Spatial hepatology: Decoding liver zonation for metabolic and regenerative therapeutics (Review)

GUIQING CHEN[1,2*], HONGYAN JIA[1*], KAI ZHAO[2*], CHANG GAO[1], YUE LIU[1], HONG ZHU[3], DAKAI YANG[1,2] and YUN CAI[2]

[1]Jiangsu Key Laboratory of Medical Science and Laboratory Medicine, School of Medicine, Jiangsu University, Zhenjiang, Jiangsu 212013, P.R. China; [2]Department of Gastroenterology, Jintan Affiliated Hospital of Jiangsu University, Changzhou, Jiangsu 213200, P.R. China; [3]Department of Life Sciences, Jingjiang College, Jiangsu University, Zhenjiang, Jiangsu 212013, P.R. China

Received November 14, 2025; Accepted April 24, 2026

DOI: 10.3892/ijmm.2026.5856

Abstract. The integration of spatial omics and single-cell technologies has redefined liver zonation as a dynamic regulator of regeneration, extending beyond its traditional role in metabolic compartmentalization. This progress has given rise to the emerging field of 'spatial hepatology', which aims to integrate spatial information with molecular features. Compared with conventional bulk or single-cell approaches, spatial hepatology incorporates tissue architecture into molecular analyses, thereby revealing region-specific regulatory mechanisms during liver regeneration and disease progression. From this perspective, the present review proposes the concept of zonation-guided therapeutic strategies. It systematically summarized recent advances in the molecular mechanisms governing liver zonation, examined the role of zonal disruption in liver disease pathogenesis, clarified the dynamic functions of zonated hepatocytes during regeneration and outlined related targeted therapeutic approaches. The present review aimed to establish a framework that integrates basic research with clinical application, providing a theoretical basis for precision hepatology.

Correspondence to: Professor Yun Cai, Department of Gastroenterology, Jintan Affiliated Hospital of Jiangsu University, 500 Avenue Jintan, Changzhou, Jiangsu 213200, P.R. China
E-mail: caiyundyh@163.com

Professor Dakai Yang, Jiangsu Key Laboratory of Medical Science and Laboratory Medicine, School of Medicine, Jiangsu University, 301 Xuefu Road, Zhenjiang, Jiangsu 212013, P.R. China
E-mail: yangdakai@126.com

*Contributed equally

Abbreviations: HIF, hypoxia-inducible factor; GH, growth hormone; mTOR, mechanistic target of rapamycin; ECM, extracellular matrix; MASLD, metabolic dysfunction-associated steatotic liver disease; MASH, metabolic dysfunction-associated steatohepatitis; YAP, Yes-associated protein; APAP, acetaminophen; NAPQI, N-acetyl-p-benzoquinone imine; HybHPs, hybrid hepatocytes; PHx, partial hepatectomy; FAO, fatty acid oxidation

Key words: liver zonation, spatial reprogramming, regenerative niche, liver disease therapy

Contents

1. Introduction

The functional diversity of the liver arises from its spatially organized zonation architecture, where hepatocytes progressively alter their metabolic and detoxification capacities along the porto-central axis (1). This intricate partitioning, sustained by blood-borne molecular gradients and intercellular crosstalk, enables the liver to balance antagonistic biochemical processes while maintaining systemic homeostasis (2). Paradoxically, this structural stability is reconfigured during severe liver injury. Hepatocyte necrosis disrupts zonal organization, which both limits remaining liver function and triggers repair responses. Recent spatial biology studies show that the liver adopts different regenerative strategies depending on context, ranging from zonal self-renewal to whole-lobule

plasticity (3-5). These processes are highly influenced by local microenvironmental signals. New technologies have begun to reveal how surviving cells use positional cues to rebuild functional structures.

Based on these studies and emerging technologies, the concept of 'spatial hepatology' has gradually emerged. This approach involves integrating multi-omics techniques in the context of tissue *in situ*, such as spatial transcriptomics, spatial proteomics and spatial metabolomics, to systematically analyze the distribution, functional states and interactions of different cell types at specific spatial locations within the liver (2). Compared with conventional single-cell sequencing or bulk transcriptomic analyses, spatial omics preserves tissue architecture while coupling molecular information with spatial coordinates, enabling precise mapping of functional liver regions and revealing dynamic molecular gradients in local microenvironments. This strategy offers significant advantages for investigating the remodeling of metabolic zonation, heterogeneity of injury responses and the regulation of cell fate during regeneration in liver disease, thereby providing a more refined and dynamic framework for understanding the pathogenesis and progression of liver disorders (4).

2. Functional zonation and dynamic adaptation in hepatic metabolic architecture

The liver lobule exhibits a clear functionally compartmentalized structure. Traditional approaches to characterizing liver zonation and metabolic compartmentalization include quantitative PCR, immunohistochemistry and *in situ* hybridization (6). These methods reveal spatial differences in protein expression and enzymatic activity between periportal and pericentral hepatocytes, forming the basis of metabolic zonation. This spatial distribution arises from sinusoidal blood flow, which generates gradients of oxygen tension, nutrient concentrations and xenobiotics along the porto-central axis (7). These gradients of these substances are key determinants of liver zonation.

Studies on zonal metabolic features show that the periportal region predominantly carries out β-oxidation, urea synthesis and gluconeogenesis, whereas the pericentral region is primarily responsible for glycolysis, lipogenesis, and detoxification processes (8-10). Although most metabolic processes exhibit strict zonal segregation and serve as key indicators of liver function, some pathways can operate independently in different zones while remaining coordinated (11). A typical example is ammonia metabolism; studies have shown that periportal hepatocytes efficiently remove ammonia through glutaminase and urea cycle enzymes, whereas pericentral hepatocytes detoxify residual ammonia via glutamine synthetase (12,13). This spatial division of labor ensures high metabolic efficiency. However, this compartmentalized organization also concentrates cytochrome P450 activity in the pericentral region, making it more susceptible to toxin-induced necrosis (14).

Notably, liver zonation is not a static structure but exhibits high dynamic plasticity through coordinated transcriptional regulation and structural remodeling (15). Nutritional status is a key driver of this dynamic change. For example, fasting enhances lobular gluconeogenesis and promotes the extension of β-oxidation, typically dominant in the periportal region, toward the midlobular zone to meet systemic energy demands (16,17). During pregnancy, this adaptation is further reinforced by the expansion of periportal gluconeogenic hepatocytes, thereby maintaining maternal-fetal glucose homeostasis (18). Together, these regulatory mechanisms indicate that the liver can dynamically reorganize its spatial architecture in response to physiological states, enabling precise control of metabolic function.

3. Molecular gradients and spatiotemporal dynamics in liver lobule compartmentalization

Recent advances in single-cell sequencing and multi-omics have improved the current understanding of liver zonation (19,20). These approaches define metabolic gradients, transcriptional networks and chromatin accessibility across hepatocyte populations, refining traditional models of lobular organization (21). Evidence shows that liver zonation is not simply binary; >80% of hepatocyte genes display continuous expression gradients along the porto-central axis (19). Based on this, the liver lobule is commonly divided into three zones: i) Periportal (zone 1); ii) midlobular (zone 2); and iii) pericentral (zone 3) (2). Higher-resolution models further divide the lobule into nine layers, corresponding to these three regions. Zone-specific markers have also been identified. Periportal markers include ASS1, Cyp2f2, Sds, Asl, Gls2 and Arg1. Midlobular markers include hepcidin antimicrobial peptide 2 (Hamp2) and Insulin-like growth factor-binding protein 2 (Igfbp2). Pericentral markers include cytochrome P450 2E1 (Cyp2e1), Cyp1a2, GS and Oat (Table I) (2,19).

Trajectory analyses of liver development have revealed the dynamic establishment of metabolic gradients. For example, hepatocytes in neonatal mice initially exhibit pericentral metabolic features, with zonation patterns progressively maturing after birth (22). This developmental plasticity is closely associated with hepatocyte polyploidization. Regarding the spatial distribution of diploid hepatocytes, early studies suggested that these cells are enriched in the periportal and decline along the lobular axis, implying a higher proliferative potential in this zone (23,24). However, subsequent investigations employing alternative lineage-tracing strategies have reported a more uniform distribution, with some even indicating relative enrichment in the pericentral zone (25,26). These discrepancies may reflect differences in species, age-related shifts in polyploidization and methodological biases in nuclear isolation. Future studies integrating spatially resolved single-cell omics will be essential to clarify the interplay between ploidy status, zonation characteristics and regenerative capacity. However, spatial specialization is not unique to hepatocytes. Non-parenchymal cell populations also exhibit pronounced zonal heterogeneity, which may be a key factor in maintaining hepatic zonation (27-29). Non-parenchymal cells distributed across different lobular regions likely perform context-dependent functions, contributing to region-specific regulation during disease progression and influencing regenerative processes. By integrating single-cell atlases with spatial transcriptomics, researchers have progressively constructed high-resolution maps of both hepatocyte and non-parenchymal cell responses during injury and regeneration, providing a theoretical foundation for understanding liver repair mechanisms (30).

Table I. Metabolic zonation in the liver.

A, Periportal (zone 1)			
Metabolic function	Metabolic markers	Key signaling pathways	(Refs.)
Fatty acid oxidation Urea cycle Gluconeogenesis Glutamine synthesis	CPT1 ASS1, Asl, Arg1 PCK1, Sds Gls2	Low Wnt/β-catenin; high glucagon signaling; high mTORC1 (fed state); low HIF	(1,2,17,33)
B, Midlobular (zone 2)			
Metabolic function	Metabolic markers	Key signaling pathways	(Refs.)
Iron metabolism Growth regulation	Hamp2 Igfbp2	Intermediate Wnt signaling	(88,89)
C, Pericentral (zone 3)			
Metabolic function	Metabolic markers	Key signaling pathways	(Refs.)
Lipogenesis Glycolysis Glutamine synthesis Xenobiotic metabolism Bile synthesis	ACC, ACL LDH, HK2 GS Cyp2e1, Cyp1a2 Cyp7a1, HSD3B7	High Wnt/β-catenin; low mTORC1 (fasting); high HIF-1α	(17,33,171)

CPT1, Carnitine palmitoyltransferase 1; ASS1, argininosuccinate synthase 1; Asl, argininosuccinate lyase; Arg1, arginase 1; PCK1, phosphoenolpyruvate carboxykinase 1; Sds, serine dehydratase; Gls2, glutaminase 2; Hamp2, hepcidin antimicrobial peptide 2; ACC, acetyl-CoA carboxylase; ACL, ATP citrate lyase; LDH, lactate dehydrogenase; HIF, hypoxia-inducible factors; HK2, hexokinase 2; GS, glutamine synthetase; Cyp2e1, cytochrome P450 family 2 subfamily E member 1; Cyp1a2, cytochrome P450 family 1 subfamily A member 2;Cyp7A1, Cytochrome P450 family 7 subfamily A member 1; HSD3B7, hydroxysteroid 17-β dehydrogenase 7.

4. Regulatory mechanisms governing hepatic zonation

Liver zonation is established through the coordinated action of systemic and local microenvironmental signals. Circulating factors, including hormonal gradients, oxygen tension and nutrient availability, activate spatially specific signaling pathways to establish metabolic compartmentalization. Among these, the Wnt/β-catenin pathway serves a central role, while other signaling networks, including Hedgehog (Hh) and oxygen-sensing pathways such as HIF, further coordinate epigenetic remodeling and metabolic reprogramming, thereby restricting hepatocyte functions to specific regions within the liver lobule (Fig. 1) (31-33).

Spatiotemporal regulation of metabolic zonation in hepatic architecture. The oxygen gradient along the porto-central axis of the hepatic lobule establishes a hypoxic microenvironment that activates hypoxia-inducible factors (HIFs) (33). Among these, HIF-1α is preferentially stabilized in pericentral hepatocytes, where it directly regulates glycolytic gene programs through binding to hypoxia-responsive elements (HREs) (33). This includes upregulation of glucose transporters GLUT1 (SLC2A1) and GLUT3 (SLC2A3), as well as key rate-limiting glycolytic enzymes such as HK2, PFK1, PKM2 and LDHA. Concurrently, HIF-1α transcriptionally activates PDK1, thereby inhibiting pyruvate entry into mitochondrial oxidative metabolism, collectively reinforcing the glycolytic phenotype characteristic of zone 3 hepatocytes (34,35). By contrast, HIF-2α primarily governs antioxidant defense and lipid metabolic regulation, directly inducing genes such as SOD2 and HMOX1 (36). However, sustained activation of HIF-2α can impair lipid homeostasis by transcriptionally suppressing PPARα-dependent fatty acid oxidation (FAO) genes, including CPT1A and ACOX1, thereby promoting steatosis (37). HIF-3α splice variants function as dominant-negative regulators by competing with HIF-1α for HRE binding or forming low-activity heterodimers, ultimately attenuating HIF-1α signaling (38,39).

Beyond oxygen gradients, the spatial distribution of hormonal signals represents another key regulatory axis shaping liver zonation. Although glucagon receptors are uniformly distributed across the hepatic lobule, glucagon itself forms a functional gradient along the porto-central axis (40). In glucagon-deficient mice, expression of the periportal marker gene Gls2 is markedly reduced, whereas the pericentral marker Glul, which is normally restricted to 1 to 3 hepatocyte layers

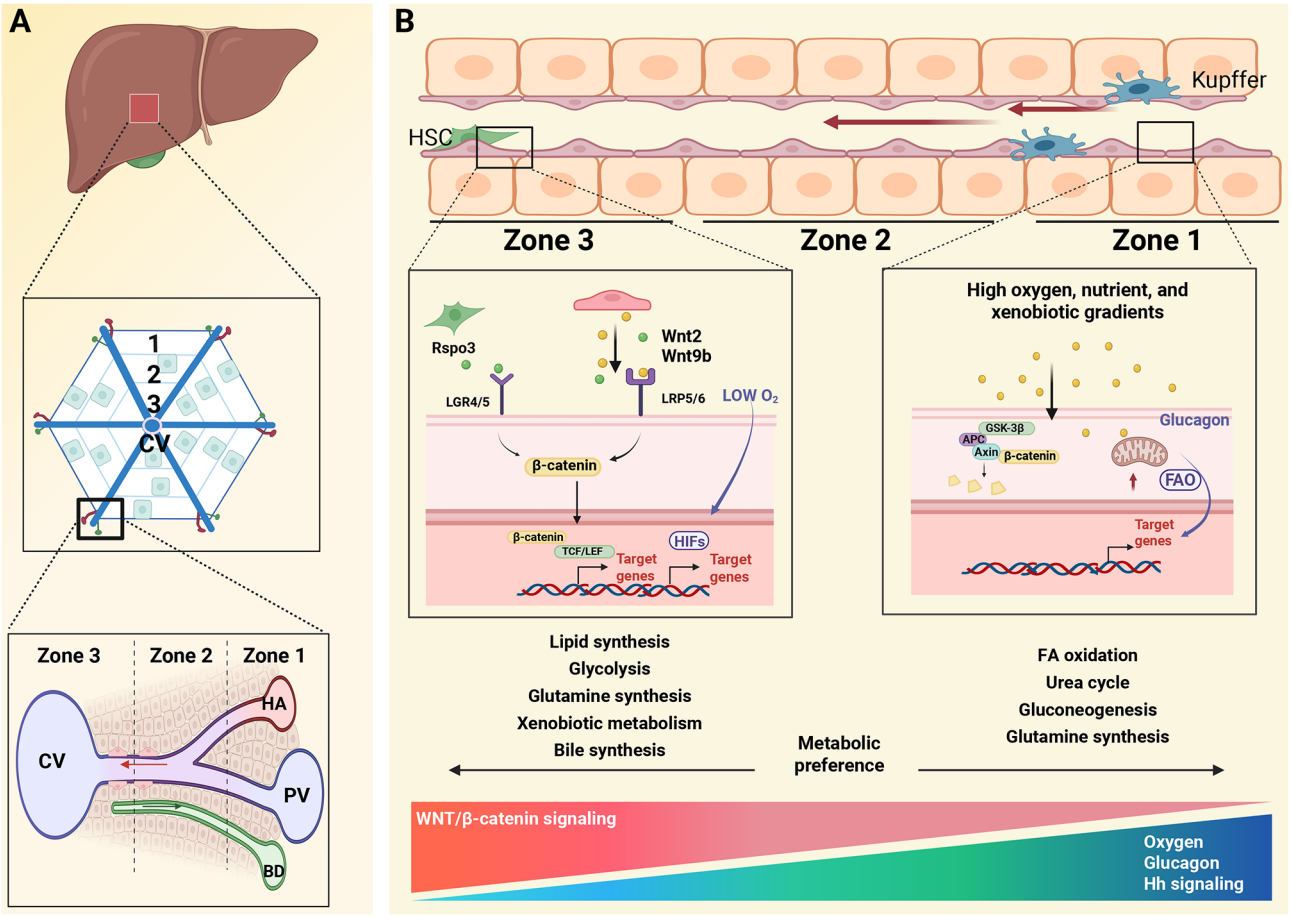

Figure 1. Spatial metabolic zonation of the liver. (A) The structural organization of liver lobules. Functionally distinct zones are defined by their position relative to the portal triad and central vein: the periportal zone (zone 1), intermediate zone (zone 2) and pericentral zone (zone 3). (B) Metabolic specialization along the portal-central axis. Zone 3 hepatocytes operate under hypoxic conditions that stabilize HIFs, promoting glycolytic metabolism. Paracrine signaling from pericentral endothelial cells and hepatic stellate cells via Wnt ligands such as Wnt2, Wnt9b and Rspo3 activates the canonical Wnt/β-catenin pathway through LRP5/6 and LGR4/5 receptors. By contrast, zone 1 hepatocytes exposed to oxygen-rich blood and glucagon signaling maintain β-catenin degradation through APC-mediated proteasomal targeting, while Hh ligand signaling further suppresses Wnt activity to preserve periportal identity. CV, central vein; HA, hepatic artery; PV, portal vein; BD, bile duct; HIFs, hypoxia-inducible factors; Rspo3, R-spondin 3; APC, adenomatous polyposis coli; LRP5/6, low-density lipoprotein receptor-related protein 5/6; LGR4/5, leucine-rich repeat-containing G-protein coupled receptor 4/5; TCF/LEF, T cell factor/lymphoid enhancer-binding factor; GSK-3β, glycogen synthase kinase 3 b; Axin, axis inhibition protein 1; FA, fatty acid; FAO, FA oxidation; Hh, Hedgehog; HSCs, hepatic stellate cells.

surrounding the central vein, expands to 5 to 6 layers (41). This abnormal pattern can be reversed by exogenous glucagon supplementation. Functionally, glucagon differentially regulates periportal urea cycle enzymes and pericentral xenobiotic metabolizing enzymes, such as members of the Cyp450 family. In addition, sexual dimorphisms further increases regulatory complexity. The sex-specific patterns of growth hormone (GH) secretion directly influence liver zonation by regulating the activity of transcription factors in distinct regions. In males, pulsatile GH in zone 1 activates SREBF1, promoting gluconeogenesis-related pathways, whereas in females, continuous GH in zone 3 supports TRIM24, enhancing hepatic progenitor cell-related pathways (42). These differential transcriptional programs restrict specific hepatocyte functions to defined zones, creating a sex-biased metabolic and regenerative landscape (42). Continuous GH infusion in males can abolish this zonal dimorphism, indicating that the mode of hormone secretion determines sex-specific liver zonation (43). These findings indicate that liver zonation functions as a dynamic interface integrating metabolic, endocrine and oxygen-dependent signals.

Wnt signaling regulates hepatic zonation programming. The Wnt/β-catenin pathway is a key regulator of pericentral hepatocyte identity (44). Its activity depends on tight regulation of the β-catenin destruction complex and phosphorylation events. Under basal conditions, β-catenin is continuously degraded by a destruction complex composed of Axin, adenomatous polyposis coli (APC), CK1 and GSK3β. CK1 initiates phosphorylation at Ser45, followed by sequential phosphorylation by GSK3β at Thr41, Ser37 and Ser33, which marks β-catenin for recognition by the β-TrCP E3 ubiquitin ligase and subsequent proteasomal degradation (45). Upon Wnt stimulation, including Wnt2 and Wnt9b secreted by central vein endothelial cells, the binding of these ligands to Frizzled receptors and the co-receptors LRP5/6 recruits CK1 and GSK3β to the membrane (46,47). This leads to phosphorylation of LRP5/6, inhibition of GSK3β activity and inactivation of the destruction complex (44). Stabilized β-catenin accumulates and translocates into the nucleus, where it binds T-cell factor/lymphoid enhancer-binding factor transcription factors to activate pericentral gene programs such as glutamine synthetase and Cyp2e1.

A critical determinant of zonal regulation is the spatially restricted expression of APC (48). APC is largely absent in the pericentral zone, permitting sustained nuclear accumulation of β-catenin, whereas its high expression in periportal hepatocytes constrains Wnt signaling (48). Genetic studies demonstrate that loss of APC leads to panlobular activation of β-catenin, accompanied by ectopic expression of pericentral markers such as Cyp2e1 in periportal hepatocytes (49). In addition, oxygen gradients modulate this axis: HIF-1α suppresses APC transcription in the pericentral zone, thereby enhancing β-catenin signaling, while reactive oxygen species in the periportal region stabilize APC, establishing a redox-dependent zonal boundary (50,51). Furthermore, Wnt5a in the periportal region antagonizes β-catenin activity through calcium-dependent signaling, which reinforces zone 1 identity (52). The Ras/MAPK/ERK pathway contributes additional modulation by promoting β-catenin phosphorylation and proteasomal degradation in periportal hepatocytes, thereby balancing regional signaling intensity (53).

Non-canonical Wnt pathways further refine zonal patterning. Hypoxia-induced Wnt11 in zone 3 activates planar cell polarity signaling via the ROR2 receptor, regulating pericentral gene expression independently of β-catenin (54-56). Wnt signaling defines hepatic zonation through coordinated regulation of the β-catenin destruction complex, spatial control of APC expression and integration of canonical and non-canonical pathways. It also interacts with multiple signaling networks to precisely specify hepatocyte identity along the porto-central axis.

Spatial regulation of Hh signaling in hepatic zonation. The Hh signaling pathway is also a key regulator of hepatic metabolic zonation. It coordinates functional specialization along the porto-central axis through spatially restricted ligand-receptor interactions (57). Spatial transcriptomics analyses have shown that Indian Hh is enriched in the pericentral region, forming a morphogen gradient that is inversely correlated with Wnt/β-catenin signaling activity in the periportal zone (31). This spatial relationship is maintained through GLI-mediated inhibition of β-catenin transcriptional activity, establishing a dynamic balance between the two pathways (58). The Hh gradient can also regulate components of the insulin-like growth factor axis (IGF-I/IGFBP-1) to coordinate zonal metabolic programming (59). Pericentral hepatocytes exhibit GLI-dependent upregulation of gluconeogenic enzymes, whereas periportal regions show enhanced lipid oxidation (32). Furthermore, hepatic stellate cells (HSCs) in the space of Disse act as spatial signal integrators; they convert Hh/Wnt crosstalk into extracellular matrix (ECM) remodeling, thereby physically maintaining the structure of metabolic zonation (60).

Spatial organization of hepatic non-parenchymal cells. Emerging evidence indicates that the spatially restricted cellular niches of non-parenchymal cells, particularly the periportal vs. pericentral zonation patterns, establish microenvironments that directly modulate hepatocyte functional specialization via ECM regulation, metabolic exchange and immune surveillance, thereby driving the establishment of hepatic metabolic zonation (61). Among these, liver sinusoidal endothelial cells (LSECs) act as central regulators of zonation. Central vein associated LSECs selectively secrete Wnt2, Wnt9b and R-spondin 3, thereby activating β-catenin signaling in adjacent hepatocytes and maintaining pericentral cell identity (47,62). Consistently, combined deletion of Wnt2 and Wnt9b abolishes zone 3 specific gene expression. In addition, LSECs modulate sinusoidal blood flow and oxygen tension through the secretion of vascular endothelial growth factors, contributing to the establishment of the porto-central metabolic axis (63). Kupffer cells exhibit a preferential periportal distribution, forming an immune zonation pattern (29). By restricting infiltrating neutrophils to the periportal region, they spatially confine pro-inflammatory responses, thereby protecting the metabolically vulnerable pericentral hepatocytes. Disruption of Kupffer cell localization leads to bacterial dissemination and panlobular inflammatory injury (64-65). HSCs also display zonal heterogeneity, comprising functionally distinct subpopulations (66). Periportal-associated stellate cells express markers such as Ngfr and Tagln and are involved in rapid immune interactions, whereas pericentral-associated stellate cells specifically express Rspo3, which enhances Wnt signaling to sustain pericentral hepatocyte homeostasis (67,68).

Epigenetic modulation as a determinant of liver zonation. Beyond extrinsic cues, hepatic zonation is also governed by intrinsic epigenetic programs (69). DNA methylation has been shown to form a gradient along the porto-central axis, progressively decreasing from the periportal to the pericentral zone. Key transcription factors exhibit spatially restricted expression patterns; USF1 is enriched in periportal hepatocytes, where it exerts negative feedback on periportal-specific genes while modulating lipid and glucose metabolism (70). By contrast, Nr2f2 is progressively enriched in the pericentral zone and activates pericentral identity genes during hepatocyte maturation (69,71). Although HNF4α is uniformly expressed, its DNA-binding activity is gated by methylation status, and its deletion leads to ectopic expression of pericentral genes in periportal hepatocytes (71,72).

Histone modifications further reinforce zonal specialization. H3K27ac, a histone acetylation marker of active enhancers, is enriched at regulatory elements of Wnt/β-catenin-associated pericentral metabolic genes, whereas H3K4me3, a histone trimethylation marker of active promoters, predominantly labels genes involved in periportal metabolic pathways such as gluconeogenesis and the urea cycle (73). The SWI/SNF chromatin remodeling component ARID1A maintains hepatocyte metabolic gene programs by regulating nucleosome positioning and chromatin accessibility (74); hepatocyte-specific loss of ARID1A reduces accessibility at metabolic loci, impairs binding of transcription factors such as PPARα and suppresses the expression of genes involved in fatty acid β-oxidation (75).

MicroRNAs (miRs) also provide an additional layer of fine-tuning. For example, liver-enriched miR-122 is required for the maintenance of zonation, and its deletion results in expansion of zone 3 characteristics (76,77). Collectively, DNA methylation, histone modifications, non-coding RNAs and transcription factor dynamics converge to form an integrated epigenetic-metabolic axis that spatially encodes hepatocyte identity.

5. Zonation homeostasis: Spatial metabolic regulation as a guardian of liver health

The spatial compartmentalization of hepatic function constitutes a critical defense mechanism against metabolic stress. Within this organizational framework, the Wnt/β-catenin pathway serves a central role, not only in maintaining pericentral hepatocyte identity but also in regulating hepatocyte turnover. Early lineage-tracing studies proposed that $Axin2^+$ hepatocytes located in the pericentral possess stem-like properties and contribute to long-term hepatocyte renewal under homeostatic conditions (78). However, accumulating evidence suggests that the contribution of these cells may have been overestimated due to labeling artifacts and signal-dependent activation of reporter systems in Axin2-Cre models (79,80). Multiple independent studies indicate that hepatocyte renewal occurs broadly throughout the liver lobule rather than being restricted to a discrete pericentral progenitor population (81-83). Accordingly, $Axin2^+$ hepatocytes are more likely to represent a Wnt responsive hepatocyte subset primarily responsible for maintaining zonal identity, rather than serving as a universal stem cell reservoir, although they may acquire regenerative potential under specific injury contexts (80). A similar paradigm applies to Leucine-rich repeat-containing G-protein-coupled receptor 5-positive ($Lgr5^+$) hepatocytes. These cells are exceedingly rare in the healthy liver but can be induced following injury (84). Under physiological conditions, the nuclear receptor farnesoid X receptor (FXR) maintains $Lgr5^+$ hepatocytes in a quiescent state; by contrast, metabolic stress or tissue injury activates a PPARα-dependent proliferative program, enabling their transient participation in tissue repair (85). Notably, aberrant activation of Lgr5 may compromise cellular identity and contribute to tumor progression.

Within this zonation dependent regulatory landscape, telomerase-positive ($Tert^{high}$) hepatocytes are predominantly localized to zones 1-2, where they function as a clonogenic reservoir supporting parenchymal homeostasis and injury repair (81,86). Spatially resolved profiling has further identified $Hamp2^+/Igfbp2^+$ zone 2 hepatocytes as a principal subpopulation driving homeostatic regeneration. CRISPR-based functional screening demonstrates that Igfbp2-induced cell cycle progression is essential for their proliferative capacity, mechanistically linking regional metabolic cues to regenerative potential (87,88). This hierarchical organization, where $Tert^{high}$ hepatocytes provide broad regenerative capacity and $Igfbp2^+$ subsets execute spatially restricted proliferation illustrates how spatial signaling networks enable precisely coordinated regeneration while preserving metabolic zonation (89).

Gestational hepatic adaptation: zonal proliferation hierarchies for maternal-fetal metabolic coupling. Based on the principles of zonated hepatocyte renewal, pregnancy induces a spatiotemporally coordinated remodeling of the liver. Hepatocyte proliferation progresses in a zonal sequence, initiating in the periportal zone 1 during early pregnancy, shifting to zone 2 in mid-gestation and culminating in pericentral zone 3 expansion in late pregnancy. This dynamic pattern parallels the progressive increase in fetal metabolic demands (20). Lineage tracing studies indicate that estrogen coordinates this hierarchical zonal program by selectively promoting the proliferation of Ccnd1 positive hepatocytes in zone 2, whereas prolactin fine-tunes liver mass through bile acid mediated hypertrophic regulation (90,91). This spatial proliferation program is closely aligned with metabolic compartmentalization. Expansion in zones 1 and 2 supports glucose and cofactor synthesis required for fetal growth, while proliferation in zone 3 enhances detoxification capacity to counteract pregnancy associated oxidative stress (90,91). Despite these advances, critical gaps remain in understanding how maternal liver zonation adapts and synchronizes with key milestones of fetal development. This highlights the need to elucidate how the placenta liver signaling axis preserves zonation fidelity during pregnancy.

6. Liver zonation in liver injury: Zonation collapse as a driver of disease progression

The spatial erosion of metabolic zonation is a hallmark of liver pathology, where hepatocyte loss disrupts lobular signaling topography and initiates self-amplifying injury circuits (Fig. 2). Notably, zone-specific hepatocyte necrosis, whether periportal oxidative stress or pericentral toxin accumulation, triggers localized inflammatory cascades that recruit neutrophils and monocytes (92,93). This inflammatory feed-forward loop not only exacerbates regional damage but also propagates zonation collapse.

Metabolic liver diseases as pathological models of hepatic zonation disruption. Metabolic dysfunction associated steatotic liver disease (MASLD) is a liver disorder linked to metabolic dysfunction and is characterized by a systemic collapse of lobular metabolic zonation architecture (94). This pathological cascade is driven by coordinated dysregulation of the three core metabolic processes, uptake, synthesis and export, ultimately disrupting the signaling gradients required to maintain functional compartmentalization (95). Hepatic steatosis, a hallmark feature of MASLD, shows a developmentally and spatially patterned distribution. In adult patients, lipid accumulation typically initiates in zone 3, the primary site of fatty acid synthesis (96). Lipotoxicity in this region triggers zone 3-predominant hepatocyte apoptosis and pericentral fibrosis (97). As the disease progresses to metabolic dysfunction-associated steatohepatitis (MASH), the spatial pattern of fibrosis is remodeled, with collagen deposition shifting toward zone 1 and forming porto-central bridging fibrosis, a key precursor of cirrhosis (98). But prepubertal MASLD exhibits an opposite spatial pattern, characterized by zone 1-dominant steatosis and periportal fibrosis without pericentral involvement, which is associated with developmental metabolic programming and microenvironment-specific immune responses (99).

Dysregulated zonal signaling is a key determinant of disease severity. Elevated Hh ligand levels and yes-associated protein (YAP) activation are associated with steatohepatitis and fibrosis progression, while Wnt/β-catenin-mediated pericentral homeostasis is disrupted (100,101). Kupffer cells, traditionally localized to the periportal region, amplify zonation-restricted inflammation in early MASLD by recruiting neutrophils and releasing pro-fibrotic cytokines, thereby exacerbating zone 1

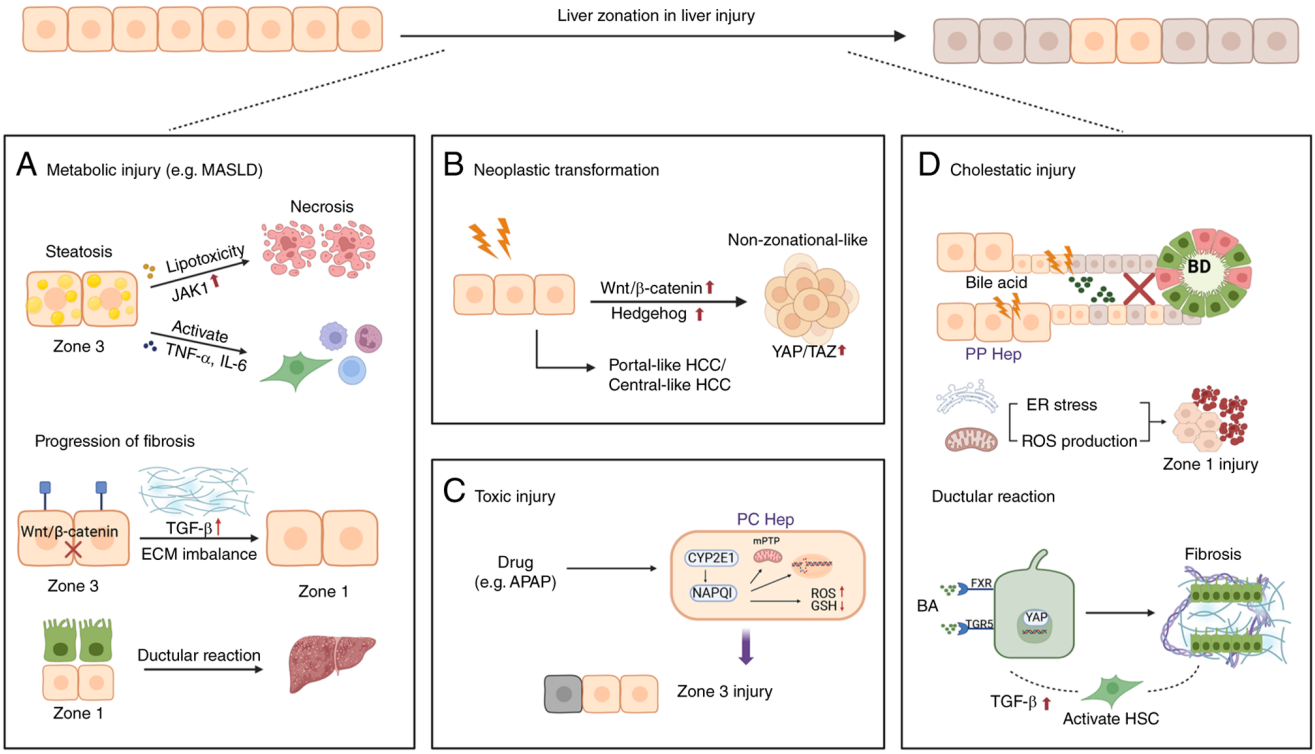

Figure 2. Liver zonation in liver injury. (A) Metabolic injury: In MASLD, centrilobular (zone 3) hepatocytes demonstrate enhanced *de novo* lipogenesis. Lipotoxicity drives pericentral hepatocyte apoptosis via JNK1 activation, initiating centrilobular fibrosis. Chronic injury activates HSCs and Kupffer cells, generating pro-inflammatory (such as TNF-α and IL-6) and pro-fibrotic (such as TGF-β) mediators that drive porto-central bridging fibrosis. (B) Neoplastic transformation: HCC subtypes show distinct zonation patterns: zonation-independent (stem-like phenotype), centrilobular-pattern (β-catenin/Wnt-activated) and periportal-pattern (Hedgehog-activated). Zonation collapse correlates with constitutive YAP/TAZ activation and metabolic reprogramming. (C) Toxic injury: Zone 3-selective cytochrome P450 such as Cyp2e1 bioactivates xenobiotics, for example, APAP into reactive NAPQI, depleting glutathione reserves and inducing mPTP opening, culminating in centrilobular coagulative necrosis. (D) Cholestatic injury: Bile acid overload disrupts zone 1 hepatocyte bile acid transporters, inducing ER stress and ROS production. Chronic cholestasis activates ductular progenitor cells through FXR/TGR5 signaling, driving periportal fibrosis via HSC-derived collagen deposition. MASLD, metabolic dysfunction-associated steatotic liver disease; NAPQI, N-acetyl-p-benzoquinone imine; GSH, glutathione; ER, endoplasmic reticulum; BA, bile acid; HSC, hepatic stellate cells; HCC, hepatocellular carcinoma; ROS, reactive oxygen species; mPTP, mitochondrial permeability transition pore; FXR, farnesoid X receptor; ECM, extracellular matrix; APAP, acetaminophen; YAP, Yes-associated protein; Cyp2e1, cytochrome P450 2E1; PP Hep, periportal hepatocytes; PC Hep, pericentral hepatocytes; BD, bile duct.

injury (102). Meanwhile, periportal ductular reactions characterized by hepatic progenitor cell expansion may contribute to porto-portal fibrosis, although their direct pro-fibrotic role remains to be fully validated (103,104). Mechanistically, early MASLD retains lobular zonation, with preserved regional gene expression and DNA methylation patterns, suggesting a therapeutic window for zonation restoration (71). This reversibility highlights the potential of targeting zone-specific drivers, including Kupffer cell-mediated spatial inflammation, HSC-derived ECM imbalance and liver sinusoidal endothelial cell-driven Wnt signaling dysregulation, to block the steatosis-fibrosis axis (105-107).

Hepatocellular carcinoma (HCC) as a zonation-entrenched malignancy. HCC arises from zonation-collapsed microenvironments in cirrhotic livers, with chronic hepatitis and metabolic dysfunction driving spatially biased oncogenesis (108). It is reported that >50% of HCCs exhibit constitutive Wnt/β-catenin activation, a pathway central to pericentral zonation, alongside Hh signaling hyperactivation, constitutive YAP/TAZ activation and glycolytic reprogramming, collectively hijacking zonation-maintaining circuits to fuel tumor initiation (109,110). While HCC cellular origins remain debated, clonal tracing reveals that regeneration-competent hepatocytes within injury niches serve as dominant precursors, acquiring malignant traits through niche-derived pressures (83,111).

Hypoxia-mediated metabolic zonation collapse further shapes HCC evolution. Pericentral hypoxia stabilizes HIF-1α, synergizing with β-catenin to select for venous zone-adapted clones with glutamine addiction and invasive potential (112). Notably, HCCs retain zonation-like phenotypic diversity, as portal-like HCC with low β-catenin expression mirrors periportal metabolism, exhibiting indolent behavior and favorable post-resection outcomes due to preserved immune surveillance. However, central-like HCC recapitulates pericentral features with high β-catenin, marked by immune-cold microenvironments and resistance to checkpoint blockade, yet maintains vulnerability to Wnt/Hh-targeted therapies (108). This zonation-based classification system overcomes the limitations of conventional histopathological subtyping and enables prognostic stratification as well as compartment-specific therapeutic strategies (113). From a therapeutic perspective, restoring Wnt signaling gradients or targeting the hypoxia-HIF-β-catenin axis may reverse treatment resistance driven by spatial dedifferentiation. In addition, portal vein-like HCC may benefit from immunomodulatory approaches that exploit residual zonal differentiation integrity (114,115).

Drug-induced liver injury (DILI) as a zonation-restorative challenge. DILI, exemplified by acetaminophen (APAP) and carbon tetrachloride models, manifests zonation-biased damage, as demonstrated by the studies that pericentral hepatocytes (zone 3) with high cytochrome P450 activity (including Cyp2e1) metabolize toxins into reactive intermediates such as NAPQI, triggering oxidative stress and mitochondrial dysfunction that culminate in zone 3-predominant necrosis (116-118). Spatial reprogramming at the injury border initiates repair where damage-adjacent hepatocytes transiently reactivate fetal genes and upregulate ribosomes and proteasomes, enabling proteome remodeling to adopt pericentral identities (82). Concurrently, zonation-restricted regeneration unfolds where surviving pericentral hepatocytes initiate proliferation via mTOR signaling, followed by zone 2 hepatocyte expansion (3). A recent study demonstrated zone 2-derived hepatocytes as a main contributor to compartmentalized replenishment, where Igfbp2$^+$ hepatocytes migrate toward necrotic zone 3 and restore metabolic zonation (89).

In addition, annexin A2-positive (ANXA2$^+$) hepatocyte subpopulations exhibit a migration-dominant wound closure mechanism during APAP-induced injury, sealing necrotic areas prior to mitotic expansion (119). This ANXA2-mediated migratory plasticity, previously implicated in cancer cell invasion, suggests a form of region-specific reparative plasticity in which spatial remodeling, rather than proliferation alone, drives functional recovery (119,120). The interplay between migration and mitosis may help maintain a dynamic balance between lobular structural integrity and metabolic demands. However, excessive migratory pressure may contribute to ductular reactions and fibrotic scar formation (121,122).

Cholestatic injury and zonal repair programs: Portal niche remodeling in ductal pathobiology. Experimental cholestasis models, including DDC diet and bile duct ligation, precisely recapitulate human cholangiopathies while unveiling zonation-defined injury patterns (123). Cholestatic injury predominantly targets zone 1 hepatocytes, as bile acids first accumulate in the periportal region following impaired biliary excretion. Hydrophobic bile acids induce membrane damage through detergent-like effects, disrupt mitochondrial function, and trigger oxidative stress and endoplasmic reticulum stress. In parallel, bile acid-activated death receptor signaling, including Fas and TRAIL pathways, further amplifies hepatocyte injury. Zone 1 hepatocytes are particularly susceptible due to their high expression of bile acid uptake transporters such as NTCP and OATPs, together with limited adaptive capacity for detoxification compared with downstream zones (124). In addition, cholestasis induces inflammatory signaling from portal stromal and immune cells, further reinforcing periportal injury and ECM remodeling.

Mechanistically, periportal hepatocytes exposed to cholestatic stress can establish a self-limiting reparative microenvironment. Within this setting, a distinct subpopulation of hepatocytes with a unique phenotype has been identified in the periportal niche. These cells express multiple biliary-enriched genes, including low levels of Sox9, while retaining the canonical hepatocyte marker HNF4α. This population, termed hybrid hepatocytes (HybHPs), is capable of contributing to parenchymal regeneration through proliferative expansion (125). However, the origin of HybHPs remains controversial. In chronic injury models, HybHPs have been proposed to arise from embryonic progenitors located in the ductal plate, as these cells can upregulate biliary markers and differentiate into cholangiocytes. However, studies using partial hepatectomy models suggest that HybHPs originate from mature hepatocytes, as they appear as early as 3 h after resection without evidence of early activation of hepatic progenitor cells (126). Furthermore, in MASLD models, analysis of fetal liver markers indicates that these biphenotypic cells do not undergo dedifferentiation or redifferentiation (127). Taken together, these findings suggest that HybHPs most likely arise from mature hepatocytes undergoing microenvironment-driven transdifferentiation in response to alterations in the periportal niche during injury, rather than from stem cell populations or developmental progenitors. In addition, chronic cholestasis activates cholangiocyte progenitor cells through the FXR/TGR5 signaling pathway, which in turn induces collagen deposition from HSCs, leading to periportal fibrosis and consequently disrupting the architecture of zone 1 (128).

7. Liver regeneration: Zonal identity plasticity and hierarchical progenitor engagement

The regenerative capacity of the liver is deeply rooted in its metabolically zonated architecture. Early lineage-tracing studies suggested that Mfsd2a positive zone 1 hepatocytes migrate toward the pericentral region (129); however, more recent evidence identifies Igfbp2 positive and Hamp2 positive zone 2 hepatocytes as the primary drivers of regeneration. Their proliferative advantage is mediated by mTOR-CCND1 signaling, a mechanism conserved with pregnancy-induced liver growth (30). In parallel, Sox9 positive hepatocytes derived from the periportal microenvironment acquire progenitor-like features during regeneration, recapitulating biliary repair programs and underscoring the dual role of zone 1 in metabolic function and regenerative adaptation (130,131). Zone 1 mobilizes hepatic progenitor cells and biliary transdifferentiation to compensate for parenchymal loss, whereas zone 3 recruits Axin2$^+$, Lgr5$^+$ and Cyp2e1$^+$ hepatocytes to support pericentral repair (132). This hierarchical strategy ensures preservation of metabolic zonation during tissue reconstruction. Importantly, this system operates through interzonal crosstalk, with zone 2 derived Igfbp2 promoting activation of zone 1 progenitor responses, while zone 3 Wnt signaling suppresses ectopic biliary reactions (133).

In addition to the hepatocyte subpopulations with regenerative potential as aforementioned, multiple liver injury models have shown that hepatocytes at the injury interface can dedifferentiate from mature hepatocytes into progenitor-like cells (118). Beyond the well-characterized HNF4α$^+$Sox9$^+$ population, these also include AFP$^+$ reprogrammed hepatocytes. These cells retain lineage restriction while acquiring controllable proliferative capacity. Their functional state is regulated through AFP-dependent metabolic reprogramming and dynamically balanced between proliferation and stress adaptation via the PPARγ signaling axis (5). Furthermore, expansion of this cell population is driven by TNF-α/AP-1 signaling originating from neutrophils in the host liver, highlighting the critical role of the immune microenvironment in regulating regenerative cell states.

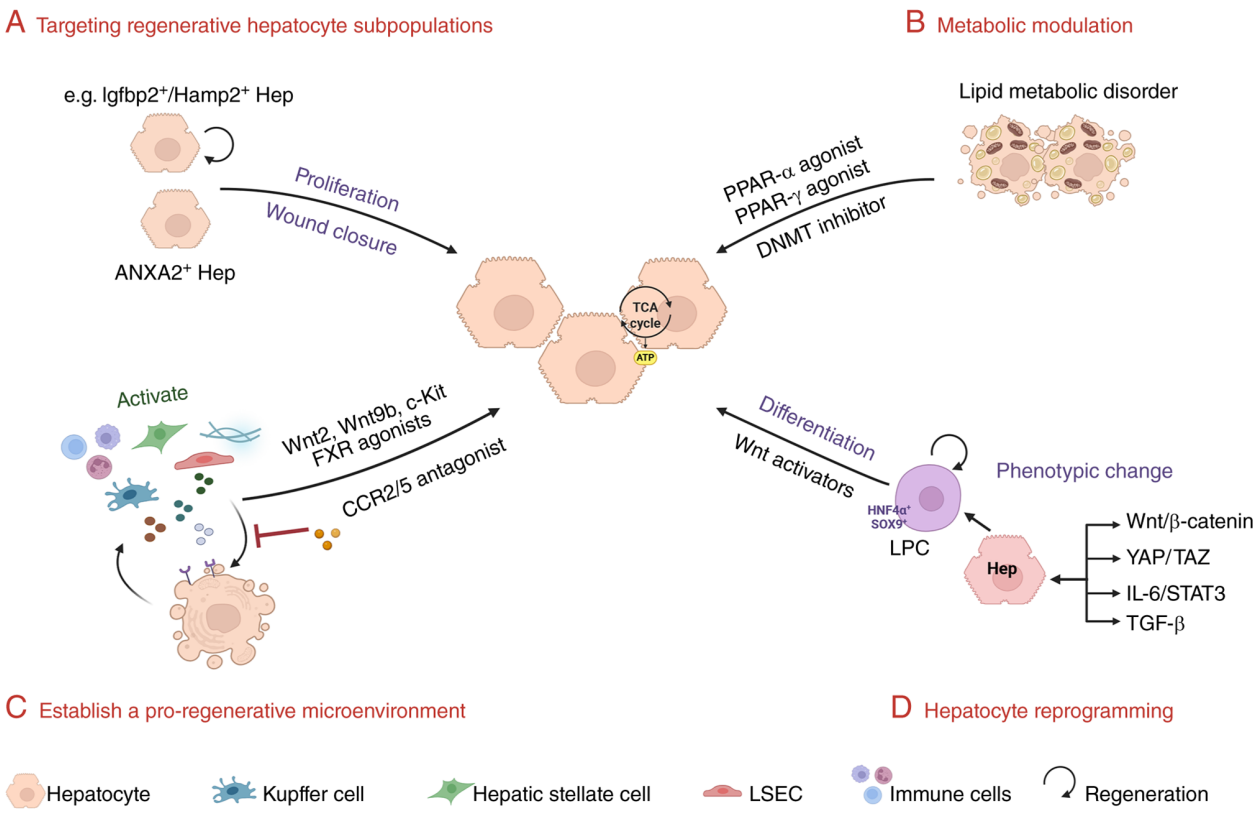

Figure 3. Precision therapeutic strategies for liver regeneration. (A) Targeting regenerative hepatocyte subpopulations: Igfbp2$^+$/Hamp2$^+$ hepatocytes demonstrate enhanced proliferative capacity during injury, while migratory ANXA2$^+$ hepatocytes restore lobular zonation through wound closure mediated architectural reorganization. (B) Metabolic modulation via PPARα agonist-driven fatty acid oxidation clears cytotoxic lipid droplets, concurrent with LSEC cycle activation to fuel ATP-dependent regeneration. (C) Niche engineering targets LSEC-derived Wnt ligands, immune modulation or ECM remodeling, to establish a pro-regenerative microenvironment. (D) Cellular reprogramming through Wnt/β-catenin, YAP/TAZ, IL-6/STAT3 and TGF-β signaling mediated expansion of SOX9+ progenitor-like hepatocytes, followed by Wnt-guided redifferentiation into functional HNF4α+ hepatocytes, enables structural and metabolic restitution. Igfbp2, Insulin-like growth factor-binding protein 2; Hamp2, hepcidin antimicrobial peptide 2; ANXA2, annexin A2; ECM, extracellular matrix; PPAR, peroxisome proliferator-activated receptors; LSEC, liver sinusoidal endothelial cells; FXR, farnesoid X receptor; DNMT, DNA methyltransferases; Hep, hepatocytes; TCA, tricarboxylic acid cycle; LPC, liver progenitor cell.

Collectively, these findings indicate that liver regeneration does not rely on a single progenitor pool but instead involves coordinated responses from multiple hepatocyte subpopulations with distinct zonal characteristics. This coordinated response forms a dynamic and adaptive regenerative network, and such cellular plasticity is essential for effective tissue repair.

8. Regenerative therapeutics: Spatial reprogramming of hepatic niche networks

Emerging paradigms in liver regeneration therapeutics converge on spatial orchestration of cellular plasticity and microenvironmental reprogramming (1). At the cellular level, strategies focus on modulating intrinsic plasticity thresholds through epigenetic and metabolic circuit editing, enabling context-dependent transdifferentiation while preserving functional zonation (Fig. 3) (134,135). Concurrently, niche-directed approaches recalibrate stromal-immune signaling gradients to license region-specific progenitor activation (136).

Metabolic reprogramming in liver regeneration: Therapeutic targets for compartmentalized repair. Liver regeneration necessitates dynamic metabolic adaptation, where zonation-defined specialization is transiently repurposed to resolve the energy-biosynthesis paradox (135). In the early phase of regeneration, HIF-1α-driven glycolytic flux predominates, while AKT/mTOR-mediated suppression of gluconeogenesis and FAO redirects substrates toward lipid droplet biogenesis, providing a strategic energy reserve to support proliferative expansion (137). This metabolic triage creates therapeutic opportunities through phased interventions. First, HIF stabilizers boost zone 1-2 glycolytic priming. Concurrently, PPARγ agonists sustain lipogenic competence, while mTOR modulators delay catabolic restoration to preserve energy reservoirs (138). Post-proliferative metabolic recovery, driven by PPARα-dependent FAO reactivation and mitochondrial rejuvenation, is spatially coordinated to reinstate functional zonation (139,140). This phase ensures pericentral detoxification via CYP450 restoration and zonal energy flux balancing, completing the metabolic reprogramming cycle essential for functional restoration.

Epigenetic control of metabolic plasticity: Therapeutic anchoring to zonal memory. Epigenetic modifiers serve as spatial regulators of metabolic reprogramming, offering druggable nodes to enhance regenerative precision. DNA methyltransferases (DNMTs) enforce FAO gene silencing during regeneration initiation, with pharmacological DNMT

inhibition extending the glycolytic proliferative window (141). Conversely, histone acetylase activators accelerate oxidative phosphorylation recovery by decompacting chromatin at mitochondrial genes, enabling timely metabolic restoration (142,143). Spatial multiomics have decoded zone-specific metabolite-epigenome crosstalk, identifying serine and ketone bodies as zone 2 resolved cofactors that prime chromatin accessibility for plasticity transcription factors, a mechanism that may be exploitable through dietary or metabolite supplementation strategies (144). For instance, zone 1 targeted HIF stabilizers amplify glycolytic flux, while zone 2 directed serine supplementation reinforces epigenetic licensing of plasticity genes. By mirroring the liver's innate spatiotemporal hierarchy where metabolic priorities shift from proliferation fuel to functional specialization, these strategies transform regenerative bottlenecks into therapeutic windows.

Non-parenchymal cell therapeutics: Spatial modulation of regenerative niches. Non-parenchymal cells have been recognized for their roles in maintaining liver zonation, and also make critical contributions to the regenerative microenvironment. Spatial transcriptomics and single-cell analyses have shown that pericentral HSC subsets enhance Wnt/β-catenin signaling through secretion of Rspo3, thereby supporting hepatocyte proliferation during regeneration (141). The loss of HSCs or Rspo3 impairs regenerative capacity, while clinical data indicate that higher Rspo3 expression is associated with improved survival and a reduced risk of hepatocellular carcinoma, supporting its potential as a therapeutic target (145).

LSECs are also key regulators of regeneration. Previous studies demonstrate that LSECs promote hepatocyte proliferation through secretion of Wnt2 and Wnt9b (47,146,147). Further work has identified c-Kit signaling in LSECs as essential for this pro-regenerative function. c-Kit$^+$ LSECs upregulate Wnt2 expression following injury, thereby stimulating proliferation of adjacent hepatocytes, whereas disruption of c-Kit signaling reduces Wnt production and significantly impairs liver regeneration (147,148). Recent advances have extended these findings into therapeutic strategies. Engineered exosome-based systems have been developed to target LSECs, combining CRLTRKRGLK peptide-mediated targeting with CD47 mediated evasion of mononuclear phagocyte clearance, enabling efficient delivery of Wnt2 mRNA (149). In APAP and dimethylnitrosamine-induced liver injury models, this approach enhances Wnt signaling, promotes hepatocyte proliferation, and improves tissue repair, demonstrating the feasibility of modulating non-parenchymal cell-derived signals to reconstruct the regenerative microenvironment (149).

In addition, a recent study has shown that injury-induced downregulation of URI1 leads to glutamate accumulation, which acts as a paracrine signal to recruit bone marrow-derived monocytes. These immune cells subsequently secrete Wnt3, activating YAP1 signaling in zone 2 hepatocytes and driving their proliferation (150). This metabolic immune regenerative axis follows a well-defined spatiotemporal sequence and has been validated across multiple models of acute and chronic liver injury. Clinical analyses further suggest that this pathway is dysregulated in chronic liver disease, characterized by reduced Wnt3 expression and disrupted metabolic signaling (150). Non-parenchymal cells thus establish a spatially organized signaling network that governs liver regeneration within the framework of liver zonation. This coordinated multicellular system indicates that liver regeneration is not driven by a single cell type but instead depends on the dynamic remodeling of zonation-defined regenerative niches. Targeting non-parenchymal cells and their spatial signaling networks therefore represents a promising strategy for enhancing liver regeneration.

Hepatocyte reprogramming: Spatial control of plasticity for regenerative therapy. Hepatocyte dedifferentiation, a spatially restricted process predominantly localized to zone 2, functions as an evolutionary safeguard mechanism for hepatic repair. This regenerative phenomenon involves surviving hepatocytes reactivating fetal transcriptional programs through conserved developmental pathways, particularly Wnt/β-catenin (151), YAP (152) and TGF-β signaling cascades (153). Beyond the classical Sox9$^+$/HNF4α$^+$ progenitor-like transition, studies have identified multiple regenerative hepatocyte subpopulations arising from dedifferentiation or enhanced cellular plasticity of mature hepatocytes (154). For instance, AFP-positive reprogrammed hepatocytes represent a state of partial dedifferentiation, retaining lineage restriction while acquiring proliferative capacity. Hepatocytes driven by YAP or Wnt/β-catenin signaling exhibit a reversible progenitor-like phenotype (152). In addition, transitional hepatocyte populations with high transcriptional plasticity, as well as hepatocytes with migratory capacity involved in wound repair, have been identified at injury borders (119). Collectively, these distinct cellular states constitute a heterogeneous pool of regenerative sources, indicating that liver regeneration relies on multilayered cell fate reprogramming rather than a single progenitor reservoir.

Critically, liver regeneration extends beyond cell-autonomous reprogramming through spatially coordinated niche interactions. For example, portal vein injury models reveal that regionally activated Kupffer cells secrete IL-6, which induces STAT3 activation in adjacent hepatocytes to directly drive reprogramming-related gene re-expression (154). This spatial regulation is further governed by metabolic zonation dynamics that compartmentalize regenerative responses across hepatic lobules.

9. Zonation-based therapies for liver diseases and barriers to clinical translation

Therapeutic strategies for liver diseases have expanded with the development of multiple targeted agents, several of which directly exploit or modulate hepatic zonation features (Table II). In MASH, obeticholic acid, an agonist of the FXR, regulates bile acid metabolism in close association with the functional specialization of pericentral hepatocytes (155). Aldafermin (NGM282), a FGF19 analog, suppresses bile acid synthesis through FGFR4 signaling and similarly targets metabolic regulation in the pericentral zone (156). PRI-724 selectively inhibits the interaction between β-catenin and CBP, thereby directly modulating Wnt-dependent zonal signaling in pericentral hepatocytes (157).

However, the clinical translation of these drugs still faces considerable challenges. Cenicriviroc, by blocking the

Table II. Completed and ongoing clinical trials targeting zonal hepatic pathology.

A, Metabolic regulation

Target/mechanism	Drug	Phase	Indication	Outcome	Trial no.	Unmet clinical needs	(Refs.)
FXR agonist	Obeticholic acid	III	NASH	Improved histology with acceptable safety	NCT02548351	Pruritus and lipid imbalance limit clinical; utility systemic activation lacks zonal specificity	(172)
FGF19 receptor agonist	Aldafermin	II	NASH/PSC	Reduced liver fat; trend toward fibrosis improvement	NCT02443116	Anti-fibrotic effect modest; requires long-term validation	(156)
IBAT inhibitor	Elobixibat	II	NAFLD/NASH	No results posted	NCT04006145	Bile acid modulation alone insufficient for fibrosis reversal	(173)
Thyroid hormone receptor-β agonist	Resmetirom	III	NASH	No results posted	NCT03900429	Metabolic improvement may not directly translate to fibrosis benefit	(174)
PPARα modulator	Pemafibrate	II	NAFLD/NASH	Reduced liver stiffness (via magnetic resonance elastography)	NCT03350165	Imaging improvement needs histological confirmation	(175)
Pan-PPAR agonist	Lanifibranor	IIb	NASH	Improved fibrosis	NCT03008070	Multi-target approach more effective than single pathway	(176)
Pan-PPAR agonist	Chiglitazar	II	NASH	No results posted	NCT05193916	Patient heterogeneity may mask efficacy	(177)

B, Inflammation/immune modulation

Target/mechanism	Drug	Phase	Indication	Outcome	Trial no.	Unmet clinical needs	(Refs.)
CCR2/5 inhibitor	Cenicriviroc	IIb	NASH	Improved results in advanced fibrosis	NCT03028740	Redundant inflammatory pathways limit efficacy	(158)
TLR4 antagonist	JKB-121	II	NASH	No results posted	NCT02442687	Single immune axis blockade insufficient	(178)
TLR7 agonist	RO7119929	I	HCC	No results posted	NCT04338685	Immune activation may be context-dependent	(179)

C, Fibrosis/ECM targeting

Target/mechanism	Drug	Phase	Indication	Outcome	Trial no.	Unmet clinical needs	(Refs.)
Galectin-3 inhibitor	Belapectin	IIb/III	NASH	No results posted	NCT04365868	ECM remodeling too advanced to reverse in late-stage patients	(161)

Table II. Continued.

D, Wnt/regeneration signalling						
Target/mechanism	Drug	Phase	Indication	Outcome	Trial no.	Unmet clinical needs	(Refs.)
CBP/β-catenin inhibitor	PRI-724	I	HCV cirrhosis	Improved histology and CP score	NCT02195440	Safety acceptable, but long-term oncogenic risk needs monitoring	(157)

FGF19, fibroblast growth factor 19; IBAT, ileal bile acid transporter; TLR4, Toll-like receptor 4; CCR2/5, C-C chemokine receptor 2/5; FXR, farnesoid X receptor; ECM, extracellular matrix; PPAR, peroxisome proliferator-activated receptor; CBP, CREB-binding protein; TLR7, Toll-like receptor 7; NASH, non-alcoholic steatohepatitis; PSC, primary sclerosing cholangitis; NAFLD, non-alcoholic fatty liver disease; HCC, hepatocellular carcinoma; HCV, hepatitis C virus.

CCR2/CCR5 signaling pathway, can reduce F4/80-positive macrophages in the portal and necrotic regions, showing some anti-fibrotic effects in a phase II trial (158). However, its efficacy was not maintained in phase III trials (158). It could be considered that single pathway blockade cannot overcome the redundancy of chemotactic signals in liver fibrosis and the dual functional roles of macrophages, nor does it directly target HSCs or ECM) deposition, resulting in limited overall anti-fibrotic efficacy (159). However, patients exhibit heterogeneous fibrosis types; early, inflammation-dominant fibrosis responds better to intervention, whereas ECM deposition driven by sustained HSC activation is difficult to reverse. Similarly, the failure of Belapectin is largely attributable to insufficient patient stratification. Galectin-3 primarily regulates HSC differentiation and serves a key role in ECM deposition (160). As its inhibitor, Belapectin can theoretically attenuate fibrosis, but without accounting for the pathological heterogeneity of patients, the overall efficacy was inadequate (161). This indicates that whether targeting immune cells or non-parenchymal cells such as HSCs, clinical success depends critically on precise patient stratification and careful consideration of the spatial and mechanistic heterogeneity of fibrosis.

Not only does the type of disease progression affect treatment outcomes, but an increasing number of studies indicate that factors such as sex, age and the gut microbiome can reshape liver zonation through specific signaling pathways, thereby influencing disease development and therapeutic responses. As aforementioned, sex-dependent hormones, particularly GH can dynamically regulate liver zonation (162). In MASLD, male GH pulses drive STAT5 activation, exacerbating lipid metabolic disorders and inflammation, making the centrilobular zone more prone to fat accumulation and fibrosis. By contrast, estrogen promotes FAO and inhibits HSC activation, providing protection in this region, although this effect is markedly reduced after menopause (163). Age is also an key confounding factor, as zonal characteristics become less distinct in the aging liver, reducing the spatial precision of drug targeting (164). Furthermore, disruption of the gut barrier allows microbes and their metabolites, such as bile acids, to enter the portal circulation and form spatial gradients along the hepatic lobule blood flow. Bile acids regulate metabolism and inflammation through FXR and TGR5 receptors, which are differentially expressed across liver zones, thereby driving region-specific fibrotic progression (165). Thus, the gut microbiome modulates pathological states in different liver regions via the 'bile acid-zonation signaling axis', influencing the efficacy of zonation-targeted therapies. Taken together, future research should focus on combination therapies, precise patient stratification and spatially targeted delivery strategies based on liver zonation biology.

10. Challenges and future perspectives

Current studies have predominately focused on engineered exosomes (145), lipid nanoparticles conjugated with targeting peptides (166) and GalNAC-based small interfering RNA systems for hepatocyte-specific delivery (167). With the progressive understanding of hepatic spatial organization, the paradigm of precision liver therapy is shifting from 'cell-targeted' approaches toward 'spatially targeted' strategies.

However, the central challenge lies in achieving precise spatio-temporal control of zonal signaling, which requires not only targeting specific zonally distributed cells but also enabling region-restricted and dynamically adjustable modulation. Furthermore, under conditions such as injury, inflammation or metabolic stress, spatial domains can shift, leading to a mismatch between predefined target regions and the actual pathological zones. As a result, simple exogenous activation or inhibition of a single pathway is often counterbalanced by intrinsic compensatory mechanisms and might trigger region-specific signal reprogramming. This can ultimately attenuate therapeutic efficacy or lead to spatial mismatch of treatment effects.

With the rapid advancement of biotechnology, the accumulation of high-throughput omics data is driving the deep integration of bioinformatics and artificial intelligence (AI) in liver disease research, providing new avenues for early diagnosis, risk stratification and prognostic prediction (168,169). Traditional machine learning and deep learning approaches can extract latent patterns from complex multidimensional data and significantly outperform conventional clinical parameters, highlighting the potential of multi-source data integration as a key tool for precision hepatology. However, current AI models largely remain at the level of prediction and lack the ability to resolve spatial heterogeneity and underlying mechanisms of disease (170).

Future efforts should shift from purely data-driven prediction toward a new paradigm that integrates mechanistic insights with spatial information. Incorporating spatial transcriptomics and single-cell sequencing into AI models would enable the modeling of zonation-specific signals within the hepatic lobule, thereby revealing region-specific contributions to disease initiation and progression. Overall, the convergence of AI and spatial hepatology is poised to reshape liver disease research and clinical management, providing a new theoretical and technological foundation for precision therapy.

11. Discussion

Liver zonation is not merely a manifestation of metabolic compartmentalization but represents a dynamic regulatory framework that integrates physiological function with disease pathogenesis. Along the porto-central axis, a continuous metabolic gradient is established through coordinated signaling interactions between zonation-specific hepatocyte subpopulations and non-parenchymal cells, thereby shaping region-specific metabolic programs and functional specialization. This highly ordered spatial organization supports efficient hepatic metabolism under homeostatic conditions, while simultaneously conferring region-specific vulnerability under pathological states. Spatial multi-omics studies further demonstrate that different hepatic zones exhibit distinct responses to injury, indicating that liver disease fundamentally arises from disruption of zonal architecture and imbalance of spatial signaling networks.

Building on this concept, the integration of spatial biology and regenerative medicine is driving a paradigm shift in therapeutic strategies from 'cell-targeted' to 'zonation-targeted' approaches. Emerging evidence shows that adeno-associated virus-mediated expression of R-spondin3 can restore Wnt/β-catenin signaling gradients, thereby re-establishing pericentral metabolic identity and ameliorating fibrosis in preclinical models. However, clinical translation of such strategies remains challenged by the difficulty of achieving precise spatial control of zonal signaling and by substantial inter-individual variability. Recently developed organoid and bioengineered systems incorporate physiologically relevant oxygen and nutrient gradients, enabling reconstruction of liver lobule-like spatial architecture *in vitro* and providing powerful platforms for dissecting zonal regulatory mechanisms and screening compartment-specific interventions. In the future, the integration of spatial omics, AI, controllable delivery systems and personalized models are expected to bridge mechanistic insights with therapeutic development, thereby advancing zonation-based precision interventions toward clinical application.

Acknowledgements

Not applicable.

Funding

The present was supported by grants from the Key Laboratory Open Project Foundation of Jiangsu University (grant no. 8161280002), the Key Laboratory Open Project Foundation of Jiangsu University (Outstanding Research Project) (grant no. JSKLM-Z-2024-011), Science and Technology Plan (Apply Basic Research) of Changzhou City (grant no. CJ20210006), Science and Technology Plan (Apply Basic Research) of Changzhou City (grant no. CJ20230002), Science and Technology Plan (Apply Basic Research) of Changzhou City (grant no. CJ20241041), Natural Science Foundation of China (grant no. 82100666), the National Natural Science Foundation for Young Scientists of Jiangsu Province (grant no. BK20200906) and the Young Scientists Initiative Foundation of Jiangsu University (grant no. 20JDG49).

Availability of data and materials

Not applicable.

Authors' contributions

DY conceived the idea and supervised the project. DY and GC wrote the manuscript. HJ, YL, CG and HZ helped with figure preparation. DY, GC and KZ revised the manuscript. HJ and YL participated in editing the manuscript. CG, HZ and YC contributed to revision of the manuscript. KZ, YC and DY finalized the manuscript and made the conceptual evaluation of the manuscript. Data authentication is not applicable. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Ben-Moshe S and Itzkovitz S: Spatial heterogeneity in the mammalian liver. Nat Rev Gastroenterol Hepatol 16: 395-410, 2019.
2. Halpern KB, Shenhav R, Matcovitch-Natan O, Toth B, Lemze D, Golan M, Massasa EE, Baydatch S, Landen S, Moor AE, *et al*: Single-cell spatial reconstruction reveals global division of labour in the mammalian liver. Nature 542: 352-356, 2017.
3. Wang S, Wang X, Shan Y, Tan Z, Su Y, Cao Y, Wang S, Dong J, Gu J and Wang Y: Region-specific cellular and molecular basis of liver regeneration after acute pericentral injury. Cell Stem Cell 31: 341-358, 2024.
4. Watson BR, Paul B, Rahman RU, Amir-Zilberstein L, Segerstolpe Å, Epstein ET, Murphy S, Geistlinger L, Lee T, Shih A, *et al*: Spatial transcriptomics of healthy and fibrotic human liver at single-cell resolution. Nat Commun 16: 319, 2025.
5. Fang T, Yang C, Qiu H, Du Y, Wang X, Li Y, Xu M, Liu C, Li X, Guo N, *et al*: Conversion of transplanted mature hepatocytes into Afp^+ reprogrammed cells for liver regeneration after injury. Adv Sci (Weinh) 13: e17126, 2026.
6. Gebhardt R: Metabolic zonation of the liver: Regulation and implications for liver function. Pharmacol Ther 53: 275-354, 1992.
7. Braeuning A, Ittrich C, Kohle C, Hailfinger S, Bonin M, Buchmann A and Schwarz M: Differential gene expression in periportal and perivenous mouse hepatocytes. FEBS J 273: 5051-5061, 2006.
8. Jungermann K and Katz N: Functional specialization of different hepatocyte populations. Physiol Rev 69: 708-764, 1989.
9. Jungermann K and Katz N: Functional hepatocellular heterogeneity. Hepatology 2: 385-395, 1982.
10. Hijmans BS, Grefhorst A, Oosterveer MH and Groen AK: Zonation of glucose and fatty acid metabolism in the liver: Mechanism and metabolic consequences. Biochimie 96: 121-129, 2014.
11. Jungermann K and Kietzmann T: Zonation of parenchymal and nonparenchymal metabolism in liver. Annu Rev Nutr 16: 179-203, 1996.
12. Haussinger D and Gerok W: Hepatocyte heterogeneity in ammonia metabolism: Impairment of glutamine synthesis in CCL4 induced liver cell necrosis with no effect on urea synthesis. Chem Biol Interact 48: 191-194, 1984.
13. Berndt N, Kolbe E, Gajowski R, Eckstein J, Ott F, Meierhofer D, Holzhutter HG and Matz-Soja M: Functional consequences of metabolic zonation in murine livers: Insights for an old story. Hepatology 73: 795-810, 2021.
14. Zanger UM and Schwab M: Cytochrome p450 enzymes in drug metabolism: Regulation of gene expression, enzyme activities, and impact of genetic variation. Pharmacol Ther 138: 103-141, 2013.
15. Parlakgul G, Arruda AP, Pang S, Cagampan E, Min N, Guney E, Lee GY, Inouye K, Hess HF, Xu CS and Hotamışlıgil GS: Regulation of liver subcellular architecture controls metabolic homeostasis. Nature 603: 736-742, 2022.
16. Parlakgul G, Pang S, Artico LL, Min N, Cagampan E, Villa R, Goncalves R, Lee GY, Xu CS, Hotamisligil GS and Arruda AP: Spatial mapping of hepatic ER and mitochondria architecture reveals zonated remodeling in fasting and obesity. Nat Commun 15: 3982, 2024.
17. Plata-Gomez AB, de Prado-Rivas L, Sanz A, Deleyto-Seldas N, Garcia F, de la Calle AC, Silva C, Caleiras E, Grana-Castro O, Pineiro-Yanez E, *et al*: Hepatic nutrient and hormone signaling to mTORC1 instructs the postnatal metabolic zonation of the liver. Nat Commun 15: 1878, 2024.
18. Kozuki S, Kabata M, Sakurai S, Iwaisako K, Nishimura T, Toi M, Yamamoto T and Toyoshima F: Periportal hepatocyte proliferation at midgestation governs maternal glucose homeostasis in mice. Commun Biol 6: 1226, 2023.
19. Rosenberger FA, Thielert M, Strauss MT, Schweizer L, Ammar C, Madler SC, Metousis A, Skowronek P, Wahle M, Madden K, *et al*: Spatial single-cell mass spectrometry defines zonation of the hepatocyte proteome. Nat Methods 20: 1530-1536, 2023.
20. He S, Guo Z, Zhou M, Wang H, Zhang Z, Shi M, Li X, Yang X and He L: Spatial-temporal proliferation of hepatocytes during pregnancy revealed by genetic lineage tracing. Cell Stem Cell 30: 1549-1558, 2023.
21. Hildebrandt F, Andersson A, Saarenpaa S, Larsson L, Van Hul N, Kanatani S, Masek J, Ellis E, Barragan A, Mollbrink A, *et al*: Spatial transcriptomics to define transcriptional patterns of zonation and structural components in the mouse liver. Nat Commun 12: 7046, 2021.
22. Liang Y, Kaneko K, Xin B, Lee J, Sun X, Zhang K and Feng GS: Temporal analyses of postnatal liver development and maturation by single-cell transcriptomics. Dev Cell 57: 398-414, 2022.
23. Yang L, Wang X, Zheng JX, Xu ZR, Li LC, Xiong YL, Zhou BC, Gao J and Xu CR: Determination of key events in mouse hepatocyte maturation at the single-cell level. Dev Cell 58: 1996-2010, 2023.
24. Matsumoto T, Wakefield L, Tarlow BD and Grompe M: In vivo lineage tracing of polyploid hepatocytes reveals extensive proliferation during liver regeneration. Cell Stem Cell 26: 34-47, 2020.
25. Katsuda T, Hosaka K, Matsuzaki J, Usuba W, Prieto-Vila M, Yamaguchi T, Tsuchiya A, Terai S and Ochiya T: Transcriptomic dissection of hepatocyte heterogeneity: Linking ploidy, zonation, and stem/progenitor cell characteristics. Cell Mol Gastroenterol Hepatol 9: 161-183, 2020.
26. Richter ML, Deligiannis IK, Yin K, Danese A, Lleshi E, Coupland P, Vallejos CA, Matchett KP, Henderson NC, Colome-Tatche M and Martinez-Jimenez CP: Single-nucleus rna-seq2 reveals functional crosstalk between liver zonation and ploidy. Nat Commun 12: 4264, 2021.
27. Su T, Yang Y, Lai S, Jeong J, Jung Y, Mcconnell M, Utsumi T and Iwakiri Y: Single-cell transcriptomics reveals zone-specific alterations of liver sinusoidal endothelial cells in cirrhosis. Cell Mol Gastroenterol Hepatol 11: 1139-1161, 2021.
28. Payen VL, Lavergne A, Alevra SN, Colonval M, Karim L, Deckers M, Najimi M, Coppieters W, Charloteaux B, Sokal EM, *et al*: Single-cell RNA sequencing of human liver reveals hepatic stellate cell heterogeneity. JHEP Rep 3: 100278, 2021.
29. Gola A, Dorrington MG, Speranza E, Sala C, Shih RM, Radtke AJ, Wong HS, Baptista AP, Hernandez JM, Castellani G, *et al*: Commensal-driven immune zonation of the liver promotes host defence. Nature 589: 131-136, 2021.
30. Wu B, Shentu X, Nan H, Guo P, Hao S, Xu J, Shangguan S, Cui L, Cen J, Deng Q, *et al*: A spatiotemporal atlas of cholestatic injury and repair in mice. Nat Genet 56: 938-952, 2024.
31. Kolbe E, Aleithe S, Rennert C, Spormann L, Ott F, Meierhofer D, Gajowski R, Stopel C, Hoehme S, Kucken M, *et al*: Mutual zonated interactions of Wnt and Hh signaling are orchestrating the metabolism of the adult liver in mice and human. Cell Rep 29: 4553-4567, 2019.
32. Matz-Soja M, Hovhannisyan A and Gebhardt R: Hedgehog signalling pathway in adult liver: A major new player in hepatocyte metabolism and zonation? Med Hypotheses 80: 589-594, 2013.
33. Jungermann K and Kietzmann T: Oxygen: Modulator of metabolic zonation and disease of the liver. Hepatology 31: 255-260, 2000.
34. Semba H, Takeda N, Isagawa T, Sugiura Y, Honda K, Wake M, Miyazawa H, Yamaguchi Y, Miura M, Jenkins DM, *et al*: HIF-1α-PDK1 axis-induced active glycolysis plays an essential role in macrophage migratory capacity. Nat Commun 7: 11635, 2016.
35. Rius J, Guma M, Schachtrup C, Akassoglou K, Zinkernagel AS, Nizet V, Johnson RS, Haddad GG and Karin M: NF-kappaB links innate immunity to the hypoxic response through transcriptional regulation of HIF-1alpha. Nature 453: 807-811, 2008.
36. Scortegagna M, Ding K, Oktay Y, Gaur A, Thurmond F, Yan LJ, Marck BT, Matsumoto AM, Shelton JM, Richardson JA, *et al*: Multiple organ pathology, metabolic abnormalities and impaired homeostasis of reactive oxygen species in Epas1-/- mice. Nat Genet 35: 331-340, 2003.
37. Rankin EB, Rha J, Selak MA, Unger TL, Keith B, Liu Q and Haase VH: Hypoxia-inducible factor 2 regulates hepatic lipid metabolism. Mol Cell Biol 29: 4527-4538, 2009.
38. Makino Y, Kanopka A, Wilson WJ, Tanaka H and Poellinger L: Inhibitory pas domain protein (IPAS) is a hypoxia-inducible splicing variant of the hypoxia-inducible factor-3alpha locus. J Biol Chem 277: 32405-32408, 2002.
39. Zhan L, Huang C, Meng XM, Song Y, Wu XQ, Yang Y and Li J: Hypoxia-inducible factor-1alpha in hepatic fibrosis: A promising therapeutic target. Biochimie 108: 1-7, 2015.

40. Aggarwal SR, Lindros KO and Palmer TN: Glucagon stimulates phosphorylation of different peptides in isolated periportal and perivenous hepatocytes. FEBS Lett 377: 439-443, 1995.
41. Kinugasa A and Thurman RG: Differential effect of glucagon on gluconeogenesis in periportal and pericentral regions of the liver lobule. Biochem J 236: 425-430, 1986.
42. Saito K, Negishi M and James SE: Sexual dimorphisms in zonal gene expression in mouse liver. Biochem Biophys Res Commun 436: 730-735, 2013.
43. Goldfarb CN, Karri K, Pyatkov M and Waxman DJ: Interplay between GH-regulated, Sex-biased liver transcriptome and hepatic zonation revealed by single-nucleus RNA sequencing. Endocrinology 163: bqac059, 2022.
44. Annunziato S, Sun T and Tchorz JS: The RSPO-LGR4/5-ZNRF3/RNF43 module in liver homeostasis, regeneration, and disease. Hepatology 76: 888-899, 2022.
45. Sun T, Annunziato S, Bergling S, Sheng C, Orsini V, Forcella P, Pikiolek M, Kancherla V, Holwerda S, Imanci D, *et al*: ZNRF3 and RNF43 cooperate to safeguard metabolic liver zonation and hepatocyte proliferation. Cell Stem Cell 28: 1822-1837, 2021.
46. Torre C, Perret C and Colnot S: Molecular determinants of liver zonation. Prog Mol Biol Transl Sci 97: 127-150, 2010.
47. Hu S, Liu S, Bian Y, Poddar M, Singh S, Cao C, Mcgaughey J, Bell A, Blazer LL, Adams JJ, *et al*: Single-cell spatial transcriptomics reveals a dynamic control of metabolic zonation and liver regeneration by endothelial cell Wnt2 and Wnt9b. Cell Rep Med 3: 100754, 2022.
48. Benhamouche S, Decaens T, Godard C, Chambrey R, Rickman DS, Moinard C, Vasseur-Cognet M, Kuo CJ, Kahn A, Perret C and Colnot S: Apc tumor suppressor gene is the 'zonation-keeper' of mouse liver. Dev Cell 10: 759-770, 2006.
49. Gerbal-Chaloin S, Dume A, Briolotti P, Klieber S, Raulet E, Duret C, Fabre J, Ramos J, Maurel P and Daujat-Chavanieu M: The Wnt/β-catenin pathway is a transcriptional regulator of CYP2E1, CYP1A2, and aryl hydrocarbon receptor gene expression in primary human hepatocytes. Mol Pharmacol 86: 624-634, 2014.
50. Kietzmann T: Liver zonation in health and disease: Hypoxia and hypoxia-inducible transcription factors as concert masters. Int J Mol Sci 20: 2347, 2019.
51. Mazumdar J, O'Brien WT, Johnson RS, Lamanna JC, Chavez JC, Klein PS and Simon MC: O2 regulates stem cells through Wnt/β-catenin signalling. Nat Cell Biol 12: 1007-1013, 2010.
52. Mori H, Yao Y, Learman BS, Kurozumi K, Ishida J, Ramakrishnan SK, Overmyer KA, Xue X, Cawthorn WP, Reid MA, *et al*: Induction of WNT11 by hypoxia and hypoxia-inducible factor-1α regulates cell proliferation, migration and invasion. Sci Rep 6: 21520, 2016.
53. Braeuning A, Menzel M, Kleinschnitz EM, Harada N, Tamai Y, Kohle C, Buchmann A and Schwarz M: Serum components and activated Ha-ras antagonize expression of perivenous marker genes stimulated by beta-catenin signaling in mouse hepatocytes. FEBS J 274: 4766-4777, 2007.
54. Mcenerney L, Duncan K, Bang BR, Elmasry S, Li M, Miki T, Ramakrishnan SK, Shah YM and Saito T: Dual modulation of human hepatic zonation via canonical and non-canonical Wnt pathways. Exp Mol Med 49: e413, 2017.
55. Wakizaka K, Kamiyama T, Kakisaka T, Orimo T, Nagatsu A, Aiyama T, Shichi S and Taketomi A: Expression of Wnt5a and ROR2, components of the noncanonical Wnt-signaling pathway, is associated with tumor differentiation in hepatocellular carcinoma. Ann Surg Oncol 31: 262-271, 2024.
56. Yang J, Cusimano A, Monga JK, Preziosi ME, Pullara F, Calero G, Lang R, Yamaguchi TP, Nejak-Bowen KN and Monga SP: Wnt5a inhibits hepatocyte proliferation and concludes beta-catenin signaling in liver regeneration. Am J Pathol 185: 2194-2205, 2015.
57. Omenetti A, Choi S, Michelotti G and Diehl AM: Hedgehog signaling in the liver. J Hepatol 54: 366-373, 2011.
58. Varnat F, Zacchetti G and Ruiz IAA: Hedgehog pathway activity is required for the lethality and intestinal phenotypes of mice with hyperactive Wnt signaling. Mech Dev 127: 73-81, 2010.
59. Matz-Soja M, Aleithe S, Marbach E, Bottger J, Arnold K, Schmidt-Heck W, Kratzsch J and Gebhardt R: Hepatic hedgehog signaling contributes to the regulation of IGF1 and IGFBP1 serum levels. Cell Commun Signal 12: 11, 2014.
60. Sicklick JK, Li YX, Choi SS, Qi Y, Chen W, Bustamante M, Huang J, Zdanowicz M, Camp T, Torbenson MS, *et al*: Role for hedgehog signaling in hepatic stellate cell activation and viability. Lab Invest 85: 1368-1380, 2005.
61. Saviano A, Henderson NC and Baumert TF: Single-cell genomics and spatial transcriptomics: Discovery of novel cell states and cellular interactions in liver physiology and disease biology. J Hepatol 73: 1219-1230, 2020.
62. Rocha AS, Vidal V, Mertz M, Kendall TJ, Charlet A, Okamoto H and Schedl A: The angiocrine factor rspondin3 is a key determinant of liver zonation. Cell Rep 13: 1757-1764, 2015.
63. Halpern KB, Shenhav R, Massalha H, Toth B, Egozi A, Massasa EE, Medgalia C, David E, Giladi A, Moor AE, *et al*: Paired-cell sequencing enables spatial gene expression mapping of liver endothelial cells. Nat Biotechnol 36: 962-970, 2018.
64. Macparland SA, Liu JC, Ma XZ, Innes BT, Bartczak AM, Gage BK, Manuel J, Khuu N, Echeverri J, Linares I, *et al*: Single cell RNA sequencing of human liver reveals distinct intrahepatic macrophage populations. Nat Commun 9: 4383, 2018.
65. Wen Y, Lambrecht J, Ju C and Tacke F: Hepatic macrophages in liver homeostasis and diseases-diversity, plasticity and therapeutic opportunities. Cell Mol Immunol 18: 45-56, 2021.
66. Trinh VQ, Lee TF, Lemoinne S, Ray KC, Ybanez MD, Tsuchida T, Carter JK, Agudo J, Brown BD, Akat KM, *et al*: Hepatic stellate cells maintain liver homeostasis through paracrine neurotrophin-3 signaling that induces hepatocyte proliferation. Sci Signal 16: eadf6696, 2023.
67. Dobie R, Wilson-Kanamori JR, Henderson B, Smith JR, Matchett KP, Portman JR, Wallenborg K, Picelli S, Zagorska A, Pendem SV, *et al*: Single-cell transcriptomics uncovers zonation of function in the mesenchyme during liver fibrosis. Cell Rep 29: 1832-1847, 2019.
68. Khan MA, Fischer J, Harrer L, Schwiering F, Groneberg D and Friebe A: Hepatic stellate cells in zone 1 engage in capillarization rather than myofibroblast formation in murine liver fibrosis. Sci Rep 14: 18840, 2024.
69. Bravo GC, Matetovici I, Hillen H, Taskiran II, Vandepoel R, Christiaens V, Sansores-Garcia L, Verboven E, Hulselmans G, Poovathingal S, *et al*: Single-cell spatial multi-omics and deep learning dissect enhancer-driven gene regulatory networks in liver zonation. Nat Cell Biol 26: 153-167, 2024.
70. Chen H, Lu S, Zhou J, Bai Z, Fu H, Xu X, Yang S, Jiao B and Sun Y: An integrated approach for the identification of USF1-centered transcriptional regulatory networks during liver regeneration. Biochim Biophys Acta 1839: 415-423, 2014.
71. Brosch M, Kattler K, Herrmann A, von Schonfels W, Nordstrom K, Seehofer D, Damm G, Becker T, Zeissig S, Nehring S, *et al*: Epigenomic map of human liver reveals principles of zonated morphogenic and metabolic control. Nat Commun 9: 4150, 2018.
72. Stanulovic VS, Kyrmizi I, Kruithof-De JM, Hoogenkamp M, Vermeulen JL, Ruijter JM, Talianidis I, Hakvoort TB and Lamers WH: Hepatic hnf4alpha deficiency induces periportal expression of glutamine synthetase and other pericentral enzymes. Hepatology 45: 433-444, 2007.
73. Lee J, Wang C, Xu S, Cho Y, Wang L, Feng X, Baldridge A, Sartorelli V, Zhuang L, Peng W, *et al*: H3K4 mono- and di-methyltransferase MLL4 is required for enhancer activation during cell differentiation. Elife 2: e1503, 2013.
74. Moore A, Wu L, Chuang J, Sun X, Luo X, Gopal P, Li L, Celen C, Zimmer M and Zhu H: Arid1a loss drives nonalcoholic steatohepatitis in mice through epigenetic dysregulation of hepatic lipogenesis and fatty acid oxidation. Hepatology 69: 1931-1945, 2019.
75. Qu Y, Deng C, Luo Q, Shang X, Wu J, Shi Y, Wang L and Han Z: Arid1a regulates insulin sensitivity and lipid metabolism. EBioMedicine 42: 481-493, 2019.
76. Hsu SH, Delgado ER, Otero PA, Teng KY, Kutay H, Meehan KM, Moroney JB, Monga JK, Hand NJ, Friedman JR, *et al*: Microrna-122 regulates polyploidization in the murine liver. Hepatology 64: 599-615, 2016.
77. Ben-Moshe S, Shapira Y, Moor AE, Manco R, Veg T, Bahar Halpern K and Itzkovitz S: Spatial sorting enables comprehensive characterization of liver zonation. Nat Metab 1: 899-911, 2019.
78. Wang B, Zhao L, Fish M, Logan CY and Nusse R: Self-renewing diploid Axin2(+) cells fuel homeostatic renewal of the liver. Nature 524: 180-185, 2015.
79. Sun T, Pikiolek M, Orsini V, Bergling S, Holwerda S, Morelli L, Hoppe PS, Planas-Paz L, Yang Y, Ruffner H, *et al*: $Axin2^{+}$ pericentral hepatocytes have limited contributions to liver homeostasis and regeneration. Cell Stem Cell 26: 97-107.e6, 2020.

80. May S, Muller M, Livingstone CR, Skalka GL, Walsh PJ, Nixon C, Hedley A, Shaw R, Clark W, Vande VJ, *et al*: Absent expansion of AXIN2+ hepatocytes and altered physiology in Axin2CreERT2 mice challenges the role of pericentral hepatocytes in homeostatic liver regeneration. J Hepatol 78: 1028-1036, 2023.
81. Lin S, Nascimento EM, Gajera CR, Chen L, Neuhofer P, Garbuzov A, Wang S and Artandi SE: Distributed hepatocytes expressing telomerase repopulate the liver in homeostasis and injury. Nature 556: 244-248, 2018.
82. Ben-Moshe S, Veg T, Manco R, Dan S, Papinutti D, Lifshitz A, Kolodziejczyk AA, Bahar HK, Elinav E and Itzkovitz S: The spatiotemporal program of zonal liver regeneration following acute injury. Cell Stem Cell 29: 973-989, 2022.
83. He L, Pu W, Liu X, Zhang Z, Han M, Li Y, Huang X, Han X, Li Y, Liu K, *et al*: Proliferation tracing reveals regional hepatocyte generation in liver homeostasis and repair. Science 371: eabc4346, 2021.
84. Prior N, Hindley CJ, Rost F, Melendez E, Lau W, Gottgens B, Rulands S, Simons BD and Huch M: Lgr5$^+$ stem and progenitor cells reside at the apex of a heterogeneous embryonic hepatoblast pool. Development 146: dev174557, 2019.
85. Qin D, Liu S, Lu Y, Yan Y, Zhang J, Cao S, Chen M, Chen N, Huang W, Wang L, *et al*: Lgr5$^+$ cell fate regulation by coordination of metabolic nuclear receptors during liver repair. Theranostics 12: 6130-6142, 2022.
86. Chen F, Jimenez RJ, Sharma K, Luu HY, Hsu BY, Ravindranathan A, Stohr BA and Willenbring H: Broad distribution of hepatocyte proliferation in liver homeostasis and regeneration. Cell Stem Cell 26: 27-33, 2020.
87. Wei Y, Wang YG, Jia Y, Li L, Yoon J, Zhang S, Wang Z, Zhang Y, Zhu M, Sharma T, *et al*: Liver homeostasis is maintained by midlobular zone 2 hepatocytes. Science 371: eabb1625, 2021.
88. Kubota N, Kubota T and Kadowaki T: Midlobular zone 2 hepatocytes: A gatekeeper of liver homeostasis. Cell Metab 33: 855-856, 2021.
89. Lin YH, Wei Y, Zeng Q, Wang Y, Pagani CA, Li L, Zhu M, Wang Z, Hsieh MH, Corbitt N, *et al*: IGFBP2 expressing midlobular hepatocytes preferentially contribute to liver homeostasis and regeneration. Cell Stem Cell 30: 665-676.e4, 2023.
90. Q BA, Vesco KK, Purnell JQ, Francisco M, Goddard E, Guan X, Debarber A, Leo MC, Baetscher E, Rooney W, *et al*: Pregnancy and weaning regulate human maternal liver size and function. Proc Natl Acad Sci USA 118: e2107269118, 2021.
91. Milona A, Owen BM, van Mil S, Dormann D, Mataki C, Boudjelal M, Cairns W, Schoonjans K, Milligan S, Parker M, *et al*: The normal mechanisms of pregnancy-induced liver growth are not maintained in mice lacking the bile acid sensor Fxr. Am J Physiol Gastrointest Liver Physiol 298: G151-G158, 2010.
92. Jaeschke H and Ramachandran A: Acetaminophen hepatotoxicity: Paradigm for understanding mechanisms of drug-induced liver injury. Ann Rev Pathol 19: 453-478, 2024.
93. Li M, Cai S and Boyer JL: Mechanisms of bile acid mediated inflammation in the liver. Mol Aspects Med 56: 45-53, 2017.
94. Younossi Z and Henry L: Contribution of alcoholic and nonalcoholic fatty liver disease to the burden of liver-related morbidity and mortality. Gastroenterology 150: 1778-1785, 2016.
95. Ibrahim SH, Hirsova P and Gores GJ: Non-alcoholic steatohepatitis pathogenesis: Sublethal hepatocyte injury as a driver of liver inflammation. Gut 67: 963-972, 2018.
96. Chalasani N, Wilson L, Kleiner DE, Cummings OW, Brunt EM and Unalp A: Relationship of steatosis grade and zonal location to histological features of steatohepatitis in adult patients with non-alcoholic fatty liver disease. J Hepatol 48: 829-834, 2008.
97. Sheka AC, Adeyi O, Thompson J, Hameed B, Crawford PA and Ikramuddin S: Nonalcoholic steatohepatitis: A review. JAMA 323: 1175-1183, 2020.
98. Leow WQ, Chan AW, Mendoza P, Lo R, Yap K and Kim H: Non-alcoholic fatty liver disease: The Pathologist's perspective. Clin Mol Hepatol 29 (Suppl): S302-S318, 2023.
99. Carter-Kent C, Yerian LM, Brunt EM, Angulo P, Kohli R, Ling SC, Xanthakos SA, Whitington PF, Charatcharoenwitthaya P, Yap J, *et al*: Nonalcoholic steatohepatitis in children: A multicenter clinicopathological study. Hepatology 50: 1113-1120, 2009.
100. Swiderska-Syn M, Suzuki A, Guy CD, Schwimmer JB, Abdelmalek MF, Lavine JE and Diehl AM: Hedgehog pathway and pediatric nonalcoholic fatty liver disease. Hepatology 57: 1814-1825, 2013.
101. Machado MV, Michelotti GA, Pereira TA, Xie G, Premont R, Cortez-Pinto H and Diehl AM: Accumulation of duct cells with activated yap parallels fibrosis progression in non-alcoholic fatty liver disease. J Hepatol 63: 962-970, 2015.
102. Gadd VL, Skoien R, Powell EE, Fagan KJ, Winterford C, Horsfall L, Irvine K and Clouston AD: The portal inflammatory infiltrate and ductular reaction in human nonalcoholic fatty liver disease. Hepatology 59: 1393-1405, 2014.
103. Richardson MM, Jonsson JR, Powell EE, Brunt EM, Neuschwander-Tetri BA, Bhathal PS, Dixon JB, Weltman MD, Tilg H, Moschen AR, *et al*: Progressive fibrosis in nonalcoholic steatohepatitis: Association with altered regeneration and a ductular reaction. Gastroenterology 133: 80-90, 2007.
104. Ko S, Russell JO, Molina LM and Monga SP: Liver progenitors and adult cell plasticity in hepatic injury and repair: Knowns and unknowns. Annu Rev Pathol 15: 23-50, 2020.
105. Hammoutene A and Rautou P: Role of liver sinusoidal endothelial cells in non-alcoholic fatty liver disease. J Hepatol 70: 1278-1291, 2019.
106. Zhou Y, Zhao Y, Carbonaro M, Chen H, Germino M, Adler C, Ni M, Zhu YO, Kim SY, Altarejos J, *et al*: Perturbed liver gene zonation in a mouse model of non-alcoholic steatohepatitis. Metabolism 154: 155830, 2024.
107. Kostallari E, Wei B, Sicard D, Li J, Cooper SA, Gao J, Dehankar M, Li Y, Cao S, Yin M, *et al*: Stiffness is associated with hepatic stellate cell heterogeneity during liver fibrosis. Am J Physiol Gastrointest Liver Physiol 322: G234-G246, 2022.
108. Llovet JM, Pinyol R, Kelley RK, El-Khoueiry A, Reeves HL, Wang XW, Gores GJ and Villanueva A: Molecular pathogenesis and systemic therapies for hepatocellular carcinoma. Nat Cancer 3: 386-401, 2022.
109. Barker N and Clevers H: Mining the Wnt pathway for cancer therapeutics. Nat Rev Drug Discov 5: 997-1014, 2006.
110. Sia D, Villanueva A, Friedman SL and Llovet JM: Liver cancer cell of origin, molecular class, and effects on patient prognosis. Gastroenterology 152: 745-761, 2017.
111. Holczbauer A, Wangensteen KJ and Shin S: Cellular origins of regenerating liver and hepatocellular carcinoma. JHEP Rep 4: 100416, 2022.
112. Perugorria MJ, Olaizola P, Labiano I, Esparza-Baquer A, Marzioni M, Marin J, Bujanda L and Banales JM: Wnt-beta-catenin signalling in liver development, health and disease. Nat Rev Gastroenterol Hepatol 16: 121-136, 2019.
113. Zhang T, Gu J, Wang X, Lu Y, Cai K, Li H, Nie Y, Chen X and Wang J: A novel liver zonation phenotype-associated molecular classification of hepatocellular carcinoma. Front Immunol 14: 1140201, 2023.
114. Desert R, Rohart F, Canal F, Sicard M, Desille M, Renaud S, Turlin B, Bellaud P, Perret C, Clement B, *et al*: Human hepatocellular carcinomas with a periportal phenotype have the lowest potential for early recurrence after curative resection. Hepatology 66: 1502-1518, 2017.
115. Ng C, Piscuoglio S and Terracciano LM: Molecular classification of hepatocellular carcinoma: The view from metabolic zonation. Hepatology 66: 1377-1380, 2017.
116. Stravitz RT and Lee WM: Acute liver failure. Lancet 394: 869-881, 2019.
117. Anundi I, Lahteenmaki T, Rundgren M, Moldeus P and Lindros KO: Zonation of acetaminophen metabolism and cytochrome P450 2E1-mediated toxicity studied in isolated periportal and perivenous hepatocytes. Biochem Pharmacol 45: 1251-1259, 1993.
118. Avasarala S, Yang L, Sun Y, Leung AW, Chan W, Cheung W and Lee SS: A temporal study on the histopathological, biochemical and molecular responses of CCL(4)-induced hepatotoxicity in Cyp2e1-null mice. Toxicology 228: 310-322, 2006.
119. Matchett KP, Wilson-Kanamori JR, Portman JR, Kapourani CA, Fercoq F, May S, Zajdel E, Beltran M, Sutherland EF, Mackey J, *et al*: Multimodal decoding of human liver regeneration. Nature 630: 158-165, 2024.
120. Kpetemey M, Dasgupta S, Rajendiran S, Das S, Gibbs LD, Shetty P, Gryczynski Z and Vishwanatha JK: MIEN1, a novel interactor of annexin A2, promotes tumor cell migration by enhancing AnxA2 cell surface expression. Mol Cancer 14: 156, 2015.
121. Gouw ASH, Clouston AD and Theise ND: Ductular reactions in human liver: Diversity at the interface. Hepatology 54: 1853-1863, 2011.
122. Miyajima A, Tanaka M and Itoh T: Stem/progenitor cells in liver development, homeostasis, regeneration, and reprogramming. Cell Stem Cell 14: 561-574, 2014.

123. Fickert P, Stoger U, Fuchsbichler A, Moustafa T, Marschall HU, Weiglein AH, Tsybrovskyy O, Jaeschke H, Zatloukal K, Denk H and Trauner M: A new xenobiotic-induced mouse model of sclerosing cholangitis and biliary fibrosis. Am J Pathol 171: 525-536, 2007.
124. Kullak-Ublick GA, Stieger B and Meier PJ: Enterohepatic bile salt transporters in normal physiology and liver disease. Gastroenterology 126: 322-342, 2004.
125. Font-Burgada J, Shalapour S, Ramaswamy S, Hsueh B, Rossell D, Umemura A, Taniguchi K, Nakagawa H, Valasek MA, Ye L, *et al*: Hybrid periportal hepatocytes regenerate the injured liver without giving rise to cancer. Cell 162: 766-779, 2015.
126. Shao C, Jing Y, Zhao S, Yang X, Hu Y, Meng Y, Huang Y, Ye F, Gao L, Liu W, *et al*: LPS/Bcl3/YAP1 signaling promotes Sox9+HNF4α+ hepatocyte-mediated liver regeneration after hepatectomy. Cell Death Dis 13: 277, 2022.
127. Gribben C, Galanakis V, Calderwood A, Williams EC, Chazarra-Gil R, Larraz M, Frau C, Puengel T, Guillot A, Rouhani FJ, *et al*: Acquisition of epithelial plasticity in human chronic liver disease. Nature 630: 166-173, 2024.
128. Lu W, Bird TG, Boulter L, Tsuchiya A, Cole AM, Hay T, Guest RV, Wojtacha D, Man TY, Mackinnon A, *et al*: Hepatic progenitor cells of biliary origin with liver repopulation capacity. Nat Cell Biol 17: 971-983, 2015.
129. Pu W, Zhang H, Huang X, Tian X, He L, Wang Y, Zhang L, Liu Q, Li Y, Li Y, *et al*: Mfsd2a+ hepatocytes repopulate the liver during injury and regeneration. Nat Commun 7: 13369, 2016.
130. Tarlow BD, Finegold MJ and Grompe M: Clonal tracing of Sox9+ liver progenitors in mouse oval cell injury. Hepatology 60: 278-289, 2014.
131. Tarlow BD, Pelz C, Naugler WE, Wakefield L, Wilson EM, Finegold MJ and Grompe M: Bipotential adult liver progenitors are derived from chronically injured mature hepatocytes. Cell Stem Cell 15: 605-618, 2014.
132. Raven A, Lu WY, Man TY, Ferreira-Gonzalez S, O'Duibhir E, Dwyer BJ, Thomson JP, Meehan RR, Bogorad R, Koteliansky V, *et al*: Cholangiocytes act as facultative liver stem cells during impaired hepatocyte regeneration. Nature 547: 350-354, 2017.
133. Pradhan-Sundd T, Kosar K, Saggi H, Zhang R, Vats R, Cornuet P, Green S, Singh S, Zeng G, Sundd P, *et al*: Wnt/β-catenin signaling plays a protective role in the Mdr2 knockout murine model of cholestatic liver disease. Hepatology 71: 1732-1749, 2020.
134. Macchi F and Sadler KC: Unraveling the epigenetic basis of liver development, regeneration and disease. Trends Genet 36: 587-597, 2020.
135. Caldez MJ, Van Hul N, Koh H, Teo XQ, Fan JJ, Tan PY, Dewhurst MR, Too PG, Talib S, Chiang BE, *et al*: Metabolic remodeling during liver regeneration. Dev Cell 47: 425-438.e5, 2018.
136. Li W, Li L and Hui L: Cell plasticity in liver regeneration. Trends Cell Biol 30: 329-338, 2020.
137. Lambrecht R, Rudolf F, Uckert A, Sladky VC, Phan TS, Jansen J, Naim S, Kaufmann T, Keogh A, Kirschnek S, *et al*: Non-canonical BIM-regulated energy metabolism determines drug-induced liver necrosis. Cell Death Differ 31: 119-131, 2024.
138. Gazit V, Huang J, Weymann A and Rudnick DA: Analysis of the role of hepatic ppargamma expression during mouse liver regeneration. Hepatology 56: 1489-1498, 2012.
139. Jiao M, Ren F, Zhou L, Zhang X, Zhang L, Wen T, Wei L, Wang X, Shi H, Bai L, *et al*: Peroxisome proliferator-activated receptor alpha activation attenuates the inflammatory response to protect the liver from acute failure by promoting the autophagy pathway. Cell Death Dis 5: e1397, 2014.
140. Fan S, Gao Y, Qu A, Jiang Y, Li H, Xie G, Yao X, Yang X, Zhu S, Yagai T, *et al*: YAP-TEAD mediates PPAR α-induced hepatomegaly and liver regeneration in mice. Hepatology 75: 74-88, 2022.
141. Kaji K, Factor VM, Andersen JB, Durkin ME, Tomokuni A, Marquardt JU, Matter MS, Hoang T, Conner EA and Thorgeirsson SS: Dnmt1 is a required genomic regulator for murine liver histogenesis and regeneration. Hepatology 64: 582-598, 2016.
142. Snykers S, Henkens T, De Rop E, Vinken M, Fraczek J, De Kock J, De Prins E, Geerts A, Rogiers V and Vanhaecke T: Role of epigenetics in liver-specific gene transcription, hepatocyte differentiation and stem cell reprogrammation. J Hepatol 51: 187-211, 2009.
143. Shi Y, Sun H, Bao J, Zhou P, Zhang J, Li L and Bu H: Activation of inactive hepatocytes through histone acetylation: A mechanism for functional compensation after massive loss of hepatocytes. Am J Pathol 179: 1138-1147, 2011.
144. Grimsrud PA, Carson JJ, Hebert AS, Hubler SL, Niemi NM, Bailey DJ, Jochem A, Stapleton DS, Keller MP, Westphall MS, *et al*: A quantitative map of the liver mitochondrial phosphoproteome reveals posttranslational control of Ketogenesis. Cell Metab 16: 672-683, 2012.
145. Sugimoto A, Saito Y, Wang G, Sun Q, Yin C, Lee KH, Geng Y, Rajbhandari P, Hernandez C, Steffani M, *et al*: Hepatic stellate cells control liver zonation, size and functions via R-spondin 3. Nature 640: 752-761, 2025.
146. Preziosi M, Okabe H, Poddar M, Singh S and Monga SP: Endothelial Wnts regulate β-catenin signaling in murine liver zonation and regeneration: A sequel to the Wnt-Wnt situation. Hepatol Commun 2: 845-860, 2018.
147. Duan JL, Zhou ZY, Ruan B, Fang ZQ, Ding J, Liu JJ, Song P, Xu H, Xu C, Yue ZS, *et al*: Notch-regulated c-kit-positive liver sinusoidal endothelial cells contribute to liver zonation and regeneration. Cell Mol Gastroenterol Hepatol 13: 1741-1756, 2022.
148. Li HY, Gao YX, Wu JC, Li JZ, Fu SW and Xu MY: Single-cell transcriptome reveals a novel mechanism of C-kit^{+}-liver sinusoidal endothelial cells in NASH. Cell Biosci 14: 31, 2024.
149. Du W, Chen C, Liu Y, Quan H, Xu M, Liu J, Song P, Fang Z, Yue Z, Xu H, *et al*: A combined 'eat me/don't eat me' strategy based on exosome for acute liver injury treatment. Cell Rep Med 6: 102033, 2025.
150. Rigual MDM, Angulo-Aguado M, Zagorac S, Álvarez-Díaz R, Benítez-Mondéjar M, Yi F, Martínez-Garay C, Santos-De-Frutos K, Kim E, Campos-Olivas R, *et al*: Macrophages harness hepatocyte glutamate to boost liver regeneration. Nature 641: 1005-1016, 2025.
151. Hu M, Kurobe M, Jeong YJ, Fuerer C, Ghole S, Nusse R and Sylvester KG: Wnt/beta-catenin signaling in murine hepatic transit amplifying progenitor cells. Gastroenterology 133: 1579-1591, 2007.
152. Yimlamai D, Christodoulou C, Galli GG, Yanger K, Pepe-Mooney B, Gurung B, Shrestha K, Cahan P, Stanger BZ and Camargo FD: Hippo pathway activity influences liver cell fate. Cell 157: 1324-1338, 2014.
153. Oh SH, Swiderska-Syn M, Jewell ML, Premont RT and Diehl AM: Liver regeneration requires Yap1-TGFβ-dependent epithelial-mesenchymal transition in hepatocytes. J Hepatol 69: 359-367, 2018.
154. Li L, Cui L, Lin P, Liu Z, Bao S, Ma X, Nan H, Zhu W, Cen J, Mao Y, *et al*: Kupffer-cell-derived Ll-6 is repurposed for hepatocyte dedifferentiation via activating progenitor genes from injury-specific enhancers. Cell Stem Cell 30: 283-299, 2023.
155. Younossi ZM, Ratziu V, Loomba R, Rinella M, Anstee QM, Goodman Z, Bedossa P, Geier A, Beckebaum S, Newsome PN, *et al*: Obeticholic acid for the treatment of non-alcoholic steatohepatitis: Interim analysis from a multicentre, randomised, placebo-controlled phase 3 trial. Lancet 394: 2184-2196, 2019.
156. Sanyal AJ, Ling L, Beuers U, Depaoli AM, Lieu HD, Harrison SA and Hirschfield GM: Potent suppression of hydrophobic bile acids by aldafermin, an FGF19 analogue, across metabolic and cholestatic liver diseases. JHEP Rep 3: 100255, 2021.
157. Kimura K, Ikoma A, Shibakawa M, Shimoda S, Harada K, Saio M, Imamura J, Osawa Y, Kimura M, Nishikawa K, *et al*: Safety, tolerability, and preliminary efficacy of the anti-fibrotic small molecule PRI-724, a CBP/β-catenin inhibitor, in patients with Hepatitis C Virus-related cirrhosis: A single-center, open-label, dose escalation phase 1 trial. EBioMedicine 23: 79-87, 2017.
158. Francque SM, Hodge A, Boursier J, Younes ZH, Rodriguez-Araujo G, Park GS, Alkhouri N and Abdelmalek MF: Phase 2, open-label, rollover study of cenicriviroc for liver fibrosis associated with metabolic dysfunction-associated steatohepatitis. Hepatol Commun 8: e335, 2024.
159. Geervliet E, Karkdijk E and Bansal R: Inhibition of intrahepatic monocyte recruitment by cenicriviroc and extracellular matrix degradation by mmp1 synergistically attenuate liver inflammation and fibrogenesis in vivo. Sci Rep 14: 16897, 2024.
160. Lurje I, Biegel DK, Zhang IW and Tacke F: The role of galectin-3 in liver inflammation and fibrosis. J Inflamm Res 19: 572637, 2026.

161. Chalasani N, Abdelmalek MF, Garcia-Tsao G, Vuppalanchi R, Alkhouri N, Rinella M, Noureddin M, Pyko M, Shiffman M, Sanyal A, *et al*: Effects of belapectin, an inhibitor of galectin-3, in patients with nonalcoholic steatohepatitis with cirrhosis and portal hypertension. Gastroenterology 158: 1334-1345, 2020.
162. Dhir RN, Dworakowski W, Thangavel C and Shapiro BH: Sexually dimorphic regulation of hepatic isoforms of human cytochrome p450 by growth hormone. J Pharmacol Exp Ther 316: 87-94, 2006.
163. Matz-Soja M, Berg T and Kietzmann T: Sex-related variations in liver homeostasis and disease: From zonation dynamics to clinical implications. J Hepatol 84: 181-193, 2026.
164. Sinha S, Ali Q, Zhang T, T-Nguyen DH, Hanna S, Sethiadi J, Hissong E and Schwartz RE: Aging disrupts hepatocyte zonation homeostasis in mice and humans. Hepatology 83: 1143-1157, 2026.
165. Zhang Y, Li Z, Gou H, Song X and Zhang L: The gut microbiota-bile acid axis: A potential therapeutic target for liver fibrosis. Front Cell Infect Microbiol 12: 945368, 2022.
166. Rizvi F, Everton E, Smith AR, Liu H, Osota E, Beattie M, Tam Y, Pardi N, Weissman D and Gouon-Evans V: Murine liver repair via transient activation of regenerative pathways in hepatocytes using lipid nanoparticle-complexed nucleoside-modified mrna. Nat Commun 12: 613, 2021.
167. Debacker AJ, Voutila J, Catley M, Blakey D and Habib N: Delivery of oligonucleotides to the liver with GalNAc: From research to registered therapeutic drug. Mol Ther 28: 1759-1771, 2020.
168. Yang Z, Song L, Chen H, Chen Y, Xie Y and Xie J: Exploring the potential anticancer effects of lobelia chinensis lour in liver cancer via multiomics analysis. Med Res 1: 483-488, 2025.
169. Dana J, Venkatasamy A, Saviano A, Lupberger J, Hoshida Y, Vilgrain V, Nahon P, Reinhold C, Gallix B and Baumert TF: Conventional and artificial intelligence-based imaging for biomarker discovery in chronic liver disease. Hepatol Int 16: 509-522, 2022.
170. Ghosh S, Zhao X, Alim M, Brudno M and Bhat M: Artificial intelligence applied to 'omics data in liver disease: Towards a personalised approach for diagnosis, prognosis and treatment. Gut 74: 295-311, 2025.
171. Bradley CA: Liver: Spatial division of hepatic metabolic labour. Nat Rev Gastroenterol Hepatol 14: 139, 2017.
172. Younossi ZM, Stepanova M, Nader F, Loomba R, Anstee QM, Ratziu V, Harrison S, Sanyal AJ, Schattenberg JM, Barritt AS, *et al*: Obeticholic acid impact on quality of life in patients with nonalcoholic steatohepatitis: REGENERATE 18-month interim analysis. Clin Gastroenterol Hepatol 20: 2050-2058, 2022.
173. Kessoku T, Kobayashi T, Ozaki A, Iwaki M, Honda Y, Ogawa Y, Imajo K, Saigusa Y, Yamamoto K, Yamanaka T, *et al*: Rationale and design of a randomised, double-blind, placebo-controlled, parallel-group, investigator-initiated phase 2a study to investigate the efficacy and safety of elobixibat in combination with cholestyramine for non-alcoholic fatty liver disease. BMJ Open 10: e37961, 2020.
174. Noureddin M, Rinella M, Taub R, Labriola D, Camacho RC, Alkhouri N, Loomba R and Bansal MB: Effects of resmetirom on metabolic-dysfunction associated steatohepatitis in patients with weight loss and/or diabetes taking glucagon-like peptide-1 receptor agonists and other diabetes therapies: A secondary analysis of the MAESTRO-NASH trial. Aliment Pharmacol Ther 62: 1089-1099, 2025.
175. Nakajima A, Eguchi Y, Yoneda M, Imajo K, Tamaki N, Suganami H, Nojima T, Tanigawa R, Iizuka M, Iida Y, *et al*: Randomised clinical trial: Pemafibrate, a novel selective peroxisome proliferator-activated receptor alpha modulator (SPPARMα), versus placebo in patients with non-alcoholic fatty liver disease. Aliment Pharmacol Ther 54: 1263-1277, 2021.
176. Cooreman MP, Butler J, Giugliano RP, Zannad F, Dzen L, Huot-Marchand P, Baudin M, Beard DR, Junien J, Broqua P, *et al*: The pan-PPAR agonist lanifibranor improves cardiometabolic health in patients with metabolic dysfunction-associated steatohepatitis. Nat Commun 15: 3962, 2024.
177. Cheng HS, Tan WR, Low ZS, Marvalim C, Lee JYH and Tan NS: Exploration and development of PPAR modulators in health and disease: An update of clinical evidence. Int J Mol Sci 20: 5055, 2019.
178. Nedrud MA, Chaudhry M, Middleton MS, Moylan CA, Lerebours R, Luo S, Farjat A, Guy C, Loomba R, Abdelmalek MF, *et al*: MRI quantification of placebo effect in nonalcoholic steatohepatitis clinical trials. Radiology 306: e220743, 2023.
179. Yoo C, Fabregat-Franco C, Lin C, Qvortrup C, Oh D, Yau T, Kim H, Castet F, Ponz Sarvise M, Hsu C, *et al*: First-in-human phase 1 study of RO7119929, an oral TLR7 agonist prodrug, in patients with advanced primary or metastatic liver cancers. J Immunother Cancer 14: e12783, 2026.