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REVIEW ARTICLE

Wnt/ β -Catenin Signalling during Liver Metabolism, Chronic Liver Disease and Hepatocarcinogenesis

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Abstract

Chronic liver diseases (CLDs) are increasing in prevalence and their end-stage complications, namely, cirrhosis, liver failure and hepatocellular carcinoma represent major global challenges. The most common initiators of progressive CLD are viral hepatitis and long-term alcohol abuse as well as steatosis and steatohepatitis. Irrespective of the underlying aetiology, a common feature of CLD is the formation of hepatic ductular reactions, involving the proliferation of liver progenitor cells (LPCs) and their signalling to fibrosis-driving hepatic stellate cells. The Wnt/ β -catenin pathway has been found to regulate development, stemness and differentiation, and alterations in its activity have been associated with tumour development. Recent data highlight the role of Wnt/ β -catenin signalling in hepatic metabolism, steatosis and cancer, and suggest targeting of this pathway as a promising molecular strategy to potentially inhibit CLD progression and hepatocarcinogenesis.

Keywords: chronic liver disease; hepatocellular carcinoma; liver progenitor cells; metabolic syndrome; Wnt/ β -catenin signalling

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Introduction

Chronic liver disease (CLD) has become one of the most common causes of death globally with an estimated 1.03 million deaths per year, as reported in 2017. Excessive alcohol consumption, viral hepatitis and hepatic steatosis are the most prevalent risk factors for the initiation and progression of CLD (1). A UK report stated that standardised CLD

mortality rates have increased by 400% since 1970, reflecting its growing burden and major challenge for global health (2). End-stage complications of CLD include cirrhosis, liver failure and malignancies, with hepatocellular carcinoma constituting 85–90% of all liver cancers (3). Current therapy options for hepatocellular carcinoma include surgical resection, radiofrequency ablation, transarterial chemoembolisation

and orthotopic liver transplantation. The multikinase inhibitors sorafenib and regorafenib are the only systemic treatments with proven survival benefits and they prolong the life expectancy of patients by 2 to 3 months (4). Immune-based approaches, including targeting of the immune checkpoint inhibitors programmed cell death (PD-1), programmed cell death ligand 1 (PD-L1) or cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), represent novel, promising therapeutic strategies to prevent or treat hepatocellular carcinoma (5).

Chronic Liver Disease and the Ductular Reaction

CLD induces molecular and cellular processes, which are initially reparative but become detrimental in the prolonged setting. Damaged liver epithelial cells release pro-inflammatory signalling molecules, which recruit immune cells to the site of injury, induce collagen deposition or fibrosis and activate liver progenitor cells (LPCs) as part of the so-called ‘ductular

reactions’ to restore lost liver tissue. The term ductular reaction describes the diverse histological phenomena occurring in response to chronic hepatic injury and encompasses the epithelial component as well as inflammatory and fibrogenic changes (6). Ductular reactions are observed in all forms of CLD with hepatocyte injury and replicative arrest. However, depending on the underlying aetiology, they display diverse morphologies, ranging from well-formed ductules to irregular strings of cells without obvious lumina (7). Irrespective of the injury stimulus, ductular reactions, activated biliary epithelial cells and LPCs are generally closely associated with inflammatory cell populations and fibrosis-driving, activated hepatic stellate cells, forming a very dynamic injury and regeneration niche (Figure 1). Although significant differences in injury and repair dynamics can be observed in different forms of CLD (8), epithelial, inflammatory and fibrogenic cells principally orchestrate liver regeneration versus disease progression through chemokine and cytokine crosstalk in all clinical settings (6, 9–13).

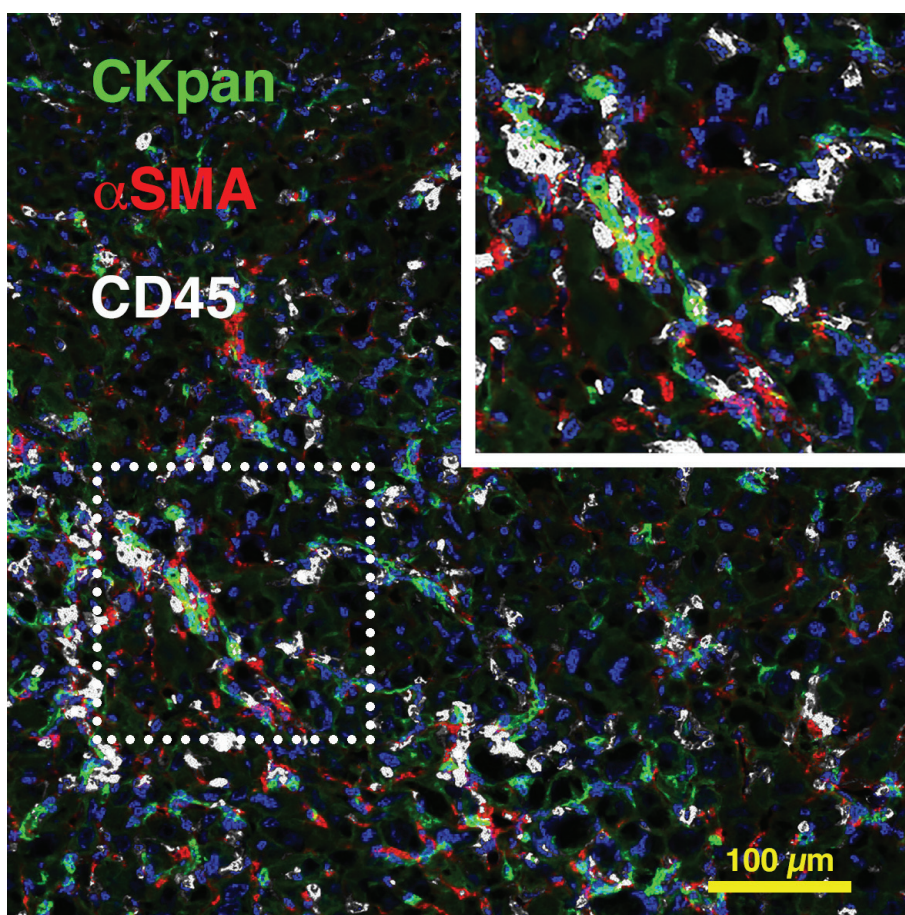


Figure 1. The injury and regeneration niche during chronic liver injury. Murine chronic liver injury induced by feeding a choline-deficient, ethionine-supplemented diet ¹⁵ leads to formation of an injury and regeneration niche, involving CKpan⁺ ductular cells and LPCs (green), αSMA⁺ hepatic stellate cells (red) and CD45⁺ inflammatory cells (white). DAPI was used for nuclear localisation.

Liver Progenitor Cells and Cancer Stem Cells

LPCs are defined as a heterogeneous pool of immature, bi-potential hepatic cells with diverse marker expression profiles and the ability to differentiate into either hepatocytes or biliary epithelial cells, depending on the underlying injury stimulus and thus tissue requirements. They are undetectable in healthy liver but upon injury emerge in portal areas near the Canals of Hering. Their origin and liver repopulation capacity have been controversially discussed (14). Studies using the choline-deficient, ethionine-supplemented model of chronic liver injury (15) reported that LPCs expressing osteopontin (16) or Foxl1 (17) contributed to hepatocellular regeneration. In addition, transplantation of clonogenic LPCs into hepatocyte-senescent murine livers, induced through deletion of the E3 ubiquitin ligase Mdm2, resulted in restoration of the hepatic parenchyma through generation of hepatocytic or biliary epithelia (18). The exact underlying mechanisms of LPC-mediated liver regeneration are not always clear; however, hepatocyte senescence seems to be a definite histological requirement (19).

The degree of LPC proliferation directly correlates with the severity of hepatocyte replicative arrest and the inflammatory and fibrogenic responses to CLD (20). Targeting of c-kit⁺ LPCs through the multikinase inhibitor imatinib mesylate during experimental chronic liver injury resulted in reduced fibrogenesis and carcinogenesis (21). Moreover, the presence of hepatobiliary LPCs, marked by epithelial cell adhesion molecule (EpCAM) and cytokeratin 7 and 19, predicted an increased risk of tumour formation in cirrhotic, hepatitis C virus-infected patients (22). This suggests that some LPCs either indirectly influence tumour development by regulating the fibrogenic potential and chemotaxis of neighbouring hepatic stellate cells (9, 12, 13, 23) or directly as tumour-initiating or cancer stem cells (CSCs).

In general, CSCs are defined as undifferentiated cells that are capable to self-renew, initiate and maintain tumour growth and may be responsible for tumour recurrence after resection. Haraguchi and colleagues first postulated the existence of liver CSCs, based on the finding that the hepatocellular carcinoma cell lines HuH7 and Hep3B contained 0.9–1.8% of side population cells with the ability to efflux the fluorescent nucleic acid-binding dye Hoechst 33342 through high activity of adenosine triphosphate-binding cassette transporters (24). Similar side population cells successfully induced xenograft tumours upon transplantation into immunodeficient NOD/SCID mice, while no tumour formation was observed when non-side population cells were transplanted (25). Subsequently, numerous studies have focussed on the identification of reliable marker expression profiles for liver CSCs. The CD133⁺ subpopulation of various hepatocellular carcinoma cell lines displayed a more immature, proliferative phenotype with greater colony formation capacity *in vitro* and upon xenotransplantation a higher tumorigenic potential compared to the CD133⁻ cellular counterpart (26–28).

Within the CD133⁺ population, cells with the expression profile CD133⁺CD44⁺ have been described as more tumorigenic and metastatic than CD133⁺CD44⁻ cells (29, 30). Other studies have suggested the mucin-like cell surface glycoprotein CD24 (31) and the glycosylphosphatidylinositol-anchored glycoprotein CD90 or Thy-1 (32) as liver CSC markers. The transmembrane glycoprotein EpCAM is expressed by normal LPCs and CSCs and regulates cell–cell adhesion, proliferation, migration, differentiation and invasion (33). EpCAM is transcriptionally activated by the Wnt/ β -catenin pathway, while inhibition of Wnt/ β -catenin signalling was shown to suppress its expression (34). Interestingly, both CD44 and CD24 are direct Wnt target genes, marking this signalling pathway a key player in CSC biology and therefore a potential therapeutic target to prevent or treat hepatocellular carcinoma.

There is strong experimental evidence for Wnt signalling directly regulating the biology of LPCs and CSCs. Using the 3,5-dithoxycarbonyl-1,4-dihydrocollidine (DDC) model of chronic liver injury, Hu *et al.* demonstrated Wnt/ β -catenin signalling activity in proliferating A6⁺ LPCs and ductular reactions. Primary LPCs showed active, nuclear β -catenin and entered the cell cycle upon Wnt3a stimulation *in vitro* (35). A constitutively active β -catenin mutant was shown to promote LPC expansion in rodents subjected to the 2-acetylaminofluorene/partial hepatectomy model. In addition, the less differentiated, LPC-like, OV6⁺ subpopulation of hepatocellular carcinoma cells displayed endogenously active Wnt/ β -catenin signalling, coupled with a more aggressive phenotype, as judged by greater tumorigenicity and chemoresistance (36). Boulter and colleagues reported Wnt3a-induced expression of the ubiquitin ligase Numb, which is required to leave the biliary differentiation path, and hepatocyte nuclear factor 4 α in the LPC line BMOL (37), together inducing its differentiation towards the hepatocyte lineage (38).

The Wnt/ β -Catenin Signalling Pathway

The Wnt signalling pathway is highly conserved and has been associated with embryogenesis, proliferation, differentiation as well as carcinogenesis (39–41). It consists of 19 Wnt ligands, 10 Wnt receptors, referred to as frizzleds (FZD), a family of co-receptors, including low-density lipoprotein receptor-related proteins 5 and 6 (LRP5 and 6), and two branches of the pathway exist (42, 43). The non-canonical pathway comprises the planar cell polarity pathway (PCP) and the Ca²⁺ pathway. These are β -catenin-independent pathways that play roles in the regulation of the actin cytoskeleton and cytoskeletal rearrangement and will not be discussed further. In contrast, the canonical pathway is β -catenin-dependent and of particular interest therapeutically, as aberrant activation of this pathway has been postulated as a key driver in many malignancies such as prostate, colorectal, ovarian and liver cancer (41).

During the inactive ‘off’ state, there is no Wnt ligand bound to the FZD receptor, which results in the multi-protein destruction complex, consisting of glycogen synthase kinase 3β (GSK3β), casein kinase 1 (CK1), adenomatous polyposis coli (APC) and Axin, to bind β-catenin. The destruction complex then phosphorylates β-catenin in a sequential pattern on residues serine 33 (S33), serine 37 (S37) and threonine (T41). Beta-catenin is then ubiquitinated by the E3-ligase beta-transducin repeat containing protein (βTRCP) and marked for proteasomal degradation, preventing it from translocating to the nucleus. The transcription repressor Groucho remains bound to T-cell factor/lymphoid enhancer factor (TCF/LEF) transcription factors, inhibiting transcription of target genes such as c-Myc and cyclin D1 (Figure 2) (40). Conversely, in

the active ‘on’ state, a Wnt ligand binds to a FZD receptor, activating the protein dishevelled (Dvl), a cytoplasmic phosphoprotein crucial for Wnt signal transduction. Axin is recruited to the plasma membrane, binding to the co-receptor LRP5/6 and inhibiting GSK3β and the destruction complex. This allows unphosphorylated β-catenin to accumulate in the cytoplasm and translocate into the nucleus, where Groucho is displaced and unbound from TCF/LEF transcription factors. In this case, β-catenin is able to bind and activate downstream signalling (Figure 2) (40). It has been estimated that Wnt/β-catenin signalling regulates the expression of more than 80 target genes involved in cell fate determination, development, regeneration, zonation, metabolism, fibrosis and carcinogenesis of the liver (44, 45).

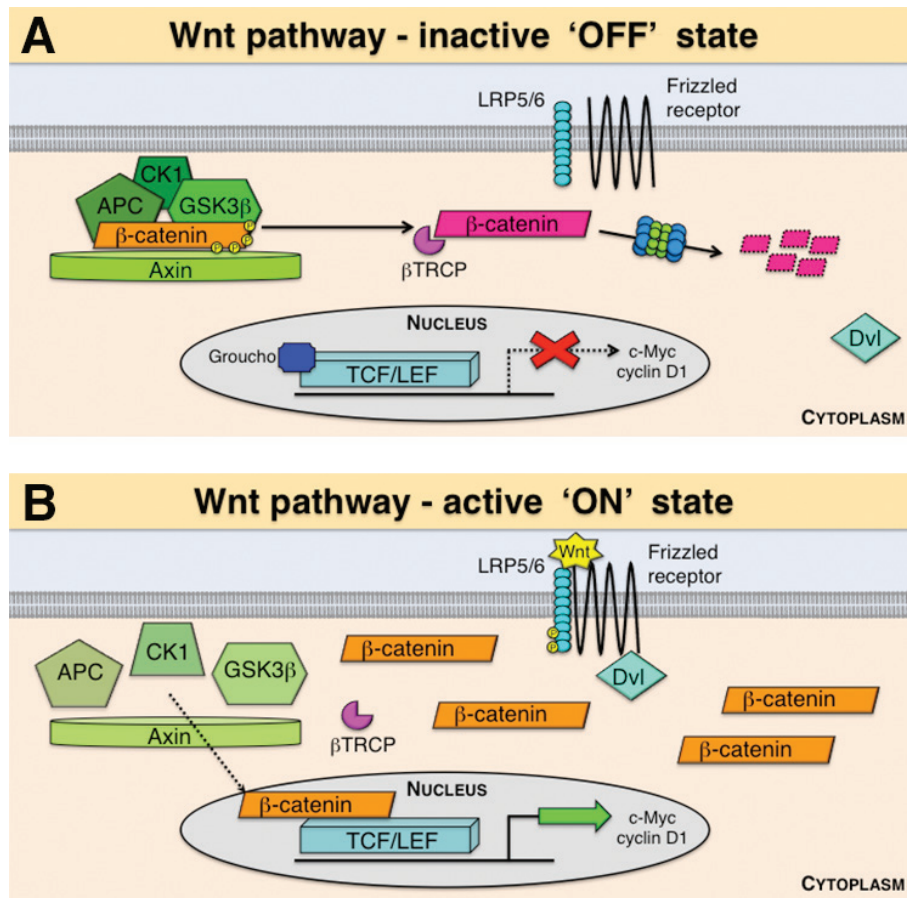


Figure 2. The canonical Wnt/β-catenin pathway. In the absence of a Wnt signal (‘OFF’ state), the destruction complex, consisting of adenomatosis polyposis coli (APC), glycogen synthase kinase 3-β (GSK3β), casein kinase 1 (CK1) and Axin, binds and phosphorylates β-catenin, marking it for ubiquitination by the E3 ubiquitin ligase subunit beta-transducin repeat containing protein (βTRCP) and degradation through the proteasome. In this case, the repressor Groucho remains bound to T-cell factor/lymphoid enhancer factor (TCF/LEF) transcription factors, inhibiting transcription of target genes such as c-Myc and cyclin D1 (A). When a Wnt protein binds a Frizzled receptor and the low-density lipoprotein receptor-related proteins 5 and 6 (LRP5/6) in the ‘ON’ state, the protein dishevelled (Dvl) activates a cascade, which eventually disrupts the destruction complex, leading to stabilisation, cytoplasmic accumulation and nuclear translocation of β-catenin and ultimately the transcription of target genes (B).

Wnt/ β -Catenin Signalling in Liver Metabolism

The liver regulates metabolic homeostasis by controlling glycogen storage, gluconeogenesis, plasma protein synthesis, lipoprotein synthesis and detoxification. To manage fluctuating metabolic demands, hepatic cells constantly alter the expression of respective regulatory pathways. Accordingly, hepatic Wnt signalling activity is modified under different physiological and pathophysiological conditions (45). In adult healthy hepatocytes, β -catenin is ubiquitously expressed, but it is more active in pericentral compared to periportal hepatocytes (44). Expression of β -catenin in periportal regions is inhibited by hepatocyte nuclear factor 4 α (46). In contrast, pericentral hepatocytes display basal activation of β -catenin signalling, controlling expression levels of glutamine synthetase, ornithine aminotransferase and the glutamate transporter GLT-1, which together regulate glutamine metabolism (47). This heterogeneous distribution of metabolic function across the lobule reflects hepatic zonation and is necessary to achieve optimal metabolic regulation. Benhamouche and colleagues established that the Wnt/ β -catenin pathway is a major control switch pathway for metabolic zonation by demonstrating that blocking of β -catenin in hepatocytes by infection with an adenovirus encoding the Wnt signalling antagonist Dickkopf-1 (Dkk-1) resulted in expansion of the periportal transcriptome and downregulation of perivenous genes. Conversely, constitutive activation of β -catenin through liver-induced disruption of the negative regulator APC reversed this gene expression profile and induced the perivenous gene expression programme (48).

The localisation and signalling activity of β -catenin becomes modified upon liver injury (Figure 3) (44, 49). Debebe and colleagues demonstrated recently that hepatic steatosis experimentally induced by feeding of a high fat diet, deletion of phosphatase and tensin homologue deleted on chromosome 10 (*Pten*) or transgenic expression of HCV core/NS5A protein, all resulted in macrophage-secreted Wnt activating CD133⁺CD49f⁺ tumour-initiating cells. These data strongly suggested a Wnt/ β -catenin-mediated link between obesity and cancer (50). In addition, β -catenin was shown to regulate hepatic gluconeogenesis during starvation and insulin-resistant conditions via interaction with the transcription factor forkhead box protein O 1 (FoxO1). This interaction leads to a change in expression of genes encoding the enzymes glucose-6-phosphatase and phosphoenolpyruvate carboxykinase, which then determine the rate of hepatic gluconeogenesis (44, 51). During oxidative stress conditions, β -catenin interacts with FOXO and enhances FOXO transcriptional activity to induce expression of targets for detoxification of reactive oxygen species (52). FOXO factors are sensitive to increased insulin levels, hence the interaction of β -catenin and FOXO is particularly important in diseases associated with insulin resistance, such as non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH) and the metabolic syndrome in general (44). The metabolic

syndrome, previously known as the insulin-resistance syndrome, has been defined as a clustering of the risk factors, namely, central obesity, hypertension, hypertriglyceridaemia, hyperglycaemia and low levels of high-density lipoprotein (53). Metabolic syndrome as well as NAFLD are associated with reduced insulin sensitivity and decreased insulin effects on glucose and lipid metabolism (54).

Numerous studies have established a role of the Wnt/ β -catenin pathway in the metabolic syndrome since it was demonstrated that Wnt signalling represents a molecular switch to control adipogenesis. Activation of the canonical Wnt/ β -catenin pathway through Wnt10b, inhibition of GSK3 β or expression of dominant stable β -catenin prevented differentiation of preadipocytes and myoblasts through inhibition of the adipogenic transcription factors CCAAT/enhancer-binding protein α (C/EBP α) and peroxisome proliferator-activated receptor γ (PPAR γ) (55, 56). Kennell and MacDougald investigated *Xenopus* Wnt8 and FZD1 or FZD2 chimeras and established a role for both β -catenin-dependent and β -catenin-independent mechanisms in mesenchymal cell fate and adipogenesis (57). Conversely, in a recent report, inhibition of Wnt signalling through the Wnt-inhibitory molecule sclerostin led to spontaneous adipogenesis of pre-adipocytes and mesenchymal precursors (58), supporting the concept of Wnt signalling controlling the adipogenic switch.

Wnt/ β -Catenin Signalling and Cancer

In hepatocellular carcinoma cells, the most frequent mutations occur in *TP53*, coding for the tumour suppressor p53, and *CTNNB1*, the β -catenin gene (59). Coste and colleagues suggested that 26% of human and 50% of mouse hepatocellular carcinomas carry activating mutations in *CTNNB1* (60). Subsequent studies supported these findings and reported up to 44% of hepatocellular carcinomas with *CTNNB1* mutations (61–64). Activation of β -catenin is hypothesised to be due to mutations in exon-3 at the serine and/or threonine sites near the NH₂ terminus in *CTNNB1*, which inhibit phosphorylation-dependent degradation of the β -catenin protein, leading to an aberrant activation of the canonical Wnt/ β -catenin pathway (44). Simultaneous mutation of the β -catenin gene and the tumour suppressor H-ras or p21 by an adenovirus-mediated liver-specific Cre-expression system resulted in 100% tumour incidence with short latency of only several weeks (65). Interestingly, mutations in the β -catenin gene in hepatocellular carcinomas induce overexpression of β -catenin targets such as the glutamine synthetase gene *GLUL* (66, 67), whereas hepatocytes from glutamine synthetase-negative tumours are often *H-ras* or *BRAF* mutated and express E-cadherin, reflecting perivenous and periportal profiles, respectively (68). Loss-of-function in *APC* and *Axin* is mutually exclusive to *CTNNB1* mutations and has been detected in 1–3% and 8–15% of hepatocellular carcinoma cases (for review, see (69)).

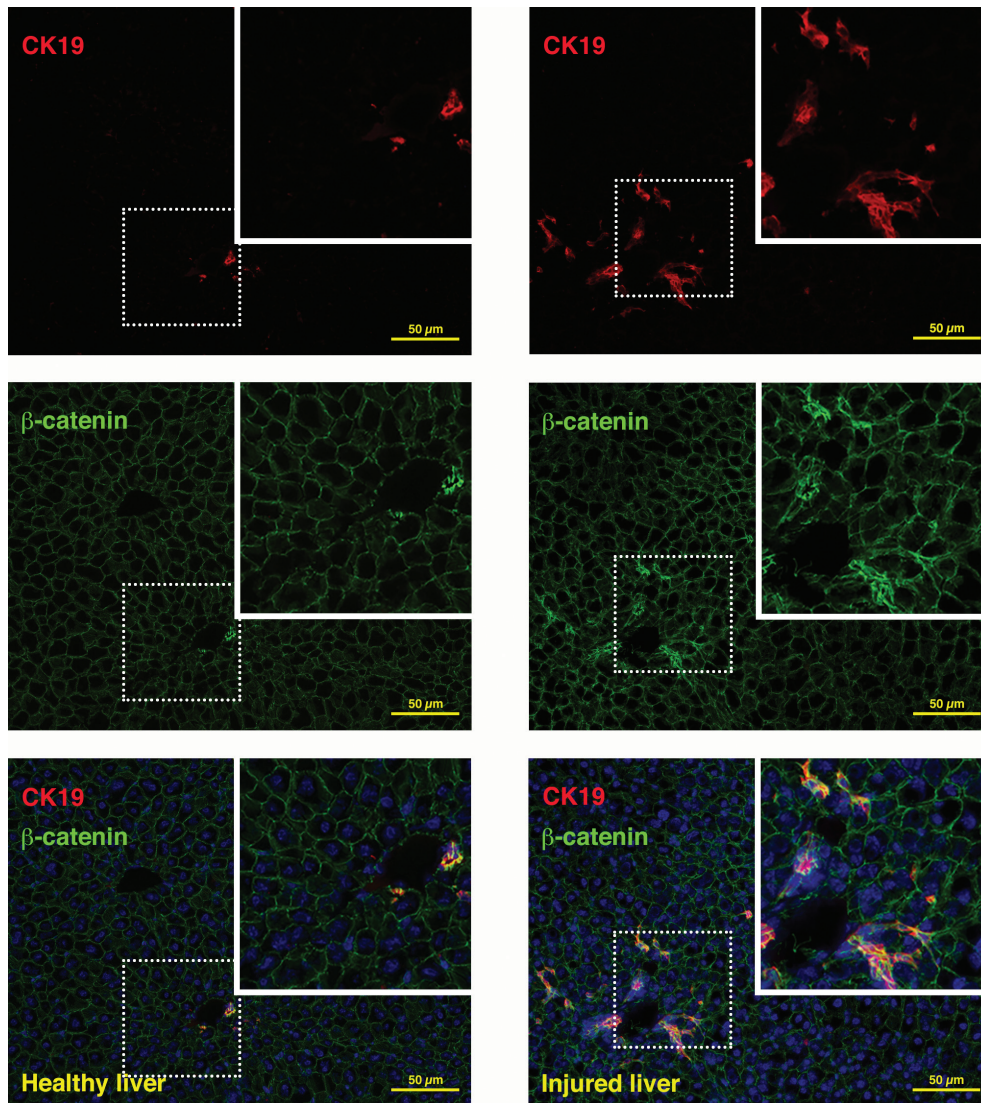


Figure 3. Beta-catenin and CK19 expression in healthy and injured liver. In healthy mouse liver, only ducts stain with an antibody targeting CK19 and show cytoplasmic and nuclear β -catenin expression, while β -catenin is exclusively membrane-bound in periportal hepatocytes (healthy liver, left panel). In injured liver (2-week treatment with a choline-deficient, ethionine-supplemented diet¹⁵), the CK19⁺ compartment expands and demonstrates strong cytoplasmic and nuclear β -catenin, signifying active signalling (injured liver, right panel).

In a 2009 study, the Wnt ligands Wnt3, Wnt9a and Wnt10b were shown to be highly expressed in most hepatocellular carcinoma cell lines, irrespective of their differentiation status. Clear profiles were, however, observed with Wnt2b, Wnt4, Wnt5a, Wnt5b and Wnt7b, which were overexpressed in poorly differentiated cell lines, while Wnt8b and Wnt9b were only expressed in well-differentiated cell lines. These data suggested canonical Wnt signalling activity in well-differentiated cells, contributing to tumour initiation and its repression in poorly differentiated cell lines, which the authors hypothesised to regulate tumour progression (70). Other Wnt

pathway components associated with hepatocellular carcinoma development include Wnt signalling antagonists such as secreted frizzled-related proteins (SFRPs), Wnt-inhibitory factor (WIF)-1 and Dickkopf (Dkk) proteins. SFRP1 has been suggested as a tumour suppressor gene, since its expression was downregulated due to promoter hypermethylation in 76.1% of hepatocellular carcinoma specimens at the RNA level and in 30% at the protein level (71). In hepatocellular carcinoma cell lines and clinical specimens, WIF-1 expression was equally found to be repressed by promoter hypermethylation, suggesting epigenetic inactivation as the primary cause

for WIF-1 loss during hepatocarcinogenesis (72). Inactivity of the negative Wnt regulators Dkk2 and Dkk3 has been reported in human gastrointestinal tumours (73). Fatima and colleagues observed significantly reduced mRNA expression of Dkk4 in almost half of all investigated hepatocellular carcinoma cases. Immunohistochemical data linked decreased Dkk4 expression to accumulation of β -catenin in hepatocellular carcinoma tissue. In addition, the authors showed that Dkk4 overexpression in hepatocellular carcinoma cell lines resulted in reduced cell proliferation, colony formation and cell migration, suggesting a tumour-suppressive role for Dkk4 (74). A recent study demonstrated that hepatocellular carcinoma cells proliferate upon stimulation in high glucose conditions as a result of Dkk4 downregulation, allowing Wnt3a-mediated β -catenin signalling and c-Myc upregulation (75), suggesting the Wnt pathway may be a therapeutic target in insulin-resistant conditions, leading to hepatocellular carcinoma.

Conclusion

Many diverse signalling pathways regulate liver development, homeostasis, regeneration and carcinogenesis. Given the strong evidence for an association of (i) progressive liver disease and LPCs, (ii) CSC-like LPCs and liver tumour formation and (iii) obesity, insulin resistance, hepatic steatosis and hepatocarcinogenesis, and the fact that the Wnt/ β -catenin signalling seems to be playing major roles in all these processes, this pathway represents a particularly promising therapeutic target to prevent or treat hepatocellular carcinoma.

Conflict of interest statement

The authors report no conflict of interest with respect to research, authorship and/or publication of this article.

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