Role and Regulation of Wnt/ β -Catenin in Hepatic Perivenous Zonation and Physiological Homeostasis

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Metabolic heterogeneity or functional zonation is a key characteristic of the liver that allows different metabolic pathways to be spatially regulated within the hepatic system and together contribute to whole body homeostasis. These metabolic pathways are segregated along the porto-central axis of the liver lobule into three hepatic zones: periportal, intermediate or midzonal, and perivenous. The liver performs complementary or opposing metabolic functions within different hepatic zones while synergistic functions are regulated by overlapping zones, thereby maintaining the overall physiological stability. The Wnt/ β -catenin signaling pathway is well known for its role in liver growth, development, and regeneration. In addition, the Wnt/ β -catenin pathway plays a fundamental and dominant role in hepatic zonation and signals to orchestrate various functions of liver metabolism and pathophysiology. The β -catenin protein is the central player in the Wnt/ β -catenin signaling cascade, and its activation is crucial for metabolic patterning of the liver. However, dysregulation of Wnt/ β -catenin signaling is also implicated in different liver pathologies, including those associated with metabolic syndrome. β -Catenin is preferentially localized in the central region of the hepatic lobule surrounding the central vein and regulates multiple functions of this region. This review outlines the role of Wnt/ β -catenin signaling pathway in controlling the different metabolic processes surrounding the central vein and its relation to liver homeostasis and dysfunction. (*Am J Pathol* 2022, 192: 4–17; <https://doi.org/10.1016/j.ajpath.2021.09.007>)

The liver is responsible for performing multiple physiological functions, including macronutrient metabolism, lipid and cholesterol homeostasis, endocrine and immune regulation, as well as breakdown of drugs to reduce toxicity. These multifunctional tasks are performed simultaneously by the liver because of its unique anatomic organization of hepatocytes, which are discretely arranged and yet synchronized to conduct all the necessary physiological processes.¹ Such specialized hepatocytic organization within the liver is called liver or metabolic zonation, and it plays a critical role in the biochemical functionality of this organ. Any perturbation of the hepatic architecture causes pathologic alterations with permanent defects, leading to metabolic diseases.

The Wnt/ β -catenin signaling pathway is well known for its role in liver development, regeneration, and maintaining homeostatic balance.² Activation of this pathway is critical

for hepatic organogenesis, as evident by its involvement in hepatoblast proliferation, survival, and maturation.³ In addition, Wnt/ β -catenin signaling is a hallmark of various hepatic pathologies, including fibrosis,⁴ steatohepatitis,⁵ tumorigenesis,⁶ and cholestasis.⁷ Such a diverse

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complexity for the role of Wnt/ β -catenin pathway in regulating homeostatic functioning and/or pathophysiology of hepatic injury has been a subject of extensive research for the past several years, although much remains unknown. This review highlights one of the fundamental roles of Wnt/ β -catenin signaling in the liver, that of metabolic zonation, and the critical functions that are regulated by targets of this signaling pathway.

Metabolic Zonation

Under steady state, hepatocytes display molecular heterogeneity depending on their localization within hepatic lobule, the hexagon-shaped structure that is the smallest functional unit in the liver (Figure 1).¹ Inside the hepatic lobule, hepatocytes are spatially segregated into three different zones: hepatocytes surrounding the portal triad (consisting of portal vein, bile ducts, and hepatic artery) constitute the periportal (afferent) zone 1; those surrounding the central vein form the perivenous or pericentral (efferent) zone 3 hepatocytes; and the intermediate hepatocytes sandwiched between the two zones are referred to as mid-zonal or intermediate perivenous zone 2.¹ These differentially zoned hepatocytes line hepatic sinusoids along the portocentral axis, and although they share morphologic similarities, they exhibit functional metabolic heterogeneity, commonly known as metabolic or liver zonation. The term metabolic zonation means that the key steps involved in various metabolic functions are distributed spatially based on where a hepatocyte is located within the hepatic lobule. These zone-specific functions of hepatocytes are driven by oxygen and nutrient gradients, allowing for interdependent metabolic pathways to be colocalized in overlapping zones while performing complementary or opposing metabolic functions in spatially separated nonoverlapping zones. Consequently, the contribution of these hepatocytes in liver homeostasis involves a tight regulation of opposing metabolic processes with the periportal zone involved in gluconeogenesis, cholesterol biosynthesis, and oxidative phosphorylation, while the perivenous hepatocytes perform glycolysis, lipogenesis, bile acid biosynthesis, glutamine synthesis, and xenobiotic metabolism.⁸

For many years, it has been known that canonical Wnt signaling is a key regulator of metabolic zonation.^{9,10} Wnt/ β -catenin signaling is most active in the hepatocytes surrounding the central vein, owing to short-range Wnt ligands secreted by central-vein endothelial cells.^{10–12} Consequently, during homeostasis, activated β -catenin is exclusively localized in the hepatocyte subpopulations surrounding the central vein.^{13,14} Gene expression in these so-called perivenous hepatocytes differs strikingly from the gene expression pattern observed in periportal hepatocytes.¹⁵ These differences have functional consequences on liver metabolism that will be discussed in detail below.

Wnt/ β -Catenin Signaling and Activation of β -Catenin

Canonical Wnt signaling is dependent on β -catenin, a transcriptional coactivator and effector of this pathway, to perpetuate the signal and regulate gene expression. Transduction through this pathway is initiated after binding of Wnt glycoproteins to the Frizzled receptor and the coreceptor low-density lipoprotein receptor-related protein (LRP) 5 or 6 on the plasma membrane.¹⁶ The Wnt-Frizzled-LRP complex triggers the recruitment of the scaffolding protein Dishevelled (Dvl) and phosphorylation of LRP5/6.¹⁷ The latter further mediates recruitment of scaffold protein Axin to the plasma membrane. Axin forms a part of the destruction complex, comprising glycogen synthase kinase-3 (GSK3), casein kinase 1 α (CK1 α), and tumor suppressor protein adenomatous polyposis coli (Apc), that together direct the phosphorylation and ubiquitination of β -catenin for its proteasomal degradation (Figure 2).¹⁸ The recruitment of Axin to the membrane disrupts the destruction complex, stabilizing β -catenin, which eventually translocates into the nucleus and binds with transcription factors T-cell factor/lymphocyte enhancer factor (TCF/LEF) to induce transcription of Wnt target genes.

Role of β -Catenin in Hepatic Perivenous Zone Gene Expression

The importance of Wnt/ β -catenin signaling in establishing liver metabolic zonation was first described in mice with liver-targeted inactivation of Apc. Deletion of this negative regulator of Wnt signaling activated β -catenin and switched the hepatocyte phenotype from periportal to perivenous.¹⁴ In the same study, it was also found that blocking hepatocyte β -catenin by overexpression of the Wnt antagonist Dickkopf-1 (Dkk1) down-regulated the expression of many perivenous genes and instead caused expansion of the periportal gene expression program. Subsequent studies confirmed the importance of β -catenin in maintaining homeostatic liver function through preservation of hepatic zonation.^{9,19,20}

More recent work has also elucidated the cell and molecular circuitry upstream of β -catenin that governs zonation. β -Catenin is activated in zone 3 by Wnts originating from the adjacent endothelial cells of the central vein.^{11,21,22} In particular, Wnt2 and Wnt9b have been reported as major drivers of β -catenin activation in zone 3 (Figure 3).^{10,13} The R-spondin ligands and their leucine-rich repeat-containing G-protein coupled receptors (LGRs) 4/5, which regulate the Wnt/ β -catenin gradient in liver, also help to control hepatic zonation.^{23,24} In addition, disruption of Wnt signaling by knockdown of liver-specific LRP5/6 leads to defective hepatic zonation and homeostasis despite intact β -catenin expression.²¹

Accordingly, perivenous hepatocytes express cytoplasmic and nuclear β -catenin along with membranous localization

Figure 1 Metabolic liver zonation, showing the different hepatic zones within the basic structural hepatic lobule and the functional responsibilities of the centrilobular hepatocytes in the perivenous zone. **A:** Overview of liver. **B:** The basic structural units of liver, called the hepatic lobules, organized into a honeycomb-like architecture. **C:** A single hexagon-like hepatic lobule with central vein located at the center and multiple portal triads at the periphery. **D:** Enlarged view of a single portal triad composed of a hepatic artery, a portal vein, and a bile duct. **E:** Outline of different perivenous zonal responsibilities in the liver. Figure was prepared using Biorender.com (Toronto, ON, Canada).

of this transcriptional coactivator. Considering the preferential activation of β -catenin within the perivenous zone and its role in mediating the zone-specific gene expression pattern, it becomes imperative to understand and delineate the responsibilities of β -catenin in regulating the various metabolic processes that are confined to this zone (Table 1).

Role of β -Catenin in Heme Biosynthesis

Heme, an iron containing porphyrin, is an essential cofactor utilized in varied cellular processes, including oxygen binding, drug detoxification, and gene regulation at transcriptional and translational levels.²⁵ Heme serves as the prosthetic group for hemoproteins, like the cytochrome P450 (CYP) family, as well as for hemoglobin, cytochrome *c*, catalases, and peroxidases.²⁶ For a hemoprotein to be fully functional, it must be bound to its prosthetic group heme, the supply of which depends on the heme synthesis regulatory mechanisms.

Synthesis of heme is differentially regulated between erythrocytes in bone marrow and hepatocytes in the hepatic perivenous zone.²⁵ Using murine models of hepatocyte-specific deletion or overexpression of β -catenin, Braeuning and Schwartz²⁶ reported four members of the heme

synthesis pathway with preferential perivenous zonal localization [namely, aminolevulinate dehydratase (ALAD, alias porphobilinogen synthase), hydroxymethylbilane synthase (HMBS), coproporphyrinogen oxidase (CPOX), and uroporphyrinogen III synthase (UROS)]. The mRNA and protein expression of these enzymes was positively regulated by β -catenin activation.²⁶ Hepatic zonation of these heme-synthesizing proteins was abolished in *Ctnnb1* or β -catenin knockout (KO) mice. Interestingly, no remarkable zonation for the first and rate-limiting enzyme of heme-synthesis pathway (i.e., aminolevulinate synthase 1 (ALAS 1)) was observed.

A defect in any of the enzymes involved in heme biosynthesis can cause accumulation of pathway intermediates called protoporphyrins. These porphyrin precursors are cytotoxic, and their excessive accumulation over time leads to porphyria-associated liver injury.²⁷ Targeted deletion of Wnt/ β -catenin components or pharmacologic inhibition of this pathway decreased porphyrin accumulation in a murine model of porphyria that normally causes accumulation of porphyrin plugs associated with intermittent bile duct blockage.²⁸ Decreased expression of heme biosynthesis enzymes aminolevulinate synthase 1 (ALAS) and aminolevulinate dehydratase (ALAD), the latter a direct target of Wnt/ β -catenin signaling, prevented accumulation of porphyrin intermediates,

Figure 2 Wnt/β-catenin signaling and β-catenin activation for target gene transcription. **A:** Wnt off situation: In the absence of Wnt glycoprotein, β-catenin, a transcriptional co-activator and the effector molecule of the pathway, is bound in a destruction complex consisting of Axin, adenomatous polyposis coli, casein kinase 1 (CK-1), and glycogen synthase kinase-3β (GSK-3β). This destruction complex directs the phosphorylation and ubiquitination of β-catenin for its proteasomal degradation with the net result that β-catenin-mediated gene transcription is not activated. **B:** Wnt on situation (canonical Wnt/β-catenin signaling): When Wnt is present, it binds to the Frizzled receptor and coreceptor low-density lipoprotein receptor-related protein (LRP) 5/6. The Wnt-Frizzled-LRP complex causes dissociation of the destruction complex, which stabilizes β-catenin. The latter then translocates to the nucleus, where it binds to the transcription factors T-cell factor (TCF)/lymphocyte enhancer factor (LEF), resulting in transcription of Wnt target genes. Figure was prepared using Biorender.com (Toronto, ON, Canada).

leading to reduced protein aggregates and less liver injury. This study demonstrated the direct effect of Wnt/β-catenin in regulating heme biosynthesis, and that this pathway can be targeted in heme-associated porphyria-mediated liver injury.

Heme oxygenase (HO) is the rate-limiting enzyme in the catabolism of heme into biliverdin, iron, and carbon monoxide. The biliverdin formed is rapidly converted by biliverdin reductase to bilirubin. In cases of excessive bilirubin production, called hyperbilirubinemia, the risk of neurologic disorders increases, causing irreversible brain damage, such as kernicterus or bilirubin encephalopathy, which occurs in newborns with severe jaundice.²⁹ Management of bilirubin levels by interfering with its formation is suggested as a better approach than employing inhibitors of heme oxygenase (HO) activity after bilirubin has reached toxic levels.³⁰ Interestingly, liver-specific β-catenin KO mice had increased expression of HO concomitant with elevated serum bilirubin levels.³¹ Multidrug resistance protein 2 (Mdr2) KO mice lacking hepatocyte β-catenin had more severe cholestatic liver injury than Mdr2 KO alone; like β-catenin KO, this mouse model also showed increased HO expression and bilirubin, which likely contributed to higher levels of oxidative stress and biliary injury.³² Therefore, activating β-catenin in patients with this condition might

inhibit HO overexpression, which, in turn, could help reduce excessive heme catabolism and regulate hyperbilirubinemia and oxidative stress. Taken together, β-catenin is important in regulating the heme biosynthetic pathway as well as heme catabolism and consequently presents as a viable therapeutic target for diseases such as porphyria and hyperbilirubinemia.

Regulation of Xenobiotic Metabolism and Cytochrome P450 Enzyme Expression by Wnt/β-Catenin

Liver is a central player in the metabolism and removal of potentially toxic compounds or xenobiotics, including drugs, pesticides, herbicides, food additives, or environmental chemicals, like carcinogens or mutagens. The cytochrome P450 monooxygenases are the major enzymes responsible for this process, and many of the isoforms, such as CYP2B1/2, CYP2E1, and CYP3A1, show a preferential perivenous hepatic zonal expression.³³ CYPs are constitutively expressed or can be induced by ligand-activated nuclear receptors [namely, constitutive androstane receptor (CAR), pregnane X receptor (PXR), and aryl hydrocarbon

Figure 3 Schematic representation for the role of Wnt/β-catenin signaling in regulating the different hepatic perivenous zonal functions and metabolic homeostasis. The Wnt proteins Wnt2 and Wnt9b, which are secreted by liver sinusoidal endothelial cells surrounding the central vein, bind to the hepatocyte cell membrane—associated receptor proteins. These Wnt proteins, via the canonical Wnt/β-catenin signaling pathway, mediate β-catenin nuclear translocation and consequent activation of Wnt target genes. The net result is the regulation of several physiological and metabolic functions of the hepatic perivenous zone by β-catenin. Figure was prepared using Biorender.com (Toronto, ON, Canada). CYP, cytochrome P450.

receptor (AHR),³⁴ or by the transcription factor peroxisome proliferator—activated receptor (PPAR)].³⁵ The expression and activity of most CYPs coincide with the zonal area where constitutive β-catenin activation is evident. Indeed, mouse livers tumors with activated β-catenin³⁶ or transgenic mice expressing a mutant form of β-catenin³⁷ have high levels of normally pericentrally localized CYP proteins. Likewise, hepatocyte-specific loss of β-catenin has been linked to loss of several CYP isoforms, including CYP2E1 and CYP1A2.^{19,38} These enzymes metabolize excessive acetaminophen (APAP) into the reactive metabolite *N*-acetyl-*p*-benzoquinone imine (NAPQI), which induces hepatic necrosis. Because both enzymes are target genes of β-catenin, liver-specific β-catenin KO mice are resistant to APAP-induced injury.¹⁹ The zonal expression of xenobiotic genes also allows a toxicant-induced injury to be limited to a specific zone, allowing hepatocytes residing in other zones to survive and restore liver mass through proliferation. For example, owing to the location of CYP2E1 and CYP1A2 to zone 3 hepatocytes, only these cells undergo necrosis following sublethal doses of APAP, allowing midzonal

hepatocytes to proliferate and restore hepatic mass and architecture in a short time frame of 24 hours after insult.³⁹ Intriguingly, as soon as zone 3 hepatocytes are destroyed by APAP overdose, midzonal hepatocytes begin to express other β-catenin zone 3 target genes, like glutamine synthetase (GS),⁴⁰ to maintain vital functions, like that of scavenging excess ammonia for conversion to glutamine. This suggests that functional compensation of the liver occurs even before the recovery of hepatic mass and architecture.⁴⁰

Wnt/β-catenin signaling is an endogenous regulator of cytochrome P450 expression both at the basal level³⁷ and in response to exposure to xenobiotic agonists of the nuclear receptors.⁴¹ Apart from CYP genes that constitute the phase I drug-metabolizing enzymes, β-catenin also modulates the activation and zonal expression of several members of the glutathione-S-transferase (GST) family that forms the phase II drug-metabolizing enzymes.⁴² A recent study demonstrated the requirement of β-catenin in *CYP2C8* gene expression, and CAR-, and PXR-mediated induction of *CYP1A*, *CYP2B6*, and *CYP3A4* genes in a human hepatocarcinoma cell line.⁴³ On the contrary, activation of the

Wnt/ β -catenin pathway inhibited PPAR α —mediated induction of *CYP1A*, *CYP2C8*, *CYP3A4*, and *CYP4A11* genes, providing a dominant-negative role of β -catenin in regulation of these genes. So far, most of the reports on the role of β -catenin in zonal CYP regulation are from murine experimental models. However, considering mice metabolize many xenobiotics differently from humans due to species differences in *CYP* genes, it is possible that regulation of xenobiotic-metabolizing enzymes can differ between mice and humans. With the use of humanized-mouse model systems and metabolomics approaches, it has now become possible to overcome the potential species differences to more accurately predict the function and regulation of these enzymes in humans.

Role of Wnt/ β -Catenin Signaling in Regulating Drug Transporters for Cancer Therapeutics

In addition to the drug-metabolizing enzymes, membrane transporters play significant roles in drug disposition. The organic anion transporting polypeptide (OATP/SLCO) is one such family of uptake transporters that mediates sodium-independent transport of a wide variety of structurally diverse endogenous compounds and xenobiotics. Of these, OATP1B1 (OATP2) and OATP1B3 (OATP8) are specifically found in the liver and expressed on the basolateral membrane of hepatocytes, wherein they participate in drug elimination.⁴⁴ In normal livers, the expression of OATP1B1/B3 increases from portal (no expression) to perivenous areas (maximal expression) along sinusoids,⁴⁵ resembling the expression pattern of β -catenin under physiological conditions.

Mutations in the *CTNNB1* gene have been implicated in approximately one-quarter of hepatocellular carcinoma (HCC) cases.⁴⁶ This particular subset of HCC has also been associated with activation of OATP1B3 expression in the perivenous region, suggesting a key role for β -catenin in regulating OATP1B3 expression.⁴⁷ Analysis of mRNA expression further revealed significant correlation of OATP1B3 expression and Wnt/ β -catenin—associated genes, including *CYP2E1*, *GS*, *OAT*, *AXIN2*, and *LGR5*.⁴⁸ In addition, GS expression and β -catenin nuclear expression strongly correlated with OATP1B3 expression score in immunohistochemical analyses. Case reports of β -catenin—activated hepatocellular adenoma also showed intense OATP1B3 expression,⁴⁹ and equivalent-to-increased expression of OATP1B1/3 throughout the tumor.⁵⁰ Thus, OATP1B3 up-regulation may be a candidate marker for Wnt/ β -catenin—activated hepatocellular lesions. Of the two subtypes of OATP1B3, (Lt)-OATP1B3 (hepatic type expressed in human liver) and (Ct) OATP1B3 (cancer type identified in cancer tissues and cell lines), the former mediates the uptake of many clinically important anticancer drugs, thereby expanding the potential clinical role of OATP1B3 in cancer treatment and drug disposition in the liver.⁵¹ Further studies will be needed to explore and

correlate the expression levels of (Ct) OATP1B3 in different types of β -catenin—mutated hepatocellular lesions.

Wnt/ β -Catenin Regulation of Glutamine Synthetase for Ammonia Detoxification

Ammonia is normally produced in the gastrointestinal tract as a by-product of amino acid metabolism or as a waste metabolite from gut bacteria and carried to the liver via portal vein where it is converted to urea for excretion by the kidneys. Within the liver, two systems participate in ammonia removal. First is the urea cycle, catalyzed by the enzyme carbamoyl phosphate synthetase I (CPSI) that is expressed in the hepatocytes of the periportal and intermediate zones, and a few adjacent layers of the perivenous zone. The second is a failsafe mechanism mediated by the enzyme GS that is exclusively localized to the first one to two layers of the hepatocytes closest to the central vein and catalyzes the ATP-dependent formation of glutamine from glutamate and ammonia.⁹ Any ammonia not taken up by urea system in the periportal zone is scavenged by GS in the perivenous hepatocytes.

The initial evidence for the role of β -catenin in ammonia detoxification came from the finding that active nuclear β -catenin signaling correlated with a significant increase in GS expression.^{52,53} Wnt/ β -catenin—regulated zonation of GS is independent of c-Myc, a downstream effector of Wnt/ β -catenin signaling.⁵⁴ Further studies showed that aberrant activation of β -catenin through loss of Apc results in expansion of the usually perivenous pattern of expression of GS toward the periportal area, as well as increased expression of other perivenous ammonia detoxification genes, such as ornithine aminotransferase (*OAT*) and glutamate transporter 1 (*GLT1*).¹⁴ In parallel to this, loss of periportal expression of CPSI, the rate-limiting step of the urea cycle, was also observed. More important, loss of Apc leads to deficient ammonia detoxification and accumulation of glutamine due to loss of CPSI and subsequent dysfunction of the high-capacity urea cycle. On the contrary, blocking the Wnt/ β -catenin pathway by Wnt antagonist DKK1, a negative regulator of β -catenin signaling, resulted in decreased expression of glutamine metabolism genes, such as *GS*, *OAT*, and *GLT1*.^{14,53} Loss of β -catenin from hepatocytes also abolished GS expression from the perivenous zone.^{9,19} The functional significance of GS loss was highlighted by the observation that although β -catenin knockout mice demonstrated normal blood ammonia levels on a standard diet, there was a marked increase in blood ammonia levels in mice fed with a high-protein diet.¹⁹ Thus, ammonia metabolism in the liver is highly dependent on tight regulation of β -catenin expression; its absence in the periportal region permits activation of the urea cycle, whereas its presence in the perivenous zone activates GS to scavenge excess ammonia.

An important implication of GS being a direct target of Wnt/ β -catenin was recently shown in HCC with β -catenin

Table 1 List of β -Catenin—Mediated Physiological Functions along with the Target Gene or Protein in the Perivenous Hepatic Zone

Physiological process	Target protein	Function	Role of β -catenin
Heme regulation	Aminolevulinate dehydratase (Alad) or porphobilinogen synthase	Heme biosynthesis	Positive regulation
	Hydroxymethylbilane synthase (Hmbs)	Heme biosynthesis	Positive regulation
	Coproporphyrinogen oxidase (Cpox)	Heme biosynthesis	Positive regulation
	Uroporphyrinogen III synthase (Uros)	Heme biosynthesis	Positive regulation
	Cytochrome P450 family	Heme biosynthesis	Positive regulation
	Heme oxygenase (HO)	Catabolism of heme	Negative regulation
Xenobiotic metabolism	Cytochrome P450 family (phase I detoxification enzymes)	Drug detoxification	Positive regulation
	CYP1A (CAR, PXR regulated), CYP2B1/2, CYP2B6 (CAR, PXR regulated), CYP2C, CYP2C8, CYP2E1, CYP3A1, CYP3A4 (CAR, PXR regulated)		
	Cytochrome P450 family (phase I detoxification enzymes)	Drug detoxification	Negative regulation
	CYP1A, CYP2C8, CYP3A4, CYP4A11 (all PPAR mediated)		
	Aminolevulinate synthase 1 (ALAS)	Cytotoxic protoporphyrin IX formation	Positive regulation
	Aminolevulinate dehydratase (ALAD)	Cytotoxic protoporphyrin IX formation	Positive regulation
Ammonia detoxification	Glutamine synthetase (GS)	Ammonia to urea conversion	Positive regulation
	Ornithine aminotransferase (OAT)	Ammonia to urea conversion	Positive regulation
	Glutamate transporter 1 (GLT1)	Ammonia metabolism	Positive regulation
Bile acid synthesis and transport	Cytochrome P450 family	Synthesis of bile	Positive regulation
	CYP7A1, CYP27		
Vitamin C synthesis	Claudin-2	Tight junction proteins	Positive regulation
	E-cadherin	Adherens junction protein	Positive regulation
	Regucalcin	Encodes L-gulonolactone oxidase for vitamin C biosynthesis	Positive regulation
Glucose metabolism	Glycolytic enzymes: glucokinase (GCK, PFK2/ FBP2, phosphofructokinase 1 (PFK1, pyruvate kinase (PK)	Glucose uptake and utilization	Positive regulation
	Glucagon-like peptide-1	Insulin secretion for glucose uptake	Positive regulation
	Kinases: Akt	Phosphorylation of FoxO proteins and decrease in gluconeogenesis	Positive regulation
	AMP-activated protein kinase (AMPK)	Proliferation of islet β -cells for insulin secretion	Positive regulation
	Pitx 2		
	Phosphoenolpyruvate carboxykinase	Decreases gluconeogenesis	Positive regulation
Lipid metabolism and homeostasis	Insulin receptor substrate-1 (IRS1)	Insulin signaling for glucose uptake	Positive regulation
	Cytochrome P450 enzyme: CYP2E1	Alcohol metabolism	Positive regulation
	Alcohol dehydrogenase	Alcohol metabolism	Positive regulation
	Aldehyde dehydrogenase	Alcohol metabolism	Positive regulation
	hepatocyte nuclear factor 4 α (HNF4 α)	Cholesterol and triglyceride metabolism	Positive regulation
	GCK	Encoding fatty acid synthase	Positive regulation
	mitochondrial glycerol-3-phosphate acyltransferase (mtGPAT) and diglyceride acyltransferase 2 (DGAT2)	Fatty acid esterification	Positive regulation

(table continues)

Table 1 (continued)

Physiological process	Target protein	Function	Role of β -catenin
Drug disposition for cancer therapy	OATP1B1 (OATP2)	Hepatic drug elimination	Positive regulation
	OATP1B3 (OATP8)	Hepatic drug elimination, uptake of anticancer drugs, candidate marker of β -catenin-activated hepatocellular lesions	Positive regulation

CAR, constitutive androstane receptor; FBP2, fructose-bisphosphatase 2; Fox = forkhead box; OATP, organic anion transporting polypeptide; Pitx = Paired-like homeodomain 1; PPAR, peroxisome proliferator-activated receptor; PXR, pregnane X receptor.

gene mutations. Owing to GS localization in most perivenous hepatocytes, and presumptively highest levels of intracellular glutamine in these cells, as well as the ability of glutamine to directly phosphorylate and activate the mechanistic target of rapamycin (mTOR) complex 1 in lysosomes, zone 3 hepatocytes in immediate proximity to the central vein were also positive for phosphorylated mTOR-serine2448.⁵⁵ This marker of active mTOR complex 1 was negative in zone 3 hepatocytes in hepatocyte-specific knockouts of β -catenin, LRP5/6, and GS. β -Catenin-mutated HCCs, which are known to be strongly GS positive, were also positive for phosphorylated mTOR-serine2448 and, hence, highly susceptible to mTOR inhibition in preclinical β -catenin-driven HCC models.

Role of Wnt/ β -Catenin Signaling in Bile Acid Metabolism

Recent evidence indicates that the Wnt/ β -catenin pathway also plays a role in regulating bile homeostasis, including bile synthesis, modification, and transport. Bile acids are synthesized from cholesterol via two pathways, which are initiated by either CYP7A1 or CYP27.⁵⁶ Although cholesterol synthesis occurs predominantly in periportal hepatocytes, CYP7A1 and CYP27 are localized in the perivenous zone of the liver lobule, coincident with β -catenin activation.⁵⁷ The close relationship between these two processes can be seen in β -catenin KO mice subjected to the methionine and choline-deficient diet, which is characterized by macrovesicular steatosis and fibrosis.⁵⁸ Liver-specific β -catenin deletion resulted in increased steatosis, higher hepatic cholesterol accumulation, and jaundice, likely due to defects in cholesterol to bile conversion mechanism and the bile export system.³¹ Intriguingly, β -catenin KO also had higher hepatic total bile acid levels on both methionine and choline-deficient and control diets, indicative of basal abnormalities in bile metabolism in the absence of β -catenin.

Disruption in bile canalicular transporter function or disruption of tight junction integrity in hepatocytes can also cause defective bile secretion and intrahepatic cholestasis.^{59,60} Loss of β -catenin from hepatocytes and cholangiocytes was associated with distorted bile canalicular

morphology seen as dilatation, tortuosity, and loss of canalicular microvilli.⁷ These mice also had bile secretory defects and reduced bile flow, resulting in higher serum and hepatic total bile acid levels.⁷ Other studies have also reported that knockdown of claudin-2, a β -catenin target gene, prevents bile canalicular formation *in vitro*.⁶¹ Furthermore, depletion of β -catenin–E-cadherin–based adherens junctions causes defective canalicular lumen remodeling.⁶²

Evidence that β -catenin has a direct effect on bile acid metabolism came from chromatin immunoprecipitation (ChIP)–sequencing studies, which showed CYP27 to be a transcriptional target of β -catenin.⁶³ Subsequent studies suggested that CYP7A1 is also either a direct or an indirect target of Wnt signaling as CYP7A1 is suppressed basally in both β -catenin KO and LRP5/6 KO.^{64,65} Interestingly, β -catenin can also impact bile acid metabolism through its physical interaction with farnesoid X receptor (FXR), a nuclear receptor that regulates expression of bile acid efflux transporters and represses CYP7A1 expression through activation of small heterodimer partner (SHP). Lack of β -catenin eliminates FXR sequestration, leading to increased availability of FXR to decrease bile acid biosynthesis and stimulate its elimination, with the result that β -catenin KO have less liver injury, fibrosis, and atypical ductular proliferation after experimental cholestasis.^{64,65} Overexpression of oncogenic β -catenin in mice leads to cholestatic liver disease, whereas in humans, hepatocellular carcinoma with β -catenin gene mutations also exhibits intratumoral cholestasis.^{66,67} Because both CYP27 and CYP7A1 are expressed in the perivenous zone along with β -catenin, it is possible that excess β -catenin activates these two bile acid synthesis enzymes either directly or indirectly through inhibition of FXR around the central vein. These studies show that β -catenin plays a complex, multifaceted role in regulating bile acid metabolism that could have mechanistic and perhaps therapeutic implications for treatment of cholestatic liver disease.

Canonical Wnt/ β -Catenin in Vitamin C Synthesis and Regulation

Vitamin C or ascorbic acid is an essential dietary nutrient that has pleiotropic functions in the body, including as an

enzyme cofactor, an antioxidant, and a component of both the innate and adaptive immune system.⁶⁸ Paradoxically, humans and most animals, including zebrafish (but excluding mice), are unable to synthesize vitamin C because of a mutation in l-gulonolactone oxidase, the enzyme that catalyzes the last and rate-limiting step in the biosynthesis of this essential nutrient.⁶⁹

The first indication that β -catenin may be playing a direct or indirect role in vitamin C biosynthesis in mice came from gene array analysis, which showed that regucalcin was one of the genes most affected by loss of β -catenin in liver.³⁸ Regucalcin, also called senescence marker protein 30, is a gluconolactonase that is important in cell homeostasis and function, and also catalyzes the penultimate step in the biosynthesis of vitamin C.⁷⁰ Livers of β -catenin knockout mice had deficiency in expression of both regucalcin and L-gulonolactone oxidase.⁷¹ These mice demonstrated low serum ascorbate levels associated with reduced basal hepatocyte survival. Additional analysis showed that regucalcin expression was more pronounced around the central veins, and molecular assays confirmed that regucalcin is a transcriptional target of β -catenin/TCF.⁷¹ Altogether, these observations support a role for β -catenin in regulating cell survival through vitamin C biosynthesis.

Although β -catenin can regulate the vitamin C pathway, it seems that the reciprocal is true as well. In a recent study, using vitamin C—dietary model of zebrafish, Liu et al⁷² demonstrated the role of β -catenin signaling in mediating the lipid-lowering effect of vitamin C. In this model, vitamin C—diet administration inhibited GSK-3 β expression, which otherwise induces phosphorylation, ubiquitination, and degradation of β -catenin in the muscle of zebrafish. Inhibition of GSK-3 β resulted in increased expression of β -catenin, ultimately leading to decreased fatty acid synthase activity and triglyceride levels. The overall result was lowering of lipid content and regulating lipid profile by vitamin C through GSK-3 β / β -catenin signaling.⁷² Although the importance of β -catenin in regulating vitamin C synthesis might be limited to pre-clinical models, the interplay between these two pathways, as well as β -catenin's role in cell survival, has implications that require further investigation.

Role of Wnt/ β -Catenin Pathway in Glucose Metabolism and Associated Injury

The presence of several enzyme-regulatory mechanisms in different hepatic zones enables the liver to perform the homeostatic function of glucose metabolism, regulating a tight switch between hepatic uptake or release of glucose to keep the blood sugar level within an optimal range. Such regulatory metabolic enzymes function in accordance to the concentration gradients of other metabolites, such as oxygen, hormones glucagon and insulin, and the morphogens Wnt and hedgehog, along the hepatic sinusoids.

Hepatocytes near the portal region are exposed to a high concentration of oxygen, which is required for anabolic processes that use ATP, such as gluconeogenesis. In contrast, hepatocytes close to the perivenous zone experience low levels of oxygen and thus are engaged in glucose uptake mechanisms, like glycolysis, an ATP-producing process that functions under conditions of oxygen deprivation. As a result, higher amounts of glycolytic enzymes glucokinase (GCK), phosphofructokinase 2/fructose-bisphosphatase 2 (PFK2/FBP2), phosphofructokinase 1 (PFK1), and pyruvate kinase (PK) are found in the perivenous region, whereas the gluconeogenetic enzymes glucose-6-phosphatase (G6PC), fructose 1,6 bisphosphatase 1 (FBP1), mitochondrial pyruvate carboxylase (PCmito), and phosphoenolpyruvate carboxykinase 1 (PEPCK) are expressed in the periportal region.⁷³ This is supported by earlier studies in rats that showed glucagon receptors involved in increasing blood glucose levels predominantly exist in the periportal region, whereas insulin receptors that participate in glucose consumption are abundantly expressed in the perivenous region.⁷⁴ In addition, hepatic insulin receptor signaling is crucial for hepatic and systemic glucose homeostasis as lack of insulin receptor in the liver is associated with severe insulin resistance and hyperglycemia after feeding.⁷⁵

Genome-wide association studies have linked the canonical Wnt/ β -catenin pathway to metabolic risk factors, with mutations in the components of this pathway associated with increased likelihood of developing metabolic syndrome and obesity phenotypes.^{76–79} A noncoding variant of transcription factor 7-like 2 (TCF7L2), commonly known as TCF4, the transcription factor that partners with β -catenin in the canonical Wnt signaling pathway, emerged as the strongest type 2 diabetes susceptibility gene that provided initial clues to the role of canonical Wnt signaling in glucose homeostasis.⁷⁸ Further studies in mice showed that TCF7L2 is a downstream effector of insulin in hepatocytes and together with β -catenin negatively regulates hepatic gluconeogenesis.⁸⁰ A single missense mutation in LRP6, the coreceptor for the Wnt signaling pathway, showed a strong genetic linkage with impaired glucose metabolism and hyperlipidemia.⁷⁶ Mice lacking the Wnt coreceptor LRP5 also exhibited impaired pancreatic insulin secretion and thus impaired glucose uptake.⁸¹

Recent studies have further suggested that β -catenin plays a direct role in regulating serum glucose concentrations and modulating hepatic insulin signaling. Proteomics analysis showed that β -catenin positively regulates enzymes involved in glycolysis, such as lactate dehydrogenase, while negatively regulating the gluconeogenesis gene FBP1.⁸² Another study showed that β -catenin indirectly regulates glucokinase function through hypoxia-inducible factor 1 alpha (HIF1 α), a transcriptional regulator of glycolysis that is subject to hypoxic regulation.⁸³ Interestingly, however, there is some indication that β -catenin positively regulates gluconeogenesis as well, albeit not

through canonical β -catenin/TCF activation. The forkhead family member FoxO1 regulates the expression of genes involved in *de novo* glucose synthesis, such as PEPCCK and G6PC.⁸⁴ Under conditions of oxidative stress, β -catenin can bind to FoxO family members, an interaction that inhibits β -catenin/TCF binding.⁸⁵ β -Catenin also preferentially binds to FoxO1 under fasting conditions, where it modulates the gluconeogenic response through regulation of PEPCCK1 and G6PC.⁸⁶ Thus, the switch in binding partners allows β -catenin to alter its functionality and target gene expression, depending on the nutritional requirements of the body. Another study showed that glucagon regulates metabolic zonation in the liver by opposing the Wnt/ β -catenin signaling pathway, enhancing periportal gene expression at the expense of the Wnt-dependent perivenous gene program.⁸⁷ Thus, hepatic Wnt signaling functions in multiple capacities to regulate glucose homeostasis in liver.

Because of the association between hepatic pathologies, such as nonalcoholic fatty liver disease (NAFLD) and surplus calorie intake, studies in recent years have focused on the role of β -catenin in glucose metabolism under conditions of high-fat diet (HFD). Liver-specific conditional loss of β -catenin lowered fasting plasma glucose and improved glucose tolerance in HFD-fed mice, likely due to the decreased expression of hepatic gluconeogenic enzymes described above, together with improvements in insulin signaling in the absence of β -catenin.⁸⁶ Another study by Behari et al⁸³ reported improved hepatic insulin sensitivity and hepatic steatosis in liver-specific β -catenin knockout on HFD. Using second-generation antisense oligonucleotides to decrease hepatic and adipose β -catenin in mice exposed to HFD, a third group reported an improvement in hepatic insulin sensitivity and insulin-stimulated whole body glucose metabolism associated with increased white adipose tissue and cardiac glucose uptake.⁸⁸ These findings were intriguing as they demonstrated the effect of reduced β -catenin in glucose homeostasis in contrast to other studies involving complete loss of liver β -catenin. As promising as these preclinical findings appear, a recent study in patients illustrates that glucose regulation is a complex process, and that β -catenin perturbation in this context may have multifactorial consequences. The insulin receptor substrate (IRS)-1, which is predominantly localized to the perivenous hepatic zone, is a direct transcriptional target of β -catenin. Decreased hepatic expression of IRS-1 and β -catenin has been associated with histologic progression, such as ballooning in a prospective cohort study on NAFLD patients with a significant risk factor of developing type 2 diabetes due to impaired glucose metabolism.⁸⁹ It seems that a coordinated regulatory mechanism involving a decrease in enzyme expression for gluconeogenesis simultaneous with an increase in insulin signaling plays a role in improving the final metabolic outcome observed in the HFD model systems and ultimately in patients as well.

Regulation of Lipid Homeostasis by Wnt/ β -Catenin Signaling

The interconnected association between glucose, insulin, and lipid homeostasis is well known, and thus it is not surprising that in addition to glucose metabolism the Wnt signaling pathway is involved in regulating fatty acid metabolism as well. In the liver, the hepatic periportal zone, which has a higher oxygen gradient, conducts energy-producing fatty acid oxidation, whereas lipogenesis and triglyceride synthesis occur primarily in the perivenous region.⁹⁰ As with glucose regulation, aberrations in Wnt/ β -catenin pathway components that affect lipid metabolism have been noted in patients. Mutations in the Wnt coreceptor LRP6 showed association with increased susceptibility toward hyperlipidemia in a family predisposed to metabolic syndrome.⁷⁶ Mice expressing this mutated form of LRP6 have a similar phenotype and develop fatty liver disease due to increased lipogenesis and cholesterol synthesis, which is a function of enhanced mTOR/Akt signaling. Administering Wnt3a partially reversed the hyperlipidemia induced by LRP6 deficiency and identified Wnt signaling as a regulator of plasma lipids.⁹¹ On the other hand, the same group showed that LRP6 heterozygous mice are protected from hepatic steatosis when fed a HFD due to decreased mTOR signaling and diminished expression of gluconeogenesis genes.⁹² The differential responses may be attributable to compensatory adaptation that is disrupted by a second hit, such as HFD, or by impaired ligand/receptor interactions in the mutated form of LRP6 compared with the half-maximal activation in the LRP6 heterozygous mice.

An important study implicating a direct role for β -catenin in lipid metabolism showed that mice transgenic for hepatocyte-specific stable β -catenin fed HFD had increased hepatic steatosis in perivenous and midzonal hepatocytes and threefold higher liver triglyceride levels compared with β -catenin KO or wild-type mice on HFD.⁸³ These mice also developed diet-induced obesity and insulin resistance. In comparison to HFD-fed KO mice, the HFD-fed β -catenin transgenic mice also showed higher protein expression of the key metabolic enzymes hepatic fatty acid synthase and glucokinase, which are involved in lipogenesis and glycolysis, respectively, both of which are located in the perivenous zone. The increased hepatic lipogenesis in HFD-fed transgenic mice was independent of PPAR γ expression, a major regulator of the lipogenic response. On the other hand, β -catenin KO mice were resistant to diet-induced obesity due to defective fatty acid oxidation, a result of lower perivenous hypoxia and decreased HIF1 α expression. These findings were supported by another study that targeted β -catenin mRNA expression level in the liver using antisense oligonucleotides (ASO), which resulted in reduced hepatic triglyceride levels as well as ameliorated hepatic steatosis and lipid-induced insulin resistance after HFD.⁸⁸ This was caused by decreased expression of mitochondrial

glycerol-3-phosphate acyltransferase (mtGPAT) and diglyceride acyltransferase 2 (DGAT2), the key enzymes of fatty acid esterification to generate triglycerides. Consequently, suppression of mtGPAT and DGAT2 prevented accumulation of triglycerides by decreasing lipogenesis and protected HFD-fed mice from hepatic steatosis. The results of these studies suggest that β -catenin has a profound effect on lipid metabolism under conditions of diet-induced fatty liver.

Other compounds, such as alcohol and hormones, can also impact the Wnt/ β -catenin-dependent regulation of lipid metabolism. Increased hepatic steatosis and liver triglycerides were observed in liver-specific β -catenin knockout mice fed liquid ethanol compared with wild-type controls. This was correlated with loss of β -catenin-regulated expression of enzymes, such as alcohol dehydrogenase, aldehyde dehydrogenase, and Cyp2E1, resulting in defective ethanol metabolism, systemic toxicity, and early mortality.⁹³ In another recent study, Tian et al⁹⁴ reported that a dominant negative form of *TCF7L2*, identified above as being a type 2 diabetes risk gene,⁷⁸ leads to elevated serum and hepatic lipid contents in male mice but not in females. The mechanism likely involves cross talk between β -catenin/TCF and the female hormone estradiol because reconstitution experiments with estradiol in wild-type females after ovariectomy attenuated HFD-induced lipid metabolic defects.

Hepatocyte nuclear factor 4 alpha (HNF4 α), the master regulator of liver development and function, is a major contributing factor in lipid metabolism and homeostasis. Down-regulation of hepatic HNF4 α has been associated with hypolipidemia and nonalcoholic steatohepatitis.^{95–97} Interestingly, HNF4 α has a dual role in metabolic zonation by stimulating periportal but down-regulating perivenous genes,⁹⁸ and may in fact cooperate with β -catenin to establish metabolic zonation in the liver. The Wnt downstream player lymphoid enhancer-binding factor 1 (LEF1) interacts with HNF4 α to dissociate it from its binding site, thus suppressing periportal gene expression in the perivenous region.⁹⁹ In the perivenous region, β -catenin also binds directly to HNF4 α , making it unavailable for activation of its target genes, whereas TCF binding to HNF4 α response elements further prevents their transcription. The reverse occurs in the periportal region; the presence of HNF4 α on β -catenin response elements prevents their transcription, while also binding to β -catenin and TCF to prevent their nuclear translocation.⁶³ Moreover, Wnt/ β -catenin signaling is strongly activated in mice deficient for HNF4 α .¹⁰⁰ Loss of HNF4 α may alter the interaction of co-activators cAMP response element-binding protein-binding protein (CBP) and p300 with β -catenin, resulting in marked deregulation of β -catenin signaling. However, the detailed signaling mechanisms by which the perivenous Wnt/ β pathway interacts with HNF4 α for regulating lipid metabolism and homeostasis under different metabolic stress conditions are not clear.

Conclusions and Future Perspectives

The extensive research findings to date present sufficient evidence for the significant and indispensable role of canonical Wnt/ β -catenin signaling in regulating liver perivenous metabolic functions and homeostasis (Figure 3). However, the detailed molecular mechanisms of many of these β -catenin-mediated perivenous tasks need more investigation. A major weakness of the current findings is they are largely based on rodent and zebrafish models, and their relevance to human liver function would require further intensive evaluation. With the advancement of humanized-mouse model systems that exclude the risk of potential species differences, as well as advanced organoid culture systems and metabolomics approaches, future studies should focus on extrapolating the findings to human subjects for ultimate therapeutic management.

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