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Brief Review

The Conundrum of the Pericentral Hepatic Niche: WNT/β-Catenin Signaling, Metabolic Zonation, and Many Open Questions

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WNT/β-catenin signaling promotes stemness, proliferation, and cell fate decisions in various tissue stem cell compartments, which maintain organs with a high turnover of cells (e.g., skin, stomach, and gut). Thus, the β-catenin target genes AXIN2 and LGR5 are widely considered as tissue stem cell markers. In contrast, AXIN2 and LGR5 are expressed in pericentral hepatocytes, which do not show overt proliferation during liver homeostasis. Given the low hepatocyte turnover, the liver does not require constant high rates of proliferation, whereas WNT/β-catenin signaling is critical for metabolic zonation. Yet, WNT/β-catenin pathway upregulation, including AXIN2 and LGR5 induction in hepatocytes throughout the liver, enables hepatocyte regeneration in response to various injuries. In this brief review, I discuss the role of WNT/β-catenin signaling in controlling metabolic zonation and the conundrum around pericentral hepatocytes that have been proposed as liver stem cells.

Key words: WNT signaling; Metabolic zonation; Homeostasis; Liver stem cell; Tissue stem cell; AXIN2; LGR5; Liver regeneration

WNT/β-CATENIN SIGNALING IN THE LIVER

The WNT/β-catenin pathway is a highly conserved master regulator controlling diverse critical processes, including organ patterning, cell fate decisions, and cell proliferation during development, homeostasis, and regeneration[1–3]. In the absence of WNT ligands, β-catenin is bound to the destruction complex, which contains the tumor suppressor adenomatous polyposis coli (APC), glycogen synthase kinase 3β (GSK3β), casein kinase 1α (CK1α), and scaffolding proteins AXIN1/2, among other components. CK1α and GSK3β phosphorylate β-catenin, targeting it for ubiquitin-dependent proteasomal degradation. When WNT ligands bind their cognate Frizzled receptor and the LRP5/6 coreceptor, phosphorylation of LRP5/6 results into decreased activity of the β-catenin destruction complex and stabilization of β-catenin. Stabilized β-catenin accumulates in the cytoplasm and can now enter the nucleus, where it binds to the TCF family of transcription factors, and enhances the transcription of β-catenin target genes[1–3]. R-spondin (RSPO)1–4 ligands potentiate WNT/β-catenin signaling following binding to their leucine-rich repeat-containing G protein-coupled receptors 4–6 (LGR4–6) by clearing the cell surface transmembrane E3 ubiquitin ligases, zinc and ring finger 3 (ZNRF3), and its homolog ring finger 43 (RNF43), which promote WNT receptor turnover, from the plasma membrane[4–11]. WNT/β-catenin signaling is also regulated via crosstalk with several other pathways, such as YAP, mTOR, HGF–cMET, Notch, SHH, USP7, and transforming growth factor-β (TGF-β) signaling[12–18].

In the liver, genetic deletion of WNT/β-catenin pathway components revealed its important role in promoting hepatic cell fate specification during early development, whereas its role during hepatoblast differentiation and late-stage liver development is less clear. Moreover, the WNT/β-catenin pathway controls proliferation and metabolic zonation during early postnatal liver development. In the adult liver, WNT/β-catenin signaling maintains liver size by controlling hepatocyte

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proliferation while preserving metabolic zonal patterning. While WNT/β-catenin activity is restricted to the pericentral area during homeostasis, its upregulation in hepatocytes throughout the liver is important to promote hepatocyte proliferation and liver regeneration in response to injury. Deregulated hepatic WNT/β-catenin signaling is also involved in various liver diseases, including liver fibrosis and tumor formation, highlighting the importance of fine-tuning and restricting hepatic WNT/β-catenin activity[19,20].

METABOLIC ZONATION

The liver is responsible for metabolizing nutrients and xenobiotics, as well as for the production and recycling of various proteins. Division of labor by hepatocytes in the three different liver zones is critical to enable liver function, since hepatocytes along the porto-central blood flow execute complementary tasks. Periportal hepatocytes perform gluconeogenesis, cholesterol biosynthesis, and urea metabolism, whereas pericentral hepatocytes perform glycolysis, bile acid biosynthesis, and glutamine synthesis. Hepatic metabolic zonation is controlled by the interplay of several pathways, often involving WNT/β-catenin signaling. A centro-portal WNT/β-catenin activity gradient is critical to establish and maintain metabolic zonation since most pericentral metabolic genes are either direct or indirect targets of β-catenin. Classical metabolic enzymes regulated by β-catenin include glutamine synthetase (GS) and CYP2E1, which are expressed only in the one to two first layers of hepatocytes around the central vein or in all pericentral and some adjacent parenchymal hepatocytes, respectively[21–30]. Several studies utilizing gene deletion of different WNT/β-catenin pathway components collectively suggest that inhibition of WNT/β-catenin signaling results in the loss of metabolic zonation due to lack of pericentral enzyme expression. Conversely, activation of the pathway caused the opposite effect and derailed metabolic zonation by expanding the pericentral metabolic program toward the portal vein[24,25,27–32]. While these studies established the importance of hepatic WNT/β-catenin signaling, they do not solve the question of how pathway activity is spatially controlled in a gradient manner. APC is expressed in a porto-central gradient, and given its inhibitory function on WNT/β-catenin activity, it may be responsible for blocking β-catenin activity in periportal hepatocytes[24]. In addition, local WNT and RSPO ligand secretion from central vein endothelial cells (CEVs) and liver sinusoidal endothelial cells (LSECs) seem to be responsible for activating WNT/β-catenin signaling in pericentral and adjacent parenchymal hepatocytes[27,30–35]. However, the exact mechanisms spatially confining expression of these ligands and thereby establishing the centro-portal WNT/β-catenin activity gradient remain elusive.

PERICENTRAL HEPATOCYTES VERSUS TISSUE STEM CELLS

The intrinsic regenerative capacity of the liver was already described in the myth of Prometheus 2,500 years ago and has been extensively studied in recent decades. While all hepatic cell types and many regenerative pathways collaboratively orchestrate liver regeneration, the WNT/β-catenin pathway stood out as a key regulator of hepatocyte proliferation[19,20,36]. Genetic labeling of cells with active WNT/β-catenin signaling allowed lineage tracing over time and revealed tissue stem cell compartments in several organs with high rates of homeostatic renewal (e.g., skin, stomach, and gut). WNT/β-catenin signaling controls proliferation, stemness, and cell fate decisions in several tissue stem cell compartments, and the β-catenin target genes AXIN2 and LGR5 were established as tissue stem cell markers[37,38].

High rates of homeostatic proliferation and the short time, in which descending cells migrate and differentiate, enabled lineage tracing tools to clearly identify tissue stem cells in other organs[37,38]. In contrast, adult hepatocytes have an average life span ranging from 200 to 300 days, and the majority is resting in a quiescent (G_0) state, while only up to 2% are actively cycling at any given time[39,40]. Such low turnover and the associated low proliferation rates make it more challenging to identify potential hepatocyte subpopulations with increased proliferative potential during liver homeostasis (Fig. 1). It is obvious to imagine that pericentral hepatocytes may be liver stem cells, since they express the tissue stem cell markers AXIN2 and LGR5 and harbor constant WNT/β-catenin signaling, similar to tissue stem cells in other organs. Likewise, several lineage tracing studies have asked the question whether AXIN2/LGR5$^+$ pericentral hepatocytes may be the long sought elusive liver stem cells[30,35,41–43]. Using AXIN2–CreERT2 lineage tracing mice, Wang and colleagues observed that AXIN2/GS$^+$ hepatocytes at the central vein expanded over time and repopulated large parts of the liver during liver homeostasis. Descendants of AXIN2/GS$^+$ hepatocytes even reached the periportal zone, collectively suggesting that they are liver stem cells refueling the hepatocyte pool under homeostatic conditions[35]. In contrast, lineage tracing of pericentral hepatocytes using LGR5–CreERT2 mice neither confirmed increased proliferative potential of pericentral hepatocytes nor did it indicate their expansion into the periportal zone[30]. However, AXIN2 inhibits WNT/β-catenin signaling and is a tumor suppressor in the liver[44–46], and AXIN2–CreERT2 lineage tracing mice are heterozygous mutants for *Axin2*[35]. Likewise, LGR5–CreERT2 mice are lacking one *Lgr5* allele, with unclear potential consequences in pericentral hepatocytes[30].

To exclude the possibility that potential haploinsufficiency of *Axin2 or Lgr5* may bias WNT/β-catenin

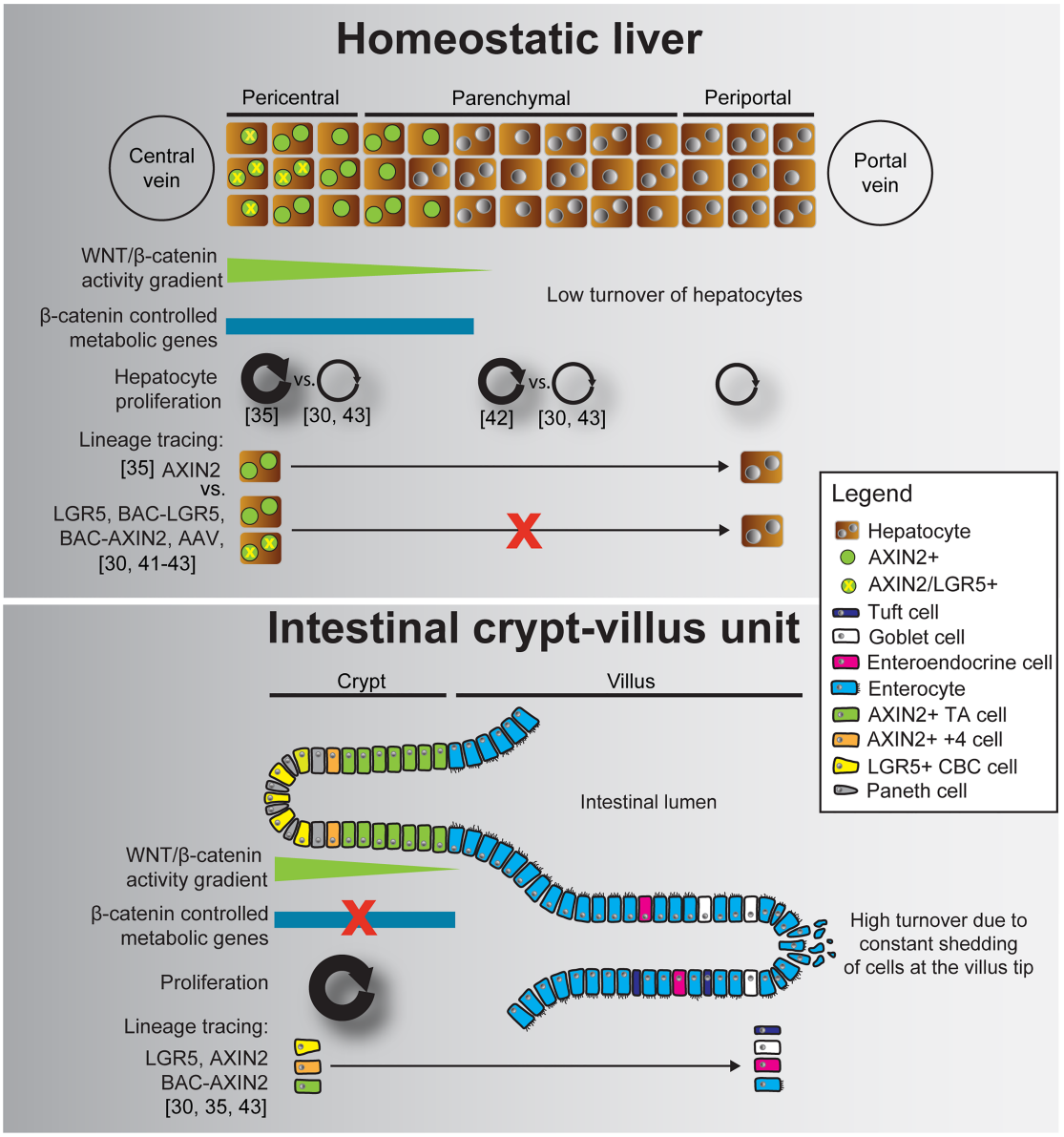

Figure 1. WNT/β-catenin signaling, metabolic zonation, proliferation, and tissue stem cell potential in the pericentral hepatocyte zone and the intestinal crypt–villus unit. While proliferation is low and similar in all three liver zones, the intestinal crypt shows high levels of proliferation that are required to compensate for constant loss of cells at the villus tip. Lineage tracing studies* collectively indicate that AXIN2/LGR5$^+$ crypt base columnar (CBC) cells and AXIN2$^+$ transit-amplifying (TA) cells repopulate the villus and give rise to more differentiated cells. In contrast, the majority of lineage tracing studies suggest that AXIN2/LGR5$^+$ pericentral hepatocytes neither repopulate the liver during homeostasis nor do their descendants stream into the periportal zone. *We only mention lineage tracing mice in this figure that have been discussed in this review. Lineage tracing of crypt cells has been pioneered with different LGR5 lineage tracing mice[51], and many more mouse lines successfully recapitulated and extended this finding.

signaling and proliferation of pericentral hepatocytes, BAC-transgenic LGR5 and AXIN2 lineage tracing mice were developed to shed additional light on the controversial role of AXIN2/LGR5$^+$ pericentral hepatocytes[41,43]. Interestingly, BAC–LGR5–CreERT2 and BAC–AXIN2–CreERT2, as well as adeno-associated virus (AAV)-based lineage tracing, indicated that pericentral hepatocytes have no superior proliferative capacity over other hepatocytes, do not stream into the periportal zone, and may therefore not represent bona fide liver stem cells[41–43] (Fig. 1). Importantly, the initial increase in BAC–AXIN2–CreERT2-labeled pericentral hepatocytes within the first week was due to additional CreERT2-mediated recombination by residual tamoxifen rather than proliferation,

with no significant further increase in labeled hepatocytes from day 7 to 10 months of lineage tracing[43]. While Wang and colleagues found more EdU incorporation in AXIN2/GS$^+$ hepatocytes when compared to AXIN2/GS$^-$ hepatocytes[35], other studies observed most EdU incorporated in midzonal hepatocytes[30,47]. However, midzonal hepatocytes also comprise the largest hepatocyte population, and normalization to hepatocyte numbers in each zone indicated similar percentage of EdU$^+$ hepatocytes in the three liver zones[30,43]. Yet, the overall low hepatocyte proliferation rates (2%–8% in hepatocytes[30,35,43], compared to almost 80% proliferating AXIN2$^+$ cells in the small intestine[43]), the high variability between the studies, and the short duration of EdU labeling make it difficult to judge the proliferative potential of hepatocytes in different hepatic zones[30,35,43]. Clonal tracing of LGR4$^+$ hepatocytes showed similar clone size in all liver zones, suggesting no zonal dominance during homeostatic hepatocyte proliferation[30]. In contrast, AAV-based random lineage tracing indicated increased proliferation of midzonal hepatocytes[42], which also showed higher baseline levels of the WNT/β-catenin target and cell cycle regulator CYCLIND1[42,48]. Additional research is required to clarify the contribution of hepatocytes in different hepatic zones to maintaining a functional hepatocyte pool during liver homeostasis.

Pericentral hepatocytes did also not display increased regenerative potential in response to various liver injury models[41,43]. Moreover, DTA-mediated ablation of AXIN2/LGR5$^+$ pericentral hepatocytes only transiently disrupted the pericentral zone, which was reestablished by conversion of GS$^-$ into GS$^+$ hepatocytes and compensatory proliferation of hepatocytes in other zones[43]. In addition, AXIN2 and LGR5 upregulation in hepatocytes outside the pericentral niche is a hallmark of many different liver injury settings where WNT/β-catenin signaling is important to drive hepatocyte proliferation[30,43,49,50]. With the majority of studies arguing against a liver stem cell role for pericentral hepatocytes, it appears that hepatocytes throughout the liver can upregulate AXIN2 and LGR5 in response to injury and contribute to liver regeneration on demand.

Another conundrum in the pericentral niche is the low rate of homeostatic hepatocyte proliferation despite constant WNT/β-catenin signaling necessary to maintain metabolic zonation. While the intestinal crypt and pericentral liver zone both consist of niche cells providing WNT and RSPO ligands and epithelial cells expressing AXIN2 and LGR5, they seem to utilize WNT/β-catenin signaling differently. Comparative transcriptomic profiling suggests that intestinal stem cells do not express β-catenin-regulated metabolic enzymes but show upregulation of cell cycle genes when compared to AXIN2$^+$ hepatocytes, and vice versa[43]. Although these are fundamentally different cell types, involving diverse cooperative signaling networks with other pathways, it would be interesting to identify mechanisms allowing these cells to differentially utilize WNT/β-catenin signaling. Most importantly, the mechanisms restricting proliferation in pericentral hepatocytes despite constant WNT/β-catenin necessary to maintain metabolic zonation remain elusive.

CONCLUDING REMARKS

Fine-tuning of WNT/β-catenin signaling is critical for maintaining metabolic zonation in the homeostatic liver, whereas overt proliferation, as seen in tissue stem cell compartments of other organs, must be prevented. Therefore, pericentral hepatocytes require mechanisms restricting their proliferation despite constant WNT/β-catenin activity. It remains to be studied whether epigenetic regulation may prevent induction of cell cycle genes in pericentral hepatocytes. Possibly we could learn from liver regeneration studies, where upregulation of WNT/β-catenin signaling is followed by increased hepatocyte proliferation and therefore signaling changes in hepatocytes reentering the cell cycle may inform about possible mechanistic cues. Given the high amount of liver tumors displaying hyperactive WNT/β-catenin signaling and the high susceptibility of pericentral hepatocytes to produce tumors in experimental models, learning more about mechanisms restricting proliferation in pericentral hepatocytes may further our understanding of liver tumor initiation. Additional lineage tracing studies utilizing different genetic tracing markers and monitoring hepatocyte proliferation over longer periods might further clarify the debated role of pericentral hepatocytes being liver stem cells and the zonal contribution of hepatocytes to liver homeostasis.

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