Role of canonical Hedgehog signaling pathway in liver

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Received: 2018.06.24; Accepted: 2018.08.01; Published: 2018.09.07

Abstract

Hedgehog (Hh) signaling pathway plays an important role in embryonic development. It becomes reactivated in many types of acute and chronic liver injuries. Hh signaling is required for liver regeneration, regulates capillarisation, controls the fates of hepatic stellate cells, promotes liver fibrosis and liver cancers. In this review, we summarize the current knowledge of the role of canonical Hh signaling pathway in adult liver. This help to understand the pathogenesis of liver diseases and find out the new effective targeted therapeutic strategies for liver diseases treatments.

Key words: Hedgehog, liver, fibrosis, HCC

Introduction

Hedgehog (Hh) signaling is a morphogenic signaling pathway that plays important roles in embryonic development. Hh signaling was first identified in Drosophila1. In 1980, Nusslein-Volhard et al. identified 15 loci that required for the establishment of segmental pattern in Drosophila, including Hedgehog1. In adult healthy liver, Hh signaling is considered to be inactive, because of mature hepatocytes barely express Hh ligands. Study shows that the basal level of Hh signaling pathway contributes to regulation of insulin-like growth factor I (IGF-I) hemostasis in healthy mature mouse hepatocytes2. Hh signaling becomes dramatically reactivated in various types of acute and chronic liver injuries (e.g., 70% partial hepatectomy (PH)3, HBV/HCV infection4, cholestatic liver injury5, 6, alcoholic liver disease7 and non-alcoholic fatty liver disease (NAFLD)8). Activation of Hh pathway promotes reconstruction of adult livers after injury. In this review, we summarize the role of canonical Hh signaling in liver regeneration, capillarisation, NAFLD, liver fibrosis and liver cancers.

1. Hh signaling pathway in vertebrate

In vertebrates, Hh signaling is initiated by Hh ligands (Sonic hedgehog (Shh), Indian hedgehog (Ihh) and Desert hedgehog (Dhh)). In addition to the key Hh signaling components, primary cilium is also required to properly transduce Hh signaling9, 10. In the absence of Hh ligands, low levels of phosphatidylinositol 4-phosphate (PI(4)P) intact with Ptched (Ptch) receptor11. Ptch resides at the base of the primary cilium that represses Smoothened (Smo) receptor activity by preventing its accumulation within cilia12. Smo associates with small ubiquitinrelated modifier (SUMO)-specific isopeptidases, such as Ubiquitin-like protease 1 (Ulp1) in Drosophila and SUMO specific peptidases (SENP) family members in mammals, leading to its ubiquitination and degradation13. The downstream of Hh signaling pathway glioma-associated oncogene transcription factors (Glis, including Gli1, Gli2 and Gli3) associate with Suppressor of Fused (SuFu) and Kif7 to form the complex in the cytoplasm associated with microtubules. Protein kinase A (PKA)14, casein kinase 1α (Ckla)15 and glycogen synthase kinase 3β (Gsk3β)16 promote phosphorylation of Glis to...
suppress their transcriptional activity. Glis were in their repressor forms (GliR). Hh signaling is inactive. (Figure 1A). In the presence of Hh ligands, the inhibition of Smo by Ptch was relieved, leading to the translocation and accumulation of Smo at cilia. About the mechanisms of Smo movement and localization, study showed that diffusion was the predominant mode of motion of Smo. Phosphorylation of Smo is required for Hh signaling pathway. Hh stimulation elevates the production of PI(4)P. PI(4)P directly binds Smo through an arginine motif, which then triggers Smo phosphorylation and activation. Sumoylation and cholesterol modification on D95 are also required for Hh signaling pathway activation. Glis dissociate the SuFu-Gli complex. Glis were in their active forms (GliA). GliA enters into the nucleus to regulate gene expression.

Hh signaling pathway is well conserved between insects and vertebrates. Ligands dependent, Smo mediated the activation of Glis (main components: Hh ligands, Ptch1 and Smo receptor, Glis transcription factors) are called canonical Hh signaling pathway. However, Glis activation may not require Smo. Glis can be activated by Hh-independent mechanisms, such as, some cytokines (TNF-α and IL-1β) activate Glis without activating Smo. The downstream of Smo may not require Glis, or mutations in components of the Hh signaling can also activate Hh signaling pathway. These collectively called noncanonical Hh signaling pathway. The rest of this review will mainly discuss the diverse functions of canonical Hh signaling in adult liver.

2. Hh signaling in liver regeneration

Adult liver has enormous regenerative capacity which requires of proliferative activity in multiple types of liver cells, such as hepatocytes and progenitor cells. In acute massive loss of liver, e.g., 70% partial hepatectomy (PH), people think liver regeneration relies largely upon increased replication of mature hepatocytes. But the expression of progenitor markers, such as alpha-fetoprotein (AFP) and Fn14 increased, suggesting progenitor cells are also involved in liver regeneration post-PH. In many types of chronic liver injury, since mature hepatocyte replication is inhibited, it is generally believed that progenitor populations mainly contribute to regeneration of chronically injured livers.
Hh signaling pathway is a major regulator of liver regeneration post 70% PH. After PH, Hh signaling dramatically activated in hepatocyte, bile ductular cells and hepatic stellate cells (HSCs)\(^3\). Activation of Hh signaling activation promotes transition of quiescent HSC to fibrogenic myofibroblast (MF), some of MFs become progenitors that regenerate the liver epithelial compartment after PH\(^3, 32\) (Figure 2). Hh signaling also regulates Hippo/Yes-associated protein 1 (Yap1) activation during liver regeneration after PH\(^33\). Yap1 is a morphogenic signaling pathway and Yap1 activation is also required for liver regeneration\(^34\). Study showed that Yap1 is a downstream effector of Hh signaling. In cultured HSC, disrupting Hh signaling blocked activation of Yap1\(^33\).

3. Hh signaling modulates capillarisation

Liver sinusoidal endothelial cells (LSECs) have a unique phenotype which differs from all mammalian endothelial cells. LSECs have typical fenestrations clustered in sieve plates, lack an organized basement membrane and only have an attenuated extracellular matrix consisting mostly of fibronectin\(^35-37\). LSECs lost this highly specialized morphology during a process called capillarisation\(^36\). Capillarisation occurs in many kinds of liver injuries and increases naturally with age\(^35, 38-40\). Vascular remodeling during liver damage involves loss of healthy LSEC phenotype via capillarisation\(^36\). Hh signaling is activated during LSEC capillarisation. Both in the mdr2\(^-/-\) mice (a model of chronic liver injury and repair that results in progressive biliary-type fibrosis) and in the 70% partial hepatectomy model (a model of acute massive liver cell loss), inhibiting Hh signaling prevented capillarisation in both chronic and acute liver injury\(^36\).

4. Hh signaling in NAFLD, NASH and liver fibrosis

Non-alcoholic fatty liver disease (NAFLD) is now one of the most prevalent liver diseases in the world\(^41\). Hepatic steatosis is the hallmark feature of NAFLD and has the potential to develop into more severe steatohepatitis (NASH), which is characterized by fat accumulation, inflammation, and hepatocyte ballooning and can progress to liver fibrosis\(^42, 43\). Liver fibrogenesis is a dynamic and highly integrated process that drives the progression of chronic liver diseases towards liver cirrhosis, hepatic failure and HCC\(^44, 45\).

In NAFLD patients, Hh pathway activation is highly correlated with the severity of liver damage (e.g., portal inflammation, ballooning, and fibrosis stage) and with metabolic syndrome parameters that are known to be predictive of advanced liver disease\(^8\). In patients with NASH, ballooned hepatocytes underwent endoplasmic reticulum (ER) stress which induced the expression of Shh\(^43\). Ballooned hepatocytes produce and release Shh and provide a paracrine pro-fibrogenic signal to the neighboring...
cells. Also, the autocrine Shh pathway in ballooned hepatocytes contributes to itself resistant to cell death due to the decreased caspase. Apoptosis can be divided into the extrinsic or death receptor pathway and the intrinsic or mitochondrial pathway. The mitochondrial pathway was initiated by the activation of caspase-9. Loss of caspases function causes cells resist to apoptosis. Ballooned hepatocytes in NASH patients showed significantly decreased caspase-9 expression and resistant to apoptosis at least partly due to the Hh autocrine signaling pathway.

The level of Hh pathway activity also significantly correlates with fibrosis stage. HSCs are thought to be the key regulators of liver fibrosis, since active HSCs secrete fibrillar collagens, induce robust induction of α-smooth muscle actin (α-SMA), matrix molecules, matrix metalloproteinases (MMPs), and express tissue inhibitors of metalloproteinases (such as, TIMP-1 and TIMP-2), resulting in the accumulation of fibrotic extracellular matrix (ECM). HSCs in adult livers express Shh and have endogenous Hh pathway activity. During fibrogenesis, Hh signaling controls the fate of HSC, in vitro inhibiting Hh signaling in HSCs blocks HSCs activation. Hh signaling promotes the transition of quiescent HSC (Q-HSC) to myofibroblastic hepatic stellate cells (MF-HSC), and provides an autocrine mechanism promoting the accumulation of MF-HSC. Many types of liver injuries trigger MF accumulation. Some of MFs become progenitors that regenerate hepatocytes, cholangiocytes and HSCs. Hh signaling activation promotes accumulation of liver progenitors through activation of quiescent myofibroblasts. Overexpression of Shh in hepatocytes activated HSCs, upregulated various fibrogenic genes and led to liver fibrosis. (Figure 3).

During fetal development, Hh pathway is a key regulator of angiogenesis and vasculogenesis. In adult liver, angiogenesis contributes to vascular remodeling during cirrhosis. Repair-related angiogenesis are regulated by asacular pericytes which promotes vascular tube formation. HSCs are liver-specific pericytes regulating angiogenesis during liver fibrosis. Inhibiting Hh signaling with Cyclopamine (Smo inhibitor) or GANT-58 (Gli1 inhibitor) reduce the expression of vascular endothelial growth factor (VEGF) and angiopoietin 1 in HSCs and suppressed HSC tubulogenesis capacity.

Osteopontin (OPN) is an important component of the extracellular matrix (ECM). OPN levels have been highlighted as a potential biomarker of liver disease and correlates with the severity of liver fibrosis and inflammation. OPN is Hh-regulated. Transcription factor Sex-determining region Y-box 9 (SOX9) lies downstream of Gli2. Hh signaling can modulate OPN through SOX9. In a mouse NAFLD model, activation of Hh signaling in hepatocytes increased the production of OPN, which subsequently enhanced the macrophage-mediated proinflammatory response through paracrine signaling. (Figure 4A). Natural killer T cells (NKT cells) have endogenous Hh pathway activity. Activated hepatic NKT cells secrete Hh ligands and associate OPN promotes HSC activation and promote liver fibrogenesis in a paracrine fashion. (Figure 4B)
Figure 4. Activation of Hh signaling in hepatocytes and NKT cells promotes OPN production. (A) Activation of Hh signaling in hepatocytes increases the production of OPN, which subsequently enhanced the macrophage-mediated proinflammatory response. (B) Activation of Hh signaling in NKT cells associates OPN promotes HSC activation and liver fibrogenesis.

Hh signaling is a potential target for the treatment of NAFLD. The NIDDK-sponsored PIVENS trial (NCT00063622) showed that Vitamin E improved NASH68. Vitamin E treatment reduced Shh+ hepatocytes and improvement in Hh-regulated processes that promote NASH progression69. In a mouse NAFLD model, administration of Cycloamine (Smo inhibitor) to high-fat diet-fed wild-type mice significantly reduced the numbers of activated macrophages and decreased the expression of proinflammatory cytokines65. Forskolin, a Hh signaling inhibitor significantly reduced CCl4-induced hepatic fibrosis70.

5. Hh signaling in liver cancers

5.1 Hh signaling in hepatocellular carcinoma

Hepatocellular carcinoma (HCC), the most common primary malignant tumor of the liver, is considered to be the third leading cause of all cancer-related deaths and fifth common cancer worldwide71,72. HCC is a complex and heterogeneous tumor with several genomic alterations. Aberrant activation of several signaling cascades (e.g., EGFR-Ras-MAPKK pathway, c-MET signaling, IGF signaling, PI3K/Akt/mTOR pathway, Wnt-β-catenin pathway, Hh signaling and apoptotic pathways) have been reported in HCC73.

The major cell populations that expanded during cirrhosis and HCC (MFs, activated endothelial cells, and progenitors) were Hh-responsive, higher levels of Hh pathway activity associated with cirrhosis and HCC42. Hh signaling pathway components (Smo, Shh, Gli1 and Gli2) were higher in both HCC cell lines and tumor tissues from HCC patients74-76. Specific inhibition of Hh signaling in HCC cell lines by Smo antagonist, KAAD-cyclopamine, or with Shh neutralizing antibodies inhibits cell growth and results in apoptosis74. Hh inhibition induces autophagy through up-regulation of Bnip3 and that this mechanism contributes to apoptosis77. Among the Gli transcription factors, Gli2 plays a predominant role in the proliferation of HCC cells, targeting of Gli2 led to decreased proliferation of various HCC cell lines75.

Hh signaling activation promotes hepatocarcinogenesis. Malignant hepatocytes produce Hh ligands. The paracrine soluble Hh ligands can stimulate glycolysis in neighboring MF resulting in release of MF-derived lactate that the malignant hepatocytes use as an energy source78. Overexpression of Shh in hepatocytes by hydrodynamic injection of Shh led to liver fibrosis, although it’s not sufficient to induce liver tumors (persistent expression of Shh for up to 13 months in mice failed to induce tumors in the liver), it enhanced hepatocarcinogenesis induced by employing mouse hepatocellular adenoma (HCA) models induced by P53(R172H) and KRAS(G12D)58.

In some tumors Hh pathway is activated in a ligand-independent manner by inactivating mutations of Ptc179,80. Ptc1, as a tumor suppressor
that inhibits Smo, were reported that exhibits a higher expression in well and moderate differentiated HCC, but a lower expression in poorly differentiated HCC. Three SNPs in Ptc1 (exon 12: T1665C and C1686T and exon 6: A1056G, no inactivating mutations of Ptc1 were found in this study) were identified in Chinese population. These SNPs showed no statistically significant for association with HCC. The findings suggest that Hh pathway in Chinese HCC is activated by ligand expression but not by mutation. Overexpression of Smo, as well as an increase in the stoichiometric ratio of Smo to Ptc1 mRNA levels, correlated with tumor size. Activation of the Smo mediates c-myc overexpression which plays a critical role in hepatocarcinogenesis. MicroRNAs are involved in carcinogenesis. Loss of miR-338-3p expression is associated with clinical aggressiveness of HCC. It has been reported that Smo was a direct target of miR-338-3p. Inhibition of miR-338-3p induces HCC cells invasion by upregulating Smo.

Surgery is the gold-standard treatment for local HCC and often complemented by radiofrequency ablation or transarterial chemoembolization. In advanced HCC, therapy options are limited and relapse and metastasis are common. Sonidegib (LDE225), N-(6-((2S,6R)-2,6-dimethylmorpholino)pyridin-3-yl)-2-methyl-4-(trifluoromethoxy)biphenyl-3-carboxamide is a potent, selective and orally bioavailable inhibitor of the Hh signaling pathway. In the phase I trial, oral sonidegib was administered to 103 patients with advanced solid tumors, including medulloblastoma and basal cell carcinoma. Results showed that sonidegib has an acceptable safety profile and exhibits antitumor activity in advanced basal cell carcinoma and relapsed medulloblastoma, both of which are strongly associated with activated Hh pathway. Sonidegib primarily metabolized by the liver. In order to make dose recommendations for patients with hepatic impairment, Hormans et al. assessed the pharmacokinetics and safety of sonidegib in subjects with varying degrees of hepatic function. This phase I, multicenter, open-label study showed that sonidegib exposures were similar or decreased in subjects with varying degrees of hepatic function. Dose adjustment is not considered necessary for subjects with mild, moderate, or severe hepatic impairment. Sonidegib has already shown survival benefits in patients with advanced HCC. Radiation-induced liver disease (RILD) is a major obstacle in treating HCC. Radiation-induced fibrosis constitutes a major problem that is commonly observed in the patients undergoing radiotherapy. Enhanced Hh signaling contributes to the RILD progression. Male mice were exposed to single dose radiotherapy (6 Gy), both of Smo, Gli2 and Hh-target genes, were upregulated at 6 and 10 weeks after irradiation. Correspondingly, when compared with radiotherapy alone, combining Cyclopamine (Shh inhibitor) with radiotherapy reduced the mean tumor size of orthotopic tumors.

5.2 Hh signaling in cholangiocarcinoma

Cholangiocarcinoma (CCA) is the second most common primary liver tumor after HCC. CCA and HCC display heterogeneity at both morphologic and molecular levels. CCA represents a diverse group of epithelial cancers arising from varying locations with the biliary tree with very poor prognosis. Based on their site of origin, CCA are classified into intrahepatic CCA (iCCA), perihilar CCA (pCCA), and distal CCA (dCCA). Mature cholangiocytes basally express Hh ligands. CCA cells produce and respond to Shh ligand. A significant activation of the Hh signaling pathway was found in human CCA. Ihh, Shh, Ptc1, Smo, Gli1, and Gli2 mRNA expressions were markedly increased in human ICC samples. An autocrine Hh signaling in human CCA promotes CCA proliferation, migration, and invasion.

Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) induces apoptosis by binding its two cognate receptors death receptor 4 (DR4) and death receptor 5 (DR5). Many malignant cells remain resistant to TRAIL cytotoxicity. Using cholangiocarcinoma cells as a model for studying TRAIL resistance, Kurita et al. found that Hh signaling may contribute to TRAIL resistance in cancer cells. Gli3 (but not Gli1 or Gli2) silences DR4 expression by binding to its promoter. Blockade of Hh signaling sensitizes human CCA cell lines to TRAIL cytotoxicity by upregulating DR4 expression by binding to its promoter. These stromal MFBs have a crucial role in promoting CCA cells proliferation, migration and invasiveness through interactive autocrine and paracrine signaling pathway. Platelet-derived growth factor (PDGF)-BB as a MFB-derived survival factor for CCA cells promotes CCA resistance to TRAIL cytotoxicity in an Hh signaling-dependent manner by inducing cAMP/PKA-mediated Smo trafficking to the plasma membrane resulting in Gli nuclear translocation and Gli transcriptional activity. Approximately 80% of human cancers (including CCA) express high levels of serine/threonine kinase pololike kinase (PLK) transcripts in tumor cells and these PLK transcripts are mostly absent in surrounding healthy tissues. Hh signaling may exert major survival signals in CCA.
by regulating PLK2. Hh signaling directly regulate PLK2 expression by binding of the Gli1 and Gli2 transcription factors to the PLK2 promoter102. Survival of cholangiocarcinoma patients depends on the stage of disease at presentation, but even in patients with localised disease, five-year survival is poor at 15% and 30% for intrahepatic cholangiocarcinoma (ICC) and extrahepatic cholangiocarcinoma (ECC) respectively103. High Gli1 or Gli2 expressers had an unfavorable overall survival (OS) prognosis and a shorter disease-free survival (DFS)96. Blocking Hh pathway inhibited EMT, decreased the viability of CCA cells and inhibited tumor growth in CCA xenografts93, 94. Capsaicin, the most abundant pungent molecule produced by pepper plants, has been reported that interferes with the growth and proliferation and viability of human CCA cells through targeting the Hh pathway104. These findings suggest that inhibition of Hh pathway may be a possible treatment option for at least in a subset of human CCA in which the Hh pathway is activated.

5.3 Hh signaling in hepatoblastoma

Hh signaling pathway may play an important role in the differentiation and malignant potential of hepatoblastoma (HB), the most common liver tumor in childhood, with most occurrences before the age of 3 years. Hh signaling pathway components (Shh, Ptch1, Smo and Gli1) were higher in patients with HB105-107. Smo or Gli1 expressions were positively correlated with tumor clinicopathological features, such as histological type, tumor grade, tumor size and clinical stage105. Inhibition of Hh signaling by Forskolin, a specific Hh signal inhibitor, suppressed the HB cell lines proliferation was associated with the down-regulation of C-Myc108.

Summary

Hh signaling is inactive in adult healthy liver, but it is activated during liver injuries of various etiologies. Hh signaling is required for liver regeneration, controls the fates of HSCs, modulates capillarisation and vasculogenesis, promotes liver fibrogenesis and liver cancers. Understanding the role of Hh signaling in liver helps us to develop new effective targeted strategies for the treatment of liver diseases.

Acknowledgements

The work was financially supported by National Natural Science Foundation of China (grant No. 81572518 & 81372750) to T.Y., and International Technology Cooperation Project of Hebei Provincial Department of Science & Technology (grant No: 13397708D) to Z.Y.Z.

Competing Interests

The authors have declared that no competing interest exists.

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