Suppression of fibrogenic signaling in hepatic stellate cells by Twist1-dependent microRNA-214 expression: Role of exosomes in horizontal transfer of Twist1

Li Chen,1 Ruju Chen,1 Sherri Kemper,1 Alyssa Charrier,1,2 and David R. Brigstock1,2,3

1The Research Institute at Nationwide Children’s Hospital, Columbus, Ohio; 2Molecular, Cellular, and Developmental Biology Program, The Ohio State University, Columbus, Ohio; 3Department of Surgery, Wexner Medical Center, The Ohio State University, Columbus, Ohio

Submitted 6 May 2015; accepted in final form 23 July 2015

Chen L, Chen R, Kemper S, Charrier A, Brigstock DR. Suppression of fibrogenic signaling in hepatic stellate cells by Twist1-dependent microRNA-214 expression: Role of exosomes in horizontal transfer of Twist1. Am J Physiol Gastrointest Liver Physiol 309: G491–G499, 2015. First published July 30, 2015; doi:10.1152/ajpgi.00140.2015.—A hallmark of liver fibrosis is the activation of hepatic stellate cells (HSC), which results in their production of fibrotic molecules, a process that is largely regulated by connective tissue growth factor (CCN2). CCN2 is increasingly expressed during HSC activation because of diminished expression of microRNA-214 (miR-214), a product of dynamin 3 opposite strand (DNM3os) that directly suppresses CCN2 mRNA. We show that an E-box in the miR-214 promoter binds the basic helix-loop-helix transcription factor, Twist1, which drives miR-214 expression and results in CCN2 suppression. Twist1 expression was suppressed in HSC of fibrotic livers or in cultured HSC undergoing activation in vitro or after treatment with ethanol. Furthermore, Twist1 decreasingly interacted with DNM3os as HSC underwent activation in vitro. Nanovesicular exosomes secreted by quiescent but not activated HSC contained high levels of Twist1, thus reflecting the suppression of cellular Twist1 during HSC activation. Exosomal Twist1 interacted differentially with HSC and stimulated expression of miR-214 in the recipient cells, causing expression of CCN2 and its downstream effectors to be suppressed. Additionally, the miR-214 E-box in HSC was also regulated by hepatocyte-derived exosomes, showing that functional transfer of exosomal Twist1 occurs between different cell types. Finally, the levels of Twist1, miR-214, or CCN2 in circulating exosomes from fibrotic mice reflected fibrosis-induced changes in the liver itself, highlighting the potential utility of these and other constituents in serum exosomes as novel circulating biomarkers for liver fibrosis. These findings reveal a unique function for cellular or exosomal Twist1 in CCN2-dependent fibrogenesis.

Address for reprint requests and other correspondence: D. Brigstock, Rm. WA2011, Research Bldg. 2, Nationwide Children’s Hospital, 700 Children’s Dr., Columbus, OH 43205 (e-mail: David.Brigstock@NationwideChildrens.Org).

http://www.ajpgi.org 0193-1857/15 Copyright © 2015 the American Physiological Society
G493

A Twist1-Mir-214 Axis Regulates Fibrogenic Signaling in HSC

Circulating exosomes were harvested using PureExo Exosome Isolation Kits (101Bio, Palo Alto, CA) from serum of mice treated for up to 5 wk with CCl4 as described above. Total RNA from exosomes in 200 μl of serum was prepared using miRNAeasy mini kits (Qiagen) as described above. Each reaction was run in triplicate, and all samples were normalized to U6a.

Statistical analysis. All experiments were performed at least three times with triplicate measurements. For controls, error bars were derived by setting the mean value as 1 and defining variance of replicates from 1. Treatment groups were then expressed as fold of means ± SE. The data from qRT-PCR or luciferase activity assays were analyzed by Student’s t-test using Sigma plot 12.0 software (SPSS, Chicago, IL), and P values <0.05 were considered statistically significant.

RESULTS

Suppression of Twist1 expression during fibrosing liver injury or during HSC activation in vivo or in vitro. Analysis of total hepatic RNA showed that hepatic Twist1 expression was high in livers recovered from control oil-treated mice but was significantly decreased in livers from CCl4-treated mice (Fig. 1A). This response was associated with suppressed expression of hepatic miR-214 and stimulated expression of CCN2, α-SMA, or collagen α1(I) (Fig. 1A). Isolated activated HSC from this 5-wk injury model showed an overall similar expression pattern in that Twist1 or miR-214 were inhibited and CCN2, α-SMA, or collagen α1(I) were enhanced (Fig. 1A).

Consistent with these findings, Western blot analysis showed that Twist1 protein levels were suppressed in fibrotic livers or in activated HSC recovered from fibrotic livers and that CCN2 protein levels increased under the same conditions (Fig. 1A). Immunostaining for Twist1 in liver sections showed that it was present in desmin-positive nonparenchymal cells (presumptive quiescent HSC) in control animals, but, after CCl4 injury, Twist1 staining was absent from activated HSC, which stained positively for α-SMA as well as desmin (Fig. 1B, top, arrows). Some parenchymal cells also strongly stained positively for Twist1, but this was only weakly reduced after CCl4 treatment (Fig. 1B, top). Nonetheless, because background hepatocyte staining might potentially confound our interpretation of Twist1 staining in HSC, we alternatively isolated HSC from the livers of control or fibrotic animals to verify their Twist1 status in vivo. As assessed by immunostaining, quiescent HSC isolated from control animals were positive for desmin or Twist1 but not for CCN2, α-SMA, or collagen α1(I). In contrast, activated HSC isolated from animals treated with CCl4 for 5 wk were positive for desmin, CCN2, α-SMA, or collagen α1(I) but not for Twist1 (Fig. 1B, bottom). Thus, because HSC activation in vivo was associated with the loss of Twist1 mRNA expression or protein production (Fig. 1, A and B), this phenomenon was the focus of the studies described herein.

In a TAA liver fibrosis model exhibiting enhanced staining in HSC for CCN2, α-SMA, or collagen α1(I), decreased expression of hepatic Twist1 mRNA or miR-214 and increased expression of hepatic CCN2 mRNA were also documented (Fig. 1C). Analysis of HSC isolated from normal livers and maintained in vitro showed that there was a large decrease in Twist1 expression between days 2 and 4 of culture and then a more gradual decline in its expression up to day 14 of culture as the cells became progressively activated and expressed decreasing levels of miR-214 and...