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MINI REVIEW

Morphogen-related therapeutic targets for liver fibrosis☆,☆☆

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Summary Recent research on hepatic stellate cells (HSCs) has spotlighted the involvement of morphogens in their cell fate determination in liver fibrosis. Temporally and spatially expressed during embryonic development, morphogens are involved in regulation of cell proliferation and differentiation, and tissue patterning. In normal adult liver, morphogens are generally expressed at low levels. However, in liver disease, myofibroblastic HSCs express morphogens such as Wnt, Shh, Necdin, DLK1, and Notch as part of their participation in fibrogenesis and wound healing. Liver regeneration involves cell proliferation and differentiation akin to embryonic liver development where the cells appear to undergo similar fates, and not surprisingly the morphogens are re-activated for the regenerative purpose in adult liver injury. Evidence also points to crosstalk of these morphogens in regulation of HSC fate determination. Genetic ablation or pharmacologic inhibition of morphogens reverts activated HSC to quiescent cells in culture and attenuates progression of hepatic fibrosis. However, positive regulation of liver regeneration by the morphogens needs to be spared. Therapeutically, manipulation of morphogen activities in a cell type and phase-specific manner should offer new modalities for chronic liver disease.
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Abbreviations: CCL4, carbon tetrachloride; C/EBPa, CCAAT/enhancer binding protein alpha; CTGF, connective tissue growth factor; Dlk1, delta-like homolog 1; ERK1/2, extracellular regulated kinases 1 and 2; FGF18, fibroblast growth factor 18; Gfap, glial fibrillary acidic protein; Gli1, GLI-Krupple family member; Hes-1, hairy and enhancer of split 1; HGF, hepatocyte growth factor; IGF, insulin-like growth factor; IL, interleukin; PAF, platelet activating factor; PDGF, platelet-derived growth factor; Pref-1, preadipocyte factor 1; Ptn, pleiotrophin; p75Ntr, neurotrophin receptor 75; RBPjk, recombination signal binding protein suppressor for immunoglobulin kappa J region; Rho, Ras homolog gene family; Shh, sonic hedgehog; Sox9, sex determining region-Y box 9; TGF, transforming growth factor; TNF, tumor necrosis factor.

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Role of hepatic stellate cells (HSCs) in liver fibrosis

Remarkably, the liver is the only internal organ with the ability to regenerate. A liver mass loss triggers well-orchestrated signaling cascades involving growth factors, cytokines, hormones, and neurotransmitters to restore the tissue to its original size and function [1,2]. However, this unique regenerative ability is often impaired in chronic liver disease resulting from sustained or repeated injury. Central to this defect is liver fibrosis characterized by accumulation of excessive extracellular matrix (ECM) proteins, which compromises normal liver regeneration, functions and intrahepatic circulation. Progression of liver fibrosis results in cirrhosis, which markedly increases the risk of developing liver failure and cancer [3].

HSCs make up approximately 5—8% of a total liver population. Residing in the perisinusoidal space of Disse, HSCs are the body's major storage site for vitamin A and serve as pericytes for hepatic sinusoids. They also fulfill the role of the major mesenchymal cell type in mesenchyme-epithelial interactions in the liver via maintenance of normal ECM milieu and production of hepatotrophic soluble factors [4]. HSCs also store neutral lipids resembling adipocytes [5] as they were once called ''fat-storing cells'' [6]. Upon injury to the liver, HSCs are transdifferentiated or ''activated'' toward a myofibroblast-like phenotype with induced expressions of cytokines, growth factors, and ECM components required for wound repair [4]. Activation of HSCs involves the coordination of multiple signaling events including down-regulation of PPARγ much like de-differentiation of mature adipocytes to preadipocytic fibroblasts [7,8].

Morphogen-associated signaling in liver regeneration

During development, morphogens are secreted from primordial cells and are recognized by specific receptors in distant cells to activate signaling cascades in support of organ growth and development [9]. Typically, morphogens form a concentration gradient to pass positional information to responding cells and guide the differentiation of cells into specific patterns and morphologies [10,11]. Embryonic tissue recombination experiments established the roles of molecular signals from the adjacent mesoderm in inducing hepatic commitment of the foregut endoderm [12]. Subsequent genetic studies revealed these molecular cues as members belonging to the fibroblast growth factors (FGF) and transforming growth factors β (TGFβ) families of morphogens [13]. FGF released from cardiac mesoderm binds to the newly formed endodermal cells and leads to induction of two FoxA transcription factors, FoxA1 and FoxA2, securing the fate of these cells to become future liver cells [14]. BMP released from septum transversum mesenchyme cooperates with FGF to support hepatic specification [15]. A recent lineage tracing definitively demonstrates that HSCs arise from mesoderm-derived septum transversum [16], suggesting a possibility that HSCs may retain this role in mesenchyme-epithelial interaction even in adult liver.

In the normal adult liver, morphogen signaling is not generally required due to the low cell turnover rate [17]. The damaged liver, on the other hand, has a vigorous injury response including activation of quiescent HSCs. Numerous mediators are released by different cell types to facilitate hepatic wound response, and those known to activate HSCs include growth factors (PDGF, TGF-α, IGF, HGF), acute phase cytokines (IL-1, TNF-α, IL-6), ECM inducers (TGF-β, CTGF), hormones (leptin, angiotensin), and lipid metabolites (PAF) [4]. Activated HSCs also serve as the source of many of these mediators to recruit inflammatory cells to the injured liver and to commence the wound healing and regeneration processes As HSC activation is considered as a manifestation of cell fate regulation which recapitulates the program known for liver development, the role of morphogens derived from HSCs can obviously be a subject of interest. Indeed, the morphogens known for liver morphogenesis are shown expressed by activated HSCs in adult liver injury.

Wnt family

The molecular name Wnt is derived from combining *Wingless*, the segment-polarity gene from *Drosophila melangonster*, and *Integrase-1*, the vertebrate homolog. The Wnt pathway is a highly conserved signaling in regulating tissue development and regeneration, and cell differentiation. The canonical pathway is activated when the Wnt protein forms a complex with the transmembrane Frizzled receptor (Fz) and the low-density lipoprotein receptor related protein (LRP) 5/6 co-receptor. The formation of the Wnt-Fz-LRP complex attracts the scaffolding protein Disheveled (Dvl) and recruits Axin and glycogen synthase kinase 3β (GSK3β) from the β-catenin destruction complex. As a consequence, this inhibits phosphorylation and subsequent degradation of β-catenin, allowing it to translocate into the nucleus for transcriptional activation of target genes with the T cell factor/lymphocyte enhancer factor (TCF/LEF) family of transcription factors. Non-canonical Wnt pathways including the Wnt/Ca^{2+} pathway and planar cell polarity (PCP) pathway are independent of regulation by β-catenin. Wnt5a, the ligand for Wnt/Ca^{2+} pathways, causes the release of intracellular Ca^{2+} through the interaction of the cytosolic protein Dvl1 and activates protein kinase C (PKC) and calmodulin kinase II (CAMKII). Wnt5a may also play a critical role in inflammatory processes associated with skeletal tissues through receptor tyrosine kinase-like orphan receptor (Ror) proteins [18]. Wnt5a/Frz5 can also activate the transcriptional factor nuclear factor-kappaB (NF-κB) that in turn stimulates the expression of chemokines and pro-inflammatory cytokines [19]. Activation of the PCP pathway supports cytoskeletal organization for cell proliferation and differentiation through the recruitment of Dvl by Frz, which then further transduces the activation of Rho family of GTPases [20].

Wnt pathway enhances the survival of activated HSC and thereby promotes hepatic fibrosis [21]. Global changes in gene expression from culture-activated rat HSCs and CCl4-treated liver reveal the upregulation of Wnt signaling components [22]. Both the components of the Wnt pathway including Wnt5a, Wnt4, Frz1 and Frz7 and genes downstream of the Wnt pathway (Gfg18, Sox9, Twist, and Fibronectin) are increased in activated HSCs compared to quiescent HSCs.