperitoneally treated with selective Janus Kinase (JAK) 2 inhibitor tyrphostin AG490 (1 mg/kg/d, Sigma, USA) in the first week. All rats were sacrificed in 16 weeks. The livers of the rats were removed, separated into lobes, and the externally visible tumors were counted and the maximal tumor size was measured [11]. These procedures were also approved by the Ethical Committee of our hospital.

2.3. Cell line culture

Human hepatic cancer cell lines (HepG2, Bel7402, MHCC97H, and HCCLM3) and a normal hepatic cell line (LO2) were cultured. The cells were treated with TGF-β1 (0, 2, 5 or 10 ng/ml; Sigma, USA), AG490 (50 μM; Sigma) or IL-6 (50 ng/ml; Sigma) for 1, 2, 4, 8 or 24 h.

2.4. IHC and immunofluorescence (IF) staining

TGF-β1 and p-STAT3 (Abcam, USA) antibodies were used in IHC