Research Article

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Hepatic expression of Sonic Hedgehog induces liver fibrosis and promotes hepatocarcinogenesis in a transgenic mouse model

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Background & Aims: Liver fibrosis is an increasing health concern worldwide and a major risk factor for hepatocellular carcinoma (HCC). Although the involvement of Hedgehog signaling in hepatic fibrosis has been known for some time, the causative role of activated Hedgehog signaling in liver fibrosis has not been verified *in vivo*.

Methods: Using hydrodynamics-based transfection, a transgenic mouse model has been developed that expresses Sonic Hedgehog (SHH), a ligand for Hedgehog signaling, in the liver. Levels of hepatic fibrosis and fibrosis-related gene expression were assessed in the model. Hepatic expression of SHH was induced in a murine model for hepatocellular adenoma (HCA) and tumor development was subsequently investigated.

Results: The transgenic mice revealed SHH expression in 2–5% of hepatocytes. Secreted SHH activated Hedgehog signaling in numerous cells of various types in the tissues. Hepatic expression of SHH led to fibrosis, activation of hepatic stellate cells, and an upregulation of various fibrogenic genes. Liver injury and hepatocyte apoptosis were observed in SHH mice. Persistent expression of SHH for up to 13 months failed to induce tumors in the liver; however, it promoted liver tumor development induced by other oncogenes. By employing a HCA model induced by $P53^{R172H}$ and $KRAS^{G12D}$, we found that the SHH expression promoted the transition from HCA to HCC.

Conclusions: SHH expression in the liver induces liver fibrosis with concurrent activation of hepatic stellate cells and fibrogenic genes. It can also enhance hepatocarcinogenesis induced by other oncogenes.

Introduction

Fibrosis of the liver is the excessive accumulation of extracellular matrix (ECM) components in the tissue as a wound healing response to chronic liver injury [1–4]. Persistent liver injury and progressive fibrosis leads to cirrhosis, which affects hundreds of millions of patients worldwide and is an increasing health concern in developed countries [4,5]. Considered a major risk factor for hepatocellular carcinoma (HCC), liver fibrosis is present in 80–90% of HCC patients. In addition, the 5-year cumulative risk of developing HCC in patients with cirrhosis is very high, ranging between 5% and 30% [6,7].

With hepatic fibrosis induced by chronic liver injury, several pathological conditions are commonly observed in the liver, including increased apoptosis of hepatocytes, activation of hepatic stellate cells (HSCs), inflammatory responses, angiogenesis, and tissue remodeling [1,4,8,9]. In particular, activation of HSCs, which is initiated by paracrine stimulation from dying hepatocytes, represents a critical event in fibrosis. Activated HSCs secrete fibrillar collagens and express tissue inhibitors of metalloproteinases (e.g., TIMP-1 and TIMP-2), resulting in the accumulation of fibrotic extracellular matrix (ECM) [3,10]. Activation of HSCs is further characterized by the overexpression of α-SMA, MMP-2, and MMP-9 [9,11,12].

Pro-inflammatory cytokines TNF-α, IL-6, PDGFs, and TGF-β1 are strongly involved in hepatic fibrogenesis and commonly upregulated in patients with liver fibrosis induced by various etiological factors [13,14]. The cytokines activate HSCs and are

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Abbreviations: α-SMA, α-smooth muscle actin; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ECM, extracellular matrix; EMT, epithelial-to-mesenchymal transition; EGFP, enhanced green fluorescent protein; Gli, glioblastoma; HCA, hepatocellular adenoma; HCC, hepatocellular carcinoma; HHIP, Hedgehog interacting protein; HSC, hepatic stellate cell; IHC, immunohistochemistry; IL, interleukin; MMP, matrix-degrading metalloproteinase; mRNA, messenger RNA; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; Ptc, Patched; ROS, reactive oxygen species; PDGF, platelet-derived growth factor; PHI, post-hydrodynamic injection; RT-PCR, reverse-transcription polymerase chain reaction; SB, Sleeping Beauty; SD, standard deviation; SEM, standard error of the mean; SHH, Sonic Hedgehog; Smo, smoothened; TGF, transforming growth factor; TIMP, tissue inhibitor of metalloproteinases; TNF, tumor necrosis factor; TUNEL, terminal deoxynucleoti dyltransferase-mediated dUTP-biotin nick end labeling; WT, wild-type.

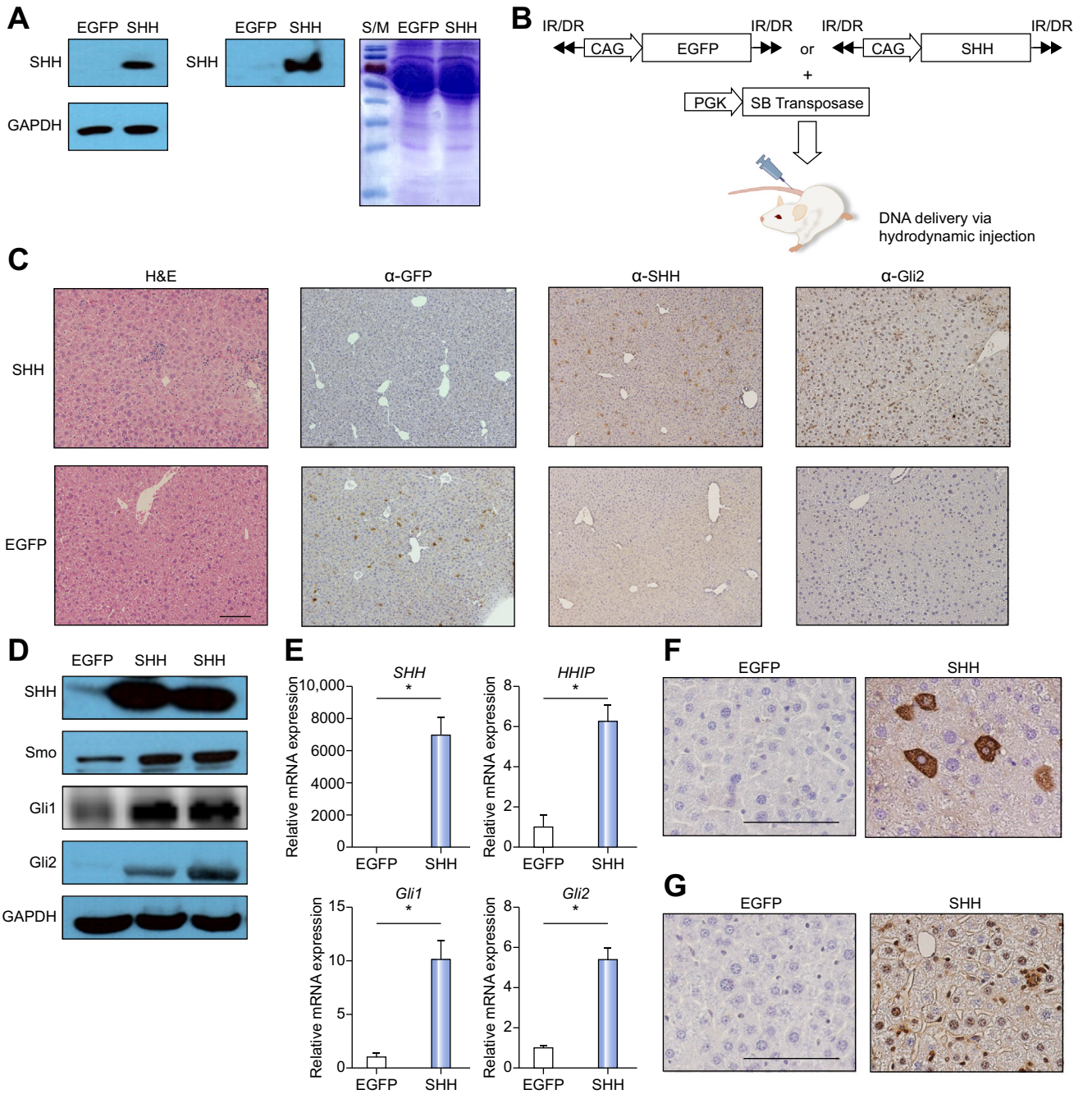

Fig. 1. Transgenic mice stably expressing Sonic Hedgehog (SHH) in the liver are generated via hydrodynamic transfection. (A) Efficient secretion of SHH in hepatoma cells transfected with pT2/SHH. Following transfection with pT2/EGFP (control plasmids) and pT2/SHH, Western blotting was performed using proteins harvested from cells (left panels) and culture media (middle panel). Coomassie blue staining of total proteins from the media was used as a loading control (right panel). S/M, a protein size marker. (B) Schematic illustration of the experimental procedure. (C) Hematoxylin and eosin (H&E) and IHC staining for GFP, SHH, and Gli2 in paraffin sections of livers hydrodynamically transfected with pT2/EGFP and pT2/SHH. Scale bar, 100 μm for H&E and Gli2 staining; 200 μm for SHH and GFP staining. (D) Protein expression levels of SHH and its downstream genes in livers transfected with EGFP (control) and SHH. (E) Quantitative RT-PCR assessing the expression levels of SHH-related genes. Data are presented as the mean ± SEM with a sample size of n = 4. (F and G) Images of a higher magnification showing IHC staining for SHH (F) and Gli2 (G) in paraffin sections of EGFP and SHH livers. Scale bars, 50 μm.

secreted from various cellular sources in injured hepatic tissue [4]. Of note, transgenic mouse models overexpressing PDGFs or TGF-β1 for several months exhibited fibrosis in the liver, suggestive of a causative role for cytokine signaling in hepatic fibrosis [15–18].

Hedgehog signaling is essential for tissue patterning during embryogenesis and for homeostasis in adult tissues [19,20]. It also modulates wound healing responses [21]. The pathway is activated when ligands such as Sonic Hedgehog (SHH) bind to patched (Ptc) receptors, leading to the release of Ptc-mediated