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From liver organoids to regulatory platforms for precision hepatology: Advances and challenges

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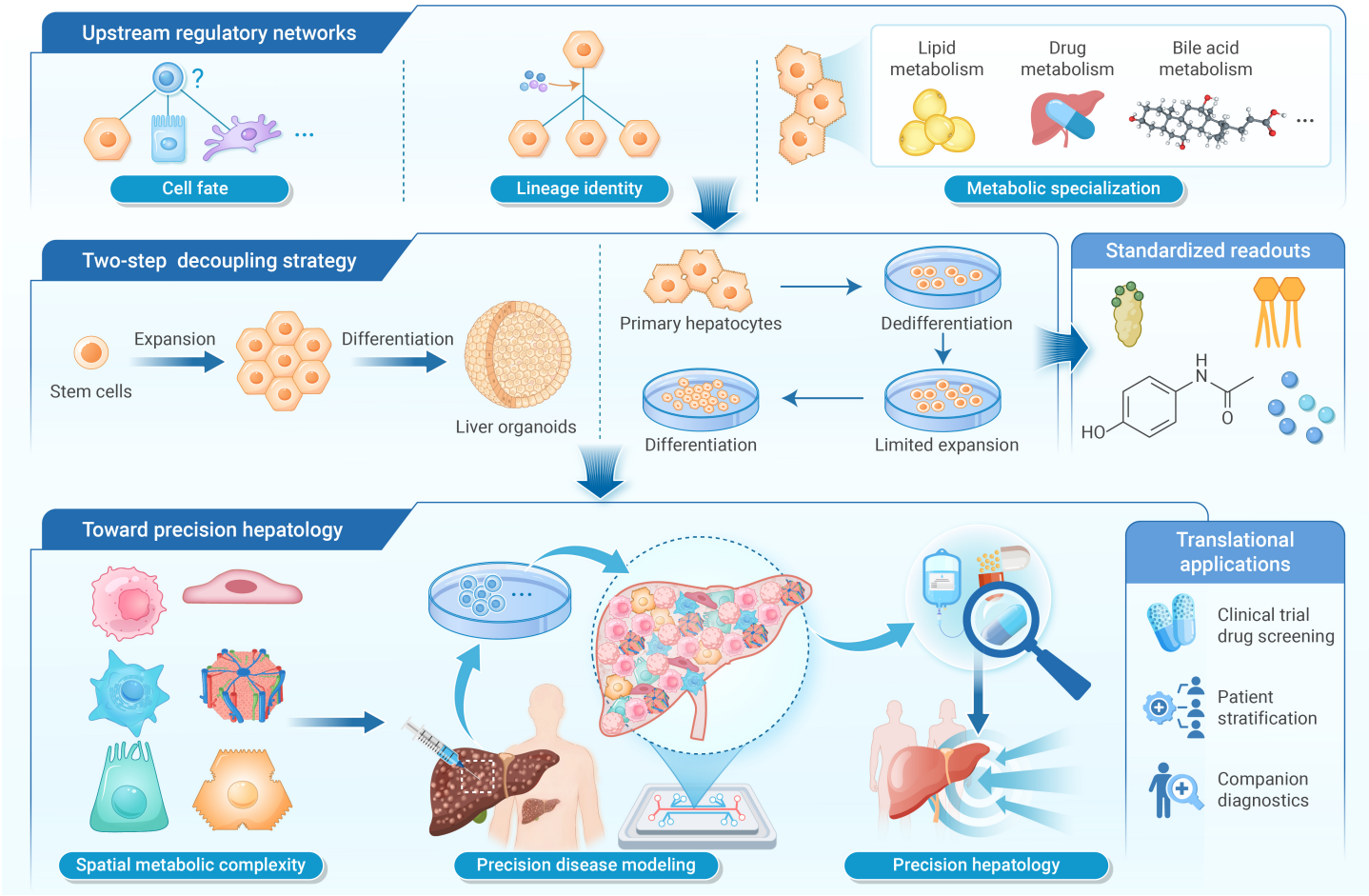
GRAPHICAL ABSTRACT



PUBLIC SUMMARY

- Liver organoids are evolving into regulatory platforms that better mimic liver metabolism and organization.
- A two-step expansion-differentiation strategy enables scalable organoid production while preserving function.
- Reconstructing upstream regulatory programs may improve mechanistic evaluation of liver organoid quality.
- Advanced liver organoids support disease modeling, drug screening, and precision hepatology research.
- Future organoids may integrate multicellular complexity and spatial metabolism to improve translation.

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From liver organoids to regulatory platforms for precision hepatology: Advances and challenges

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Liver organoids have rapidly advanced as human-relevant systems for modeling liver development, metabolic disease, and drug responses. Recent studies have established expandable adult hepatocyte organoids with sustained proliferative capacity, engineered induced pluripotent stem cell-derived organoids exhibiting metabolic zonation through controlled signaling gradients, and constructed multicellular assembloids that incorporate non-parenchymal cell types to recapitulate physiological periportal architecture and model cholestatic fibrotic microenvironments. These developments have enhanced scalability, structural organization, and disease modeling fidelity, supporting applications in translational research. Despite these advances, several challenges remain. Current evaluation of liver organoids largely relies on terminal functional outputs, while the upstream regulatory hierarchies that specify hepatic identity, zonation, and metabolic competence remain insufficiently reconstructed and validated. Limitations in long-term stability, spatial precision, and standardization further constrain predictive performance. This Review summarizes recent technological progress, discusses strategies to improve regulatory fidelity and functional benchmarking, and outlines future directions toward developing liver organoids as more reliable platforms for disease modeling and precision hepatology.

INTRODUCTION

The liver is a central metabolic organ whose functions are orchestrated through tightly regulated molecular, cellular, and spatial control systems.[1] Along the portocentral axis, gradients of oxygen, nutrients, and morphogens contribute to the organization of anabolic, catabolic, and detoxification activities.[1] Beyond lobular zonation, hepatic metabolic homeostasis is also influenced by organelle-associated metabolic pathways that coordinate lipid oxidation and energy balance.[2] These functional programs are encoded not only by regionally enriched metabolic enzymes but also by integrated upstream regulatory hierarchies, including cell fate specification regulators, lineage identity stabilization regulators, and metabolic sensing and functional specialization regulators.[3,4] Disruption of this integrated regulatory architecture contributes to a wide spectrum of liver diseases, ranging from metabolic dysfunction-associated steatohepatitis (MASH) to drug-induced liver injury (DILI) and hepatocellular carcinoma (HCC).[5] Mechanistic understanding of hepatic physiology and disease therefore requires experimental systems capable of reconstructing this integrated regulatory architecture.

However, faithfully recapitulating such regulatory architecture in experimental models remains challenging. Primary hepatocytes rapidly dedifferentiate *in vitro*, losing zonated identity, while animal models are constrained by interspecies differences in metabolic regulation.[6,7] Liver organoids derived from pluripotent or adult stem cells provide a human-relevant platform with long-term expansion and structural fidelity.[8] Yet, most organoid systems are evaluated primarily by downstream outputs such as albumin secretion or basal cytochrome P450 (CYP) activity, leaving largely unresolved whether the upstream networks that specify and maintain metabolic zonation *in vivo* are functionally restored.[9,10] Without mechanistic validation of these upstream regulatory programs, liver organoids may reproduce complex phenotypes without fully capturing the underlying mechanisms governing metabolic regulation.

This critical gap coincides with a pressing translational need.[11] Regulatory agencies are actively promoting the use of human-relevant new approach methodologies (NAMs).[12] Recent initiatives, such as the FDA's roadmap to reduce animal testing and the NIH's establishment of a Standardized Organoid Modeling Center, alongside the first FDA investigational new drug (IND) approval based on organoid-derived efficacy data, underscore a paradigm shift toward human-based predictive models.[13-15] For liver organoids to fulfill their role in precision hepatology, they must evolve beyond phenotypic mimicry into mechanistic discovery platforms capable of modeling patient-specific disease mechanisms and predicting therapeutic outcomes.

Addressing this gap requires reframing organoids as regulatory platforms rather than phenotypic surrogates. We synthesize a two-step expansion-differentiation decoupling strategy to reconcile scalability with metabolic fidelity, and emphasize deliberate reconstruction and functional validation of upstream metabolic control networks. Integrated with spatial engineering and multicellular design, this approach positions liver organoids to mechanistically decode disease-specific metabolic dysregulation and advance precision hepatology.

FROM EARLY MODELS TO METABOLIC ZONATION: THE EVOLUTION OF ENGINEERED LIVER ORGANOIDS

Over the past two decades, liver organoid technologies have evolved from progenitor-based cultures toward increasingly physiologic multicellular microtissues.[16] A seminal breakthrough by Huch *et al.* demonstrated long-term expansion of bipotent liver epithelial organoids from $Lgr5^+$ adult progenitors using a Wnt/R-spondin enriched niche.[17] Building on this framework, subsequent studies extended Wnt-dependent regenerative cues to enable long-term expansion of hepatocyte organoids, marking a critical advance beyond progenitor-restricted models.[18,19] In parallel, Takebe and colleagues pioneered multicellular liver buds by co-assembling iPSC-derived hepatic endoderm with endothelial and mesenchymal cells, demonstrating self-organization into vascularized liver-like microtissues.[20] Collectively, these seminal studies established foundational platforms for maintaining hepatic identity and multicellular organization *in vitro*, yet primarily emphasized tissue viability and bulk metabolic function rather than spatially organized metabolic programs.

However, the journey toward fully recapitulating human liver physiology remains far from complete. Most liver organoids undergo progressive dedifferentiation with declining CYP activity and metabolic capacity, compounded by limited access to high-quality primary hepatocytes, incomplete maturation of iPSC-derived hepatocyte-like cells, and insufficient multicellular diversity or spatial organization.[21-23] Consequently, most current liver organoid systems lack the capacity to reconstruct and interrogate disease-relevant spatial metabolic states, rendering them insufficient as discovery platforms for complex disorders such as MASH and the microenvironment of HCC.[16,24] Notably, hepatic metabolism is inherently organized by spatial zonation along the preportal-precentral axis, enabling functionally specialized hepatocyte subpopulations to execute distinct anabolic, catabolic, and detoxification programs.[25] However, despite multiple attempts using primary and iPSC-derived hepatocytes, the emergence of zone-dependent functions was poorly defined in human liver models.[21] The long-standing limitations of conven-

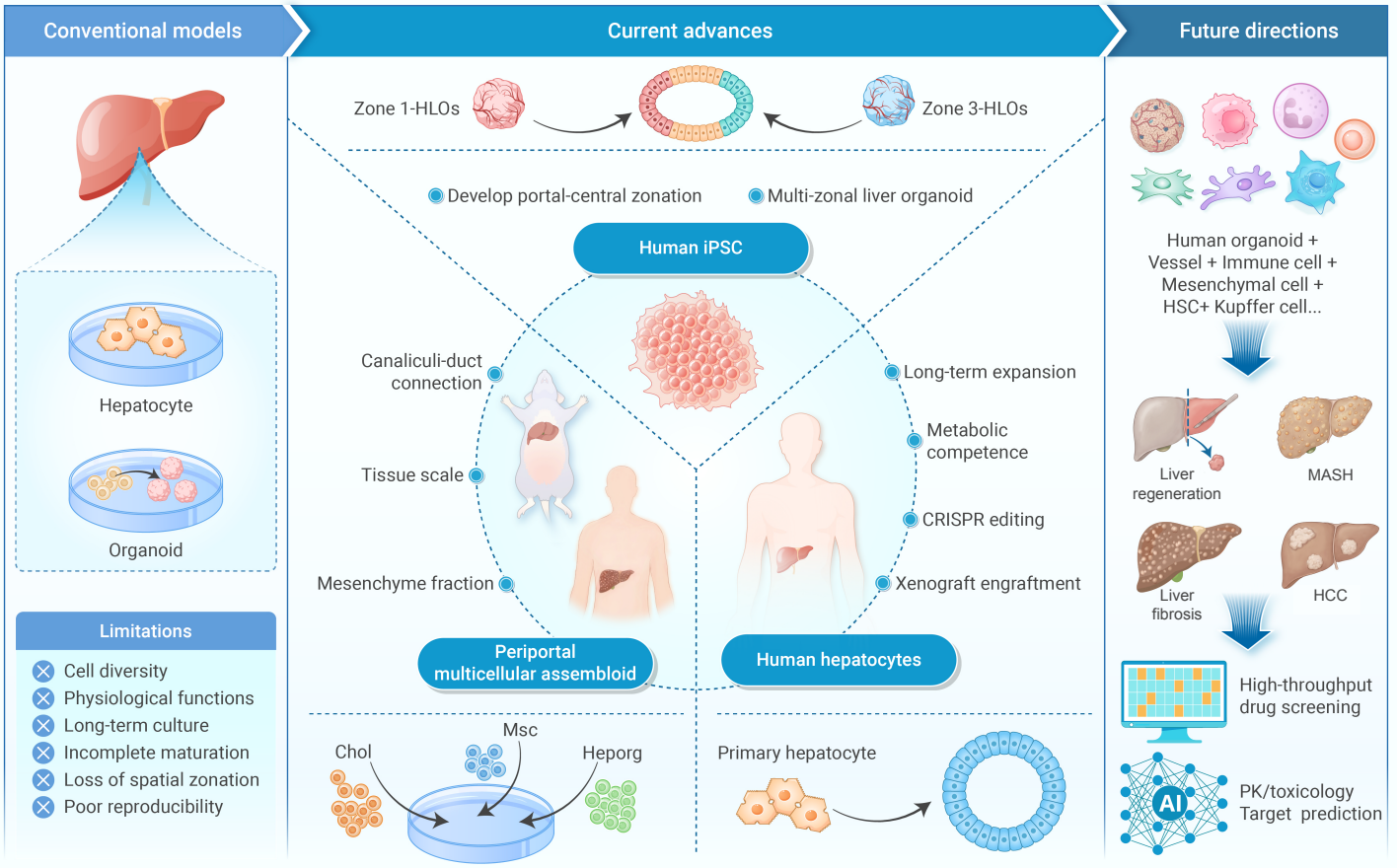

Figure 1. Evolution of liver organoid systems from conventional models to advanced platforms for mechanistic interrogation and translational applications Left: Conventional models are limited by poor cell diversity, incomplete maturation, loss of spatial zonation, and restricted long-term functional stability. Middle: Current advances include three representative liver organoid platforms, namely expandable human hepatocyte organoids, multi-zonal human iPSC-derived liver organoids, and periportal multicellular assembloids, each addressing distinct aspects of hepatic metabolic, spatial, and microenvironmental complexity. Right: Future directions are expected to further integrate vascular, stellate, Kupffer, mesenchymal, and immune components, together with scalable screening and AI-assisted analysis, to support disease modeling, pharmacokinetic and toxicology studies. Msc, mesenchymal cells; Chol, cholangiocytes; HepOrg, hepatocyte organoids; HSCs, hepatic stellate cells; MASH, metabolic dysfunction-associated steatohepatitis; HCC, hepatocellular carcinoma; AI, artificial intelligence; PK, pharmacokinetics.

tional hepatocyte and simple organoid models are summarized in Figure 1 (left panel). Despite these challenges of dedifferentiation, limited cell sourcing, and lack of spatial organization, the representative platforms of the three core functional capacities have led to great progress in overcoming these bottlenecks.[26-29]

RECONSTRUCTING HEPATIC SPATIAL METABOLISM: LIVER ORGANOIDS AS REGULATORY PLATFORMS

Expandable, metabolically competent human hepatocyte organoids: How STAT3 and Wnt signaling achieve balance

STAT3 and Wnt signaling play central roles in regulating proliferation and metabolic homeostasis in expandable hepatocyte organoids.[26] STAT3 mediates cytokine-driven control of proliferation, survival, and metabolic homeostasis, whereas Wnt signaling governs liver development, regeneration, stem cell maintenance, and hepatocyte fate.[30,31] However, despite the importance of these pathways in maintaining hepatocyte identity, precisely reconstructing their physiological balance *in vitro* within organoid systems remains a challenge. Pro-proliferative cues, such as Wnt or YAP activation, readily induce dedifferentiation or cholangiocytic conversion, whereas insufficient homeostasis-associated signaling, such as STAT3, limits expansion potential.[30,32] This dilemma has long hindered robust human hepatocyte expansion.

Early five-small-molecule strategies based on combined modulation of Wnt, Notch, BMP, and cAMP-related signaling transiently preserve primary human hepatocyte function but severely limit proliferation.[33] While organoid technologies have enabled the expansion of functional adult mouse hepatocytes and human fetal hepatic progenitors, successful long-term expansion of adult human hepatocytes remain limited.[18,19] Early studies showed that oncostatin M (OSM) activates STAT3 to promote proliferation while suppressing YAP-driven ductal transdifferentiation, preserving hepatocyte identity.[34] Building on this mechanism, Igarashi *et al.* recently developed a chemically refined expansion medium that incorporates OSM into a Wnt ligand-based formulation to activate STAT3.[26] This system enables long-term expansion of metabolically competent adult human hepatocyte organoids but it lacks spatial organization. Together, these findings highlight the need to balance Wnt-driven proliferation with lineage maintenance.

Upon withdrawal of expansion factors and supplementation with maturation-inducing hormones, adult human hepatocyte organoids reorganize into cord-like structures featuring bile canaliculi.[26] Early work demonstrated that hepatocyte organoids could perform key hepatic functions such as bile acid synthesis and urea production.[18,19,33] However, hepatocytes within adult human liver organoids exhibit broad metabolic functions, including bile acid, lipid, and glucose metabolism, urea cycling, CYP-mediated drug metabolism, and albumin secretion.[26] These features collectively reconstruct a metabolic profile that more closely resembles that of native adult liver tissue.

In summary, coordinated Wnt and STAT3 activation enables long-term expansion of adult human hepatocyte organoids while preserving identity and restoring near-physiological metabolic function.[26] Nonetheless, important limitations remain. Expansion capacity shows marked donor dependence, with fetal-derived hepatocytes capable of proliferating for more than 100 days, whereas hepatocytes from adult donors older than 20 years often cease proliferation after approximately 30 days.[26] In addition, although these systems can achieve extensive *in vitro* expansion, hepatocyte engraftment capacity progressively declines after prolonged expansion. Future efforts will

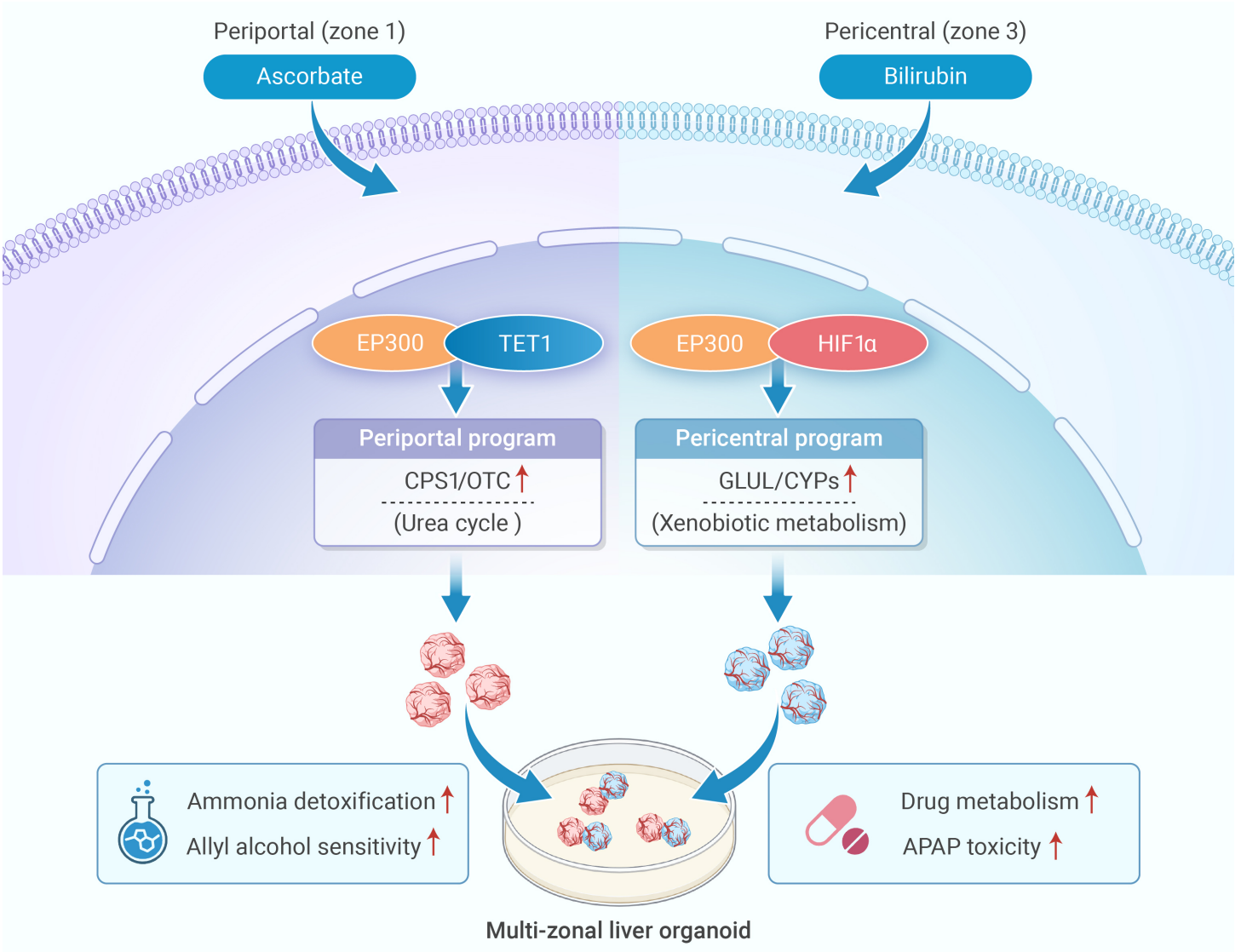

Figure 2. Endogenous metabolites act as instructive cues to specify zonal identity in liver organoids Ascorbate promotes periportal-like programs by facilitating EP300-TET1 interactions, leading to activation of urea cycle genes such as CPS1 and OTC. In contrast, bilirubin enhances EP300-HIF1α association, inducing pericentral-like functions including GLUL-mediated glutamine synthesis and CYP-dependent xenobiotic metabolism. These regulatory pathways establish spatially distinct gene expression programs that underlie zonated metabolic functions. Functionally, periportal-like regions exhibit enhanced ammonia detoxification and increased sensitivity to allyl alcohol, whereas pericentral-like regions display elevated drug metabolism capacity and heightened susceptibility to acetaminophen-induced toxicity. Together, these findings illustrate how integration of metabolic and oxygen-sensing signals through EP300-centered networks enables reconstruction of liver zonation *in vitro*.

likely integrate mature or patient-derived hepatocyte expansion systems with multicellular modules to achieve more physiologically relevant liver organoid models.

Multi-zonal human iPSC-derived liver organoids: encoding spatial metabolism through metabolite-driven zonation

The spatial division of labor segregates periportal and pericentral hepatic functions, a fundamental feature governing hepatic metabolism and drug response.[1] Accordingly, reconstructing metabolic zonation is both essential for mechanistic insights and a major challenge in liver organoid research.[21] Morphogen and oxygen gradients are important to maintain metabolic zonation.[3] However, conventional hepatocyte and organoid systems lack stable oxygen and morphogen gradients, preventing faithful recapitulation of *in vivo* zonated architecture and metabolic specialization.[35] Furthermore, the absence of integrated, perfusable vasculature further compromises gradient stability and physiological relevance.[20]

Interestingly, zonal fate specification can be driven by endogenous metabolites, with ascorbate promoting periportal-like identity and bilirubin inducing pericentral-like differentiation.[36,37] Using ascorbate and bilirubin as spatiotemporal cues, Al Reza *et al.* established a multi-zonal human iPSC-derived organoid platform generating both periportal- and pericentral-like domains.[27] Early studies have demonstrated that EP300 acts as a central transcriptional coactivator integrating metabolic and oxygen-sensing cues to regulate zonal gene expression.[38] Accordingly, ascorbate promotes EP300-TET1 interactions to activate periportal programs (e.g., CPS1, OTC), whereas bilirubin enhances EP300-HIF1α association, inducing pericentral functions such as GLUL-mediated glutamine synthesis and CYP-dependent metabolism.[27] The molecular cascade underlying metabolite-regulated zonation is summarized in Figure 2. These observations naturally raise the possibility that HIF signaling occupies a central position in coupling oxygen availability to zonal metabolic identity.[39] While HIF activity is well established as a transcriptional responder to hepatic oxygen gradients,[40] whether it also exerts an instructive role in specifying or stabilizing zonal programs remains unresolved. Multi-zonal organoids offer a unique platform to address this question by enabling independent, spatial control of oxygen and metabolic cues.

Conventional organoids typically recapitulate only a single zonal profile and fail to capture periportal-pericentral metabolic heterogeneity.[41] Using metabolically zonated human organoids, Al Reza *et al.* not only reproduced compartmentalized metabolic functions across spatial domains, but also revealed zone-specific susceptibility to hepatotoxic compounds that faith-

fully recapitulated *in vivo* zonated toxicity patterns, with periportal-like regions being more sensitive to allyl alcohol and pericentral-like regions preferentially injured by acetaminophen in this zonated organoid system,[27] which phenocopies the characters of these two hepatotoxins *in vivo*.[42] This zonated toxicity reflects region-specific metabolism, with CYP2E1-enriched pericentral hepatocytes mediating acetaminophen toxicity, whereas allyl alcohol toxicity depends on alcohol dehydrogenase-mediated metabolism and preferentially causes periportal injury.[43,44] Quantitative comparisons with single-zonation organoids show that multi-zonated organoids exhibit higher ammonia clearance and urea production than pericentral-like organoids, while retaining glutamine synthesis absent in periportal-like organoids.[27] However, despite the preservation of zonal features in short-term culture and after transplantation,[27] the long-term stability and scalability of hepatic zonation during passaging remain insufficiently characterized.

Conceptually, advances in human multi-zonal organoids represent not only breakthroughs in key technologies but also signify the evolution of organoid research toward a new paradigm of spatial engineering, aligning closely with the core design principles of metabolic bionics.[45] However, several limitations of the iPSC-derived organoid model remain, including incomplete metabolic maturation and suboptimal mitochondrial function in human iPSC-derived hepatocytes.[27,46] Although multi-zonal organoids advance the field from developmental mimicry toward physiological reconstruction, incorporation of vascular and immune components remains essential for modeling spatially organized hepatic regulation in a physiologically relevant context. Addressing this structural gap, recent micropatterned human pluripotent stem cell-derived gastruloid systems demonstrated that vascular-inducing signaling cues can drive the formation of spatially organized and branched vascular networks within hepatic organoids, suggesting that conserved embryonic vascularization programs may be harnessed to generate more physiologically vascularized liver organoid models.[47] In addition to vascular architecture, emerging evidence suggests that biomechanical cues such as fluid shear stress generated by sinusoidal blood flow represents an additional layer of regulation that may contribute to hepatic zonation.[48] In this context, microfluidic organ-on-a-chip platforms provide a powerful approach to precisely control fluid dynamics, including shear stress and oxygen gradients, thereby offering an additional dimension for reconstructing physiologically relevant zonation.[49] Integration of these biomechanical cues with metabolite-driven strategies may enable more faithful recapitulation of *in vivo* hepatic zonation and improve the predictive performance of liver organoid systems.

Periportal multicellular assembloids: Reconstructing periportal niches through stromal-epithelial crosstalk

Current liver organoid models are predominantly composed of a single cell type, typically hepatocytes or cholangiocytes.[50] Although valuable for cell expansion and basic functional studies, the lack of portal fibroblasts and other stromal components limits reconstruction of the periportal microenvironment. For example, although Liu *et al.* established metabolically competent adult-like hepatocyte organoids, their monocellular design precluded modeling early events such as cholangiocyte injury and stromal activation.[51] Such limitations underscore the need for multicellular liver organoids capable of capturing the complex microenvironmental dynamics underlying cholestatic injury and fibrogenesis.[23]

Recent advances have shifted liver organoid research from hepatocellular monocultures toward modular multicellular systems that recapitulate periportal niches. Dowbaj *et al.* developed a two-step assembly of hepatocyte and cholangiocyte organoids with portal fibroblasts isolated from adult mouse liver, enabling reconstruction of a periportal-like microenvironment and providing a framework for studying stromal-epithelial interactions and modeling cholestatic injury and biliary fibrosis.[28] Similarly, Yuan *et al.* extended the multicellular assembloid strategy to human systems by incorporating patient-derived primary hepatocytes together with cholangiocytes and portal fibroblasts, with all three cellular components preferentially obtained from the same donor to preserve cellular compatibility, thereby more faithfully reconstructing human periportal liver pathophysiology.[29] More generally, non-parenchymal components in multicellular liver organoid systems are derived from multiple sources, including primary tissue isolation, pluripotent stem cell differentiation, and commercially available cell types, each differing in physiological relevance, scalability, and standardization.[52,53] The reliance on patient-derived human materials requires clear documentation of both parenchymal and non-parenchymal cell sources, typically obtained from surgical or biopsy specimens under ethical approval, but also introduces constraints in tissue accessibility, donor matching, and standardization.

Variations in fibroblast abundance or activation state have been shown to influence ductular expansion, highlighting stromal composition as a key regulator of epithelial plasticity.[54] In earlier studies, Islam *et al.* treated human cholangiocyte organoids with high concentrations of bile acids to simulate cholestatic injury.[55] By assembling parenchymal and non-parenchymal cells at physiological ratios, Dowbaj *et al.* and Yuan *et al.* reconstructed bile canaliculi-duct connectivity in mouse and human assembloids, in which increased portal fibroblast abundance induced cholestatic phenotypes and recapitulated selected features of biliary fibrosis.[28,29] These systems provide a tractable platform to investigate stromal-epithelial interactions and fibrogenic responses associated with biliary injury. Nevertheless, several physiological components remain missing from the periportal multicellular assembloids. The assembloid lacks key non-parenchymal cell types, including hepatic stellate cells, sinusoidal endothelial cells, and resident immune cells, all of which modulate hepatocyte metabolism and stress responses.[56] Notably, the omission of Kupffer cells limits modeling of immune-driven hepatotoxicity, given their central role[57] in inflammatory responses to drugs and toxins. Similarly, stellate cell-driven activation and immune responses central to fibrotic diseases such as MASH[58] are not captured. Portal fibroblasts largely mediate periductal scarring typical of cholangiopathies, whereas stellate cells orchestrate the diffuse fibrogenic response in metabolic and toxic injuries.[59] Integrating stellate cells and Kupffer cells will be essential to bridge periportal and lobular fibrogenic mechanisms.

FROM DIVERSE PLATFORMS TO A UNIFYING TWO-STEP DECOUPLING STRATEGY

Although each platform emphasizes distinct design priorities, current studies suggest complementary strengths across different aspects of liver physiology.[26-29] Expandable human hepatocyte organoids exhibit robust metabolic activity and high genetic accessibility, making them suitable for studies of drug metabolism and gene regulation.[26] Multi-zonal human iPSC-derived liver organoids recapitulate periportal-pericentral patterning and enable investigation of zone-specific metabolism and toxic responses.[27] Periportal multicellular assembloids incorporating stromal components have been applied to explore matrix induction, ductular reactions, and early fibrogenic signaling.[28,29] Collectively, the three representative liver organoid platforms capture different dimensions of hepatic metabolic, structural, and spatial organization, and may, to some extent, complement one another (Figure 1, middle panel; Table 1). In principle, integrating key features from these representative platforms could broaden *in vitro* applications while preserving essential aspects of liver biology.

Despite substantial progress, liver organoid research remains constrained by pronounced methodological heterogeneity across laboratories. Divergent growth factor combinations, staging schemes, and culture conditions have generated protocols that are effective locally but difficult to compare, optimize, or standardize.[10] This lack of coherence hampers the development of controllable, reproducible, and regulatory-grade organoid platforms.

Against this backdrop, a central challenge is the trade-off between large-scale expansion and maintenance of hepatic functionality.[60] Protocols favoring rapid proliferation often lead to dedifferentiation and metabolic decline, whereas early maturation strategies yield limited cell numbers inadequate for biochemical or pharmacological applications.[50,61] Inspired by the unifying methodological theme across the four recent representative studies[26-29] and many other studies that developed organoids,[51,62,63] we here conceptualize this recurring pattern as a "two-step expansion-differentiation decoupling strategy," a shared methodological framework that systematically separates proliferative expansion from functional maturation, which we believe could help resolve the inherent trade-off between expansion and differentiation. Rather than referring to a newly introduced experimental protocol, this framework summarizes a recurring and commonly adopted underlying culture logic across liver organoid systems derived from different hepatocyte sources. By

Table 1. Comparative features and functional attributes of advanced liver organoid models

	Human hepatocyte organoids	Periportal assembloids		Multi-zonal liver organoids
Species	Human	Mouse	Human	Human
Composition	Hepatocytes	Hepatocytes, Cholangiocytes, Portal mesenchymal cells		Hepatocytes
Zonation	No	Limited		Yes
Expansion Medium	EGF, HGF, FGF10, Wnt/R-spondin, A83-01, Noggin, OSM, IL-6, Forskolin	EGF, HGF, FGF10, FGF7, Wnt/R-spondin, A83-01, Gastrin, Nicotinamide, CHIR9902, Y-27632	EGF, HGF, WntS, TRULI , Y-27632, Nicotinamide	Not required (post-differentiation zonation)
Differentiation Medium	GH, prolactin, cortisol, DAPT	WNT3a-conditioned medium, Y-27632	Added: Dexamethasone Excluded: YAP activator, FGFR2 activator	Ascorbate (Zone 1); Bilirubin (Zone 3)
Structural features	Cord-like hepatocyte organization; bile canaliculi networks	Periportal-like architecture; canaliculi-bile duct connectivity; mesenchyme-supported bile ducts		Single organoid contains Zones 1-3
Functional validation	Long-term expansion; metabolic functions; liver repopulation; CRISPR compatibility	Canaliculi to ducts bile drainage; fibrosis-like phenotype		Zone-specific functions; transplantation rescue in bile duct-ligated rats
Main innovations	Long-term expansion of adult PHHs with retained metabolic competence; compatibility with gene editing and xenotransplantation	Recreation of periportal multicellular interactions and canaliculi-duct connectivity; fibrosis modeling via mesenchyme ratio tuning		Reproducible *in-vitro* human liver model with periportal-pericentral zonation
Limitations	Donor age dependence; passaging-associated decline; long-term genomic stability undefined	Absence of immune and vascular cells; restricted to periportal zone		Incomplete maturation; limited long-term function maintenance
Optimal application scenarios	Drug metabolism; PK/Tox assays; genetic liver disease modeling	Cholestatic disease modeling; periportal fibrogenic response modeling; canaliculus-duct interaction analysis		Hepatic zonation research; zone-specific toxicity testing

EGF, epidermal growth factor; HGF, hepatocyte growth factor; FGF, fibroblast growth factor; OSM, oncostatin M; IL-6, interleukin 6; GH, growth hormone; PK, pharmacokinetics; Tox, Toxicology.

formalizing this commonly used but previously non-standardized practice into a theoretical framework, we aim to facilitate understanding of the shared methodological logic underlying inter-model variations and functional optimization in liver organoid cultures (Figure 3A). Similar staged expansion-maturation strategies have also been observed in multiple non-hepatic organoid systems,[64–66] suggesting that the methodological logic may have broader relevance for organoid engineering across different tissues.

The two-step expansion-differentiation decoupling strategy is employed in two consecutive and separate phases (Figure 3A). In the first phase, proliferative capacity is maximized under defined culture conditions that suppress ductal metaplasia and lineage drift, maintaining a stable progenitor-like state. During this stage, pathways associated with proliferation and progenitor maintenance, including Wnt signaling and growth factor-dependent cues, are activated to sustain cell expansion and prevent premature differentiation. In the second phase, proliferation-associated signals are attenuated, while carefully titrated hormonal and metabolic cues, such as glucocorticoids and thyroid hormones, are introduced to activate transcriptional programs characteristic of mature hepatocytes. By decoupling expansion from maturation, the strategy reconciles scalability with functional fidelity, generating organoid biomass suitable for biochemical, pharmacological, and genetic assays. Importantly, this temporal separation enables phase-specific modulation of upstream regulatory inputs, thereby providing an experimental framework for systematically dissecting how distinct upstream regulatory programs govern hepatocyte identity, functional maturation, and metabolic specialization across different stages of organoid development.

Interestingly, the two-step decoupling principle represents a broadly applicable regenerative logic across multiple liver organoid sources, including pluripotent stem cells, adult stem cells, and primary hepatocytes (Figure 3B). Although often described as multistage differentiation schemes, these protocols largely follow the same underlying methodological logic.[10] For instance, the differentiation of iPSCs into hepatocytes is typically divided into three stages, definitive endoderm induction, hepatic progenitor/hepatoblast specification, and final maturation.[67] Functionally, however, the first two stages together represent an expansion phase that establishes proliferative progenitor-like intermediates, while the final step corresponds to the differentiation phase. The same expansion-maturation paradigm underlies other cellular sources as well. Embryonic stem cell-derived liver organoids similarly undergo progenitor expansion followed by hormone- and metabolism-driven maturation.[68,69] Adult stem cell-derived liver organoids are established from $Lgr5^{+}$ progenitors and are initially expanded under progenitor-supportive conditions that sustain epithelial proliferation and suppress differentiation, most prominently through Wnt pathway activation.[17] Organoids derived from primary hepatocytes follow a distinct yet conceptually related route, involving transient dedifferentiation into a progenitor-like state followed by hormone-driven redifferentiation to restore mature hepatic functions.[18,51]

Importantly, the two-step expansion-differentiation decoupling strategy is currently applied primarily to enable scalable expansion and functional maturation of hepatocytes, thereby addressing the limited availability of functional hepatocyte populations. Although multicellular assembloid organoids contain multiple cell types, they are generally established by first expanding and maturing hepatocytes or hepatic progenitor cells, followed by controlled assembly with non-parenchymal components.[28,29] In contrast, hepatic non-parenchymal cell types, including cholangiocytes, hepatic stellate cells, and liver sinusoidal endothelial cells, are typically generated through directed differentiation protocols without a dedicated expansion-maturation framework.[70] Until now, it is still less explored whether the two-step decoupling strategy is applicable to generating non-parenchymal cell components.

Beyond technical optimization, the two-step expansion-differentiation

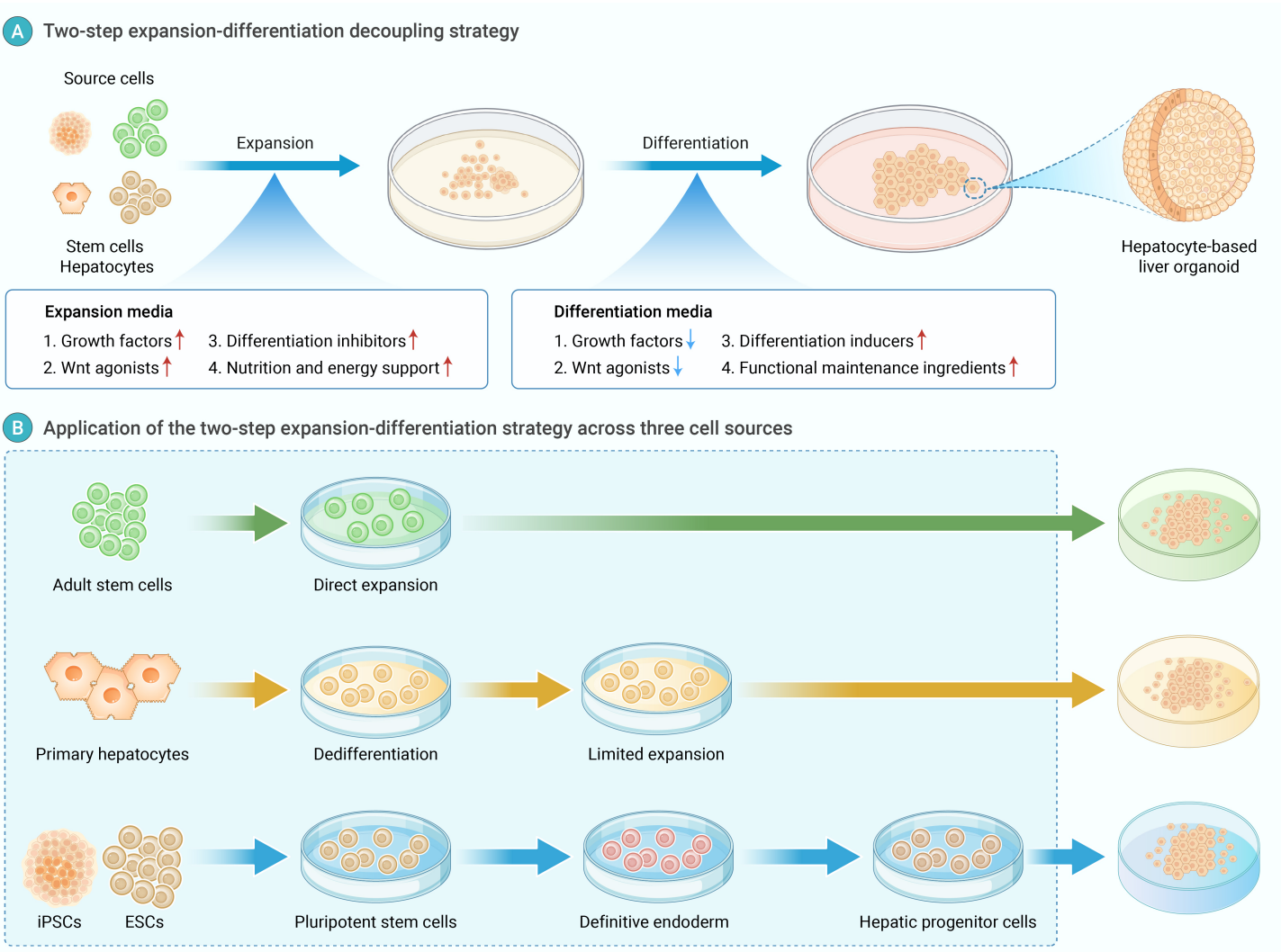

Figure 3. Two-step expansion-differentiation decoupling strategy and its applicability across multiple cell sources (A) Schematic illustration of the two-step expansion-differentiation decoupling strategy, which defines a conceptual framework in which proliferative expansion and functional maturation are temporally separated through stage-specific culture conditions. (B) Application of this framework across distinct cell sources, including adult stem cells, primary hepatocytes, and pluripotent stem cells. The expansion phase maximizes cell proliferation under defined conditions, whereas the differentiation phase introduces cues that activate hepatocyte-specific transcriptional programs. Despite differences in lineage origin and intermediate states, these systems follow the same operational principle, in which expansion precedes differentiation, enabling scalable generation of functional hepatocyte-based organoids. iPSc, induced pluripotent stem cell; ESCs, embryonic stem cells.

strategy also enhances physiologically relevant hepatic functions.[71] Organoid systems incorporating stage-specific metabolic and hormonal cues reconstitute coordinated hepatic functional programs that are largely absent in earlier models.[63] Although direct comparison across different organoid systems remains limited by variations in cell source, culture conditions, and assay systems, representative studies consistently support the advantages of temporally separating expansion and differentiation. Representative functional features and reported performance metrics are summarized in Table S1.

In summary, the two-step expansion-differentiation strategy provides a coherent and increasingly standardized framework for generating scalable yet physiologically informative liver organoids (Figure 3), thereby supporting the development of experimentally tractable and mechanistically grounded liver organoid platforms.

BEYOND FUNCTIONAL OUTPUTS: RESTORING UPSTREAM REGULATORY HIERARCHIES TO ACHIEVE MECHANISTIC FIDELITY

Currently, liver organoid performance is primarily assessed using downstream metabolic outputs, such as glucose, lipid, and drug metabolism, as proxies for maturity and physiological relevance.[51,72,73] However, these readouts provide only a partial view of hepatocyte function and do not necessarily reflect whether the underlying regulatory mechanisms governing hepatocyte identity and metabolic coordination have been faithfully reconstructed.[74] Hepatocyte function depends on coordinated programs controlling lineage identity, metabolic sensing, and functional specialization in response to nutrients, xenobiotics, and injury.[75] Therefore, recapitulating liver pathophysiology *in vitro* requires more than restoring a limited set of metabolic outputs; it also requires reconstruction of the upstream regulatory architecture that governs these functions. Recent advances in organoid engineering now support a shift from descriptive downstream readouts toward systems-level reconstruction of upstream regulatory control.[76] However, the extent to which upstream regulatory programs govern organoid formation, functional maturation, and metabolic organization remains largely unexplored, representing an important direction for future research. In this section, we outline technical directions for developing mechanistically informed liver organoid platforms, with a focus on restoring upstream regulatory architecture, including cell fate specification regulators, cell identity stabilization regulators, and metabolic sensing and functional specialization regulators, to support functional maintenance and standardized quality control.

The ultimate test of a liver organoid system lies in the ability to recapitulate human liver pathophysiology. Although hepatocyte functions are governed by coordinated regulatory programs controlling lineage identity,

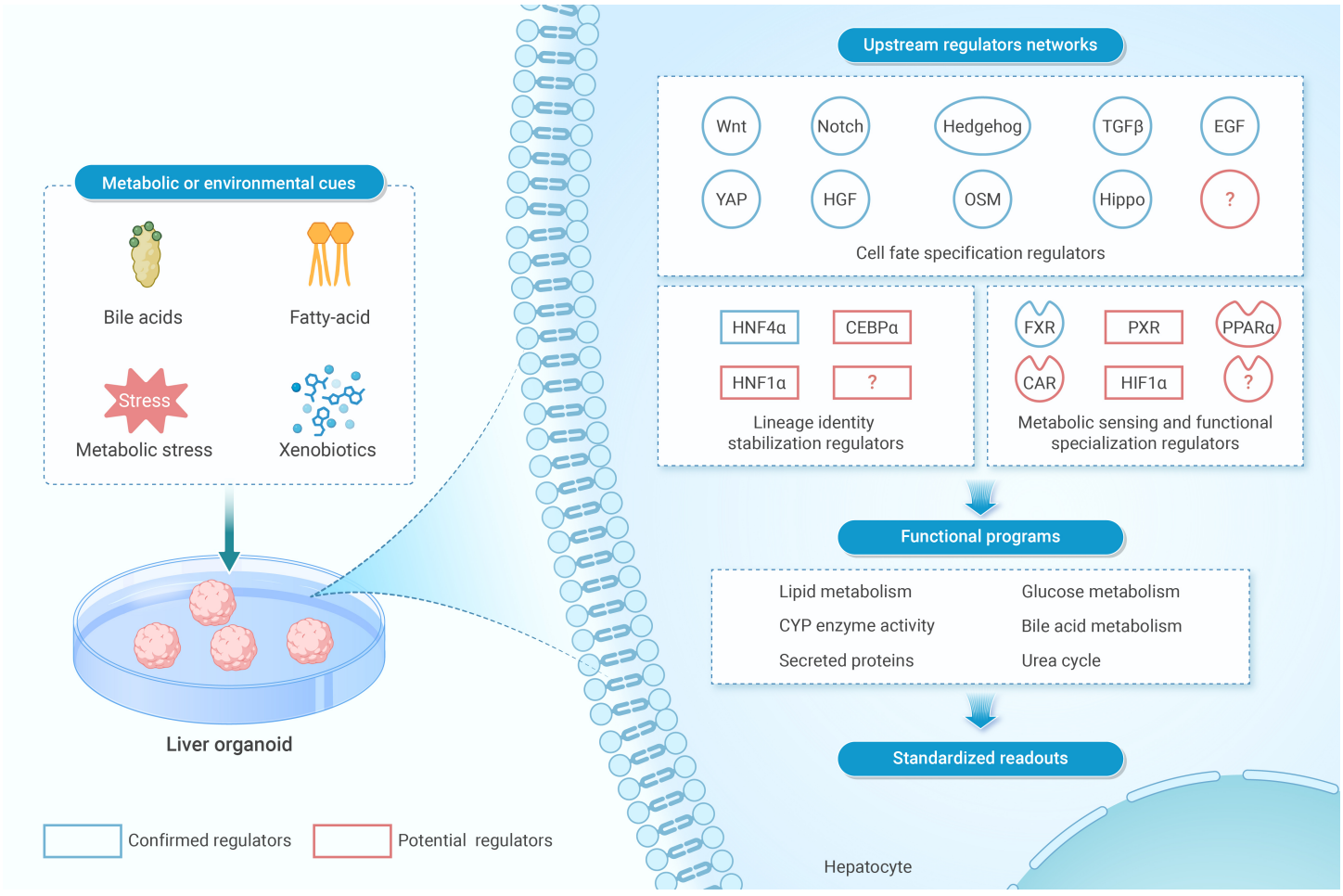

Figure 4. Hierarchical framework for decoding upstream regulatory networks governing hepatic metabolic states in liver organoids This schematic illustrates how metabolic and environmental cues (left) are interpreted through multilayered upstream regulatory networks to establish hepatocyte functional programs and standardized readouts (right). Within this framework, upstream regulators are organized into three functional layers. Cell fate specification regulators, including developmental signaling pathways (e.g., Wnt, Notch, Hedgehog, TGFβ, and Hippo), are well established and commonly incorporated into organoid systems. Lineage identity stabilization regulators (e.g., HNF4α, HNF1α, and CEBPα) maintain hepatocyte-specific transcriptional programs. Metabolic sensing and functional specialization regulators, including nuclear receptor networks (e.g., FXR, PXR, CAR, and PPARα) and oxygen/metabolic sensors such as HIF1α, integrate environmental and metabolic signals to shape hepatic functions. Together, these regulatory layers control lipid and glucose metabolism, bile acid homeostasis, xenobiotic metabolism, and protein secretion, which can be captured as standardized readouts. Regulators highlighted in blue have been experimentally validated in liver organoid systems, whereas those outlined in orange represent putative regulators with established roles in liver biology but lacking direct organoid validation. Question marks indicate unidentified components within the current regulatory network.

metabolic specialization, and environmental responsiveness,[77-79] how these upstream regulatory mechanisms collectively shape spatial organization and metabolic complexity in liver organoids remains largely unexplored. Current organoid systems can partially restore downstream hepatic functions,[51] and some platforms have begun to reconstruct spatially organized metabolic features.[27] However, restoration of these downstream outputs does not necessarily indicate that the upstream regulatory programs governing hepatocyte identity, metabolic specialization, and environmental responsiveness have been fully reconstructed. Indeed, many organoid models still exhibit unstable metabolic responsiveness, incomplete bile acid metabolism, and only partially maintained lineage identity programs.[70,72] These observations suggest that improvements in downstream metabolic outputs may occur independently of full reconstruction of upstream regulatory architecture, highlighting the limitations of commonly used functional assays in evaluating mechanistic fidelity. Importantly, cell fate specification regulators, including developmental pathways such as Wnt, Notch, and Hippo, are already routinely manipulated to steer hepatocyte differentiation, polarity, and maturation in liver organoid systems,[17,18] demonstrating that upstream regulatory modules are experimentally accessible and may therefore serve as mechanistically informative readouts beyond conventional metabolic assays.

From this perspective, next-generation liver organoid evaluation may extend beyond downstream functional readouts to encompass the full upstream regulatory hierarchy (Figure 4). Rather than a simple collection of regulators, this architecture consists of multiple interconnected but functionally distinct layers that collectively determine hepatocyte fate, identity, and functional output. Notably, cell fate specification regulators, including developmental signaling pathways (e.g., Wnt, Notch, and Hippo), could control lineage commitment and early differentiation.[80-82] Lineage identity stabilization regulators, including HNF1, HNF4, and CEBP family members, could maintain hepatocyte-specific transcriptional programs.[83-86] Metabolic sensing and functional specialization regulators, including nuclear receptor networks (e.g., FXR, PPAR, PXR, AHR, CAR, LXR/LRH, RXR, RAR, and SHP families), and HIF signaling, could integrate environmental and metabolic cues to shape functional outputs.[87-90] Importantly, the upstream regulatory layers do not form a strictly linear hierarchy, but instead operate in a coordinated and interdependent manner as an integrated regulatory network.[91] Here, we propose a conceptual perspective shift that redefines the evaluation of liver organoids from isolated functional outputs to the extent of coordinated reconstruction of upstream regulatory layers. While developmental specification pathways are routinely incorporated into current liver organoid systems,[26-29] the coordinated reconstruction of lineage identity and metabolic sensing layers remains incomplete, highlighting a key gap in the liver organoid field.

Lineage identity stabilization regulators represent a key upstream layer that maintains hepatocyte identity, and recent liver organoid studies have provided direct functional evidence supporting their role in preserving hepatocyte-specific transcriptional programs and maturation states. In human liver organoids, forced expression of *HNF4α* is sufficient to preserve hepato-

cyte-specific transcriptional programs and liver functions under inflammatory stress while suppressing the activation of alternative transcriptional states.[92] CRISPR screening platforms based on adult stem cell-derived liver organoids have enabled systematic identification of lineage determinants, followed by functional validation demonstrating that regulators such as FOS and UBR5 directly control hepatocyte maturation, with *Fos* knockout promoting and *Ubr5* knockout delaying maturation in organoid systems.[93] Consistent with these findings, immature or stressed liver organoids exhibit reduced activity of lineage stability regulators, which is associated with impaired metabolic regulation and progressive dedifferentiation.[94] Collectively, these findings highlight lineage identity regulators, particularly transcription factors, as key candidates for future investigation to define causal regulatory circuits and to enable targeted manipulation of hepatocyte identity and maturation in liver organoid systems.

Complementing identity stabilization, metabolic sensing and functional specialization regulators constitute another critical layer of upstream control, acting as transcriptional hubs that integrate metabolic, xenobiotic, and inflammatory cues.[4,95] In multicellular liver organoids recapitulating MASLD-like phenotypes, the FXR agonist obeticholic acid functionally activates FXR, thereby attenuating oxidative stress and fibrogenic phenotypes.[96] In parallel, the bile acid derivative tauro-obeticholic acid activates FXR signaling in iPSC-derived liver organoids, thereby sustaining long-term expansion and hepatic identity through regulation of downstream targets such as SHP.[72] Furthermore, YAP is a key regulator of cell fate and lineage identity, as it promotes biliary differentiation of hepatic progenitor cells during development and drives hepatocyte-to-cholangiocyte transdifferentiation under pathological conditions.[81,97] YAP-mediated repression of FXR signaling in liver organoids results in bile acid accumulation, which can be reversed by treatment with FXR agonists such as GW4064 and obeticholic acid.[98] This regulatory relationship indicates that the cell fate and lineage regulator YAP modulates the metabolic sensing and functional specialization regulator FXR, highlighting coordinated crosstalk among cell fate, lineage regulation, and metabolic specialization programs. Such causal regulation further demonstrates that perturbation of upstream metabolic regulators can directly shape downstream metabolic outputs, underscoring the importance of upstream regulatory nodes in governing functional readouts and quality control in liver organoid systems.

In the context of hepatic metabolic control, additional metabolic sensing and functional specialization regulators can be considered beyond FXR. For example, PXR primarily regulates xenobiotic metabolism through control of drug-metabolizing enzymes and drug transporters, CAR governs detoxification responses and chemical-induced metabolic adaptation, and PPARα regulates fatty acid β-oxidation and lipid metabolism.[99-101] Collectively, these regulators represent key nodes linking environmental and metabolic cues to hepatocyte functional specialization. However, direct functional validation of these transcription factors in liver organoid systems remains limited and warrants further investigation.

Together, cell fate specification regulators, lineage identity stabilization regulators, and metabolic sensing and functional specialization regulators constitute an integrated upstream regulatory architecture that more accurately reflects hepatocyte functional maturity than downstream metabolic assays alone. To implement upstream regulatory network reconstruction as a standardized readout, future studies may establish a defined upstream regulatory panel rather than relying solely on conventional functional assays such as albumin secretion, CYP activity, or urea production. Such a framework should consider whether developmental programs are appropriately activated or suppressed, whether hepatocyte identity-maintaining programs remain stable, and whether metabolic sensing programs, such as FXR-, PPAR-, PXR-, or HIF-associated pathways, can respond appropriately to bile acids, lipids, xenobiotics, or oxygen-related cues. Importantly, the goal should not be limited to evaluating individual genes, but rather to assessing coordinated regulatory target groups and their collective reconstruction in comparison with corresponding physiological or disease-associated liver states.

FROM CHRONIC LIVER DISEASES TO CANCER: LIVER ORGANOIDS AS PLATFORMS FOR DECODING DISEASE-SPECIFIC REGULATION

Improved culture systems and functional maturation have expanded the capacity of liver organoids to model pathological features, shifting research from descriptive phenotyping toward mechanistic dissection of disease-specific metabolism. Here, we synthesize recent advances in liver organoid disease modeling (Table S2) to evaluate how existing platforms enable causal interrogation of pathogenic metabolic programs, and discuss limitations and future directions for establishing liver organoids as discovery platforms.

MASH

MASH results from metabolic dysregulation and non-parenchymal signaling, driving organoid studies from descriptive lipid-loading to hierarchical regulation.[102-104] Early hepatocyte-only organoid models recapitulated steatosis and mitochondrial dysfunction upon fatty acid exposure,[105,106] but lacked inflammatory and fibrotic components. Subsequent studies showed that specific lipid species induce distinct stress responses, with palmitic acid triggering lipotoxic and fibrogenic programs, whereas oleic acid promotes lipid accumulation with limited fibrogenic activation.[107]

To capture the roles of hepatic non-parenchymal cells in modulating MASH, reconstructing liver organoids harboring multicellular complexity is essential. Upon free fatty acid exposure, these multicellular organoids recapitulate steatosis, inflammation, and fibrosis in a sequential manner, exhibit increased tissue stiffness correlating with fibrosis severity, and, in lysosomal acid lipase-deficient patient-derived organoids, reproduce severe steatohepatitis phenotypes that can be alleviated by FXR agonist treatment.[96] Complementary models composed of hepatocyte and cholangiocyte lineages reproduce bile canaliculi architecture and show disruption of canalicular networks and induction of ductular reactions upon fatty acid exposure, recapitulating key features of human MASH pathology.[106] Despite these advances, current organoid systems remain limited in capturing zonated metabolism and long-term disease progression. Bioengineered platforms such as liver-on-chip systems may further enable chronic exposure and controlled perturbation in future studies.[108,109] Future work may require multi-organ microphysiological systems to model gut-liver axis interactions[110] or other interorgan crosstalk.

Alcohol-associated steatohepatitis (ASH)

ASH is driven by ethanol metabolism-derived acetaldehyde and ROS, triggering steatosis, mitochondrial dysfunction, and inflammation.[111] In human ESC-derived expandable hepatic organoids, ethanol exposure induces dose-dependent metabolic changes, oxidative stress, and activation of alcohol-metabolizing enzymes such as CYP2E1 and CYP3A4.[112] In human hepatic 3D organoids, CYP2E1 has been identified as a key driver of mitochondrial dysfunction and stress-responsive retrograde signaling that promotes steatogenesis, and this causal metabolic circuit is validated by CYP2E1 knockdown or inhibition of downstream calcineurin and DRP1 pathways.[113]

Notably, most hepatocyte-only organoid models primarily recapitulate metabolic features such as steatosis and oxidative stress, but are limited in modeling inflammatory and fibrogenic processes due to the absence of non-parenchymal components.[16] In contrast, human multicellular organoid systems incorporating hepatocytes together with non-parenchymal cell populations derived from stromal/endodermal progenitors exhibit enhanced functional maturation, as reflected by increased expression of alcohol-metabolizing enzymes (ADH1B and ALDH1B1), oxidative stress responses, and extracellular matrix deposition, and upon ethanol exposure recapitulate key features of alcohol-associated liver disease, including steatosis, inflammation, and fibrosis-like phenotypes.[114] Despite these advances, current organoid models mainly rely on acute ethanol exposure paradigms, which constrain their ability to model chronic disease progression and sustained inflammatory and fibrogenic signaling. Developing models that capture long-term ethanol-induced pathology therefore remains an important direction for future studies.

DILI

DILI modeling remains a challenge due to the inability of conventional systems to capture human-specific metabolism and patient susceptibility.[115] Human iPSC-derived liver organoids have been applied to high-throughput DILI risk assessment, where organoids cultured in 384-well formats enable measurement of drug-specific cytotoxicity (IC50) and distinct morphology-

based injury phenotypes, while integration into liver-on-chip systems increases albumin secretion, CYP expression, and alanine aminotransferase/aspartate aminotransferase (ALT/AST) levels and allows prediction of clinically observed synergistic hepatotoxicity, such as the tenofovir-inarigivir interaction.[116] Microfluidic liver-on-chip platforms further extend this capability by maintaining organoid viability and liver-specific functions for up to 28 days, sustaining CYP activity, and enabling detection of hepatotoxicity at micromolar drug concentrations, including acetaminophen and fialuridine, under prolonged exposure conditions.[117]

Beyond acute injury, human liver organoids recapitulate multiple DILI-associated phenotypes, including steatosis, fibrosis, and immune responses, with drug-induced changes showing concordance with clinical observations, and additionally support anti-fibrotic drug screening against chemically induced fibrogenesis.[118] In parallel, amino acid-based metabolic profiling in human hepatic organoids provides a non-destructive and quantitative readout for hepatotoxicity, distinguishing hepatotoxic from non-hepatotoxic compounds.[119] Together, human liver organoid models of DILI are emerging as mechanistic platforms for studying drug-induced hepatic injury in precision hepatology.

Infectious liver diseases

Liver organoids support sustained viral infection in a human-relevant context by maintaining hepatic function and donor-specific genetic backgrounds. In human iPSC-derived liver organoids, HBV infection can be maintained over extended periods, producing infectious virus and inducing hepatic dysfunction, including downregulation of hepatic gene expression, release of acute liver failure markers, and ultrastructural alterations.[120] Primary human liver organoids similarly support the complete HBV life cycle, including covalently closed circular DNA formation, viral antigen production, and release of infectious particles, and can be used for antiviral drug testing and toxicity assessment.[121] Transcriptomic analysis of HBV-infected patient-derived organoids from cirrhotic liver explants reveals an early oncogenic gene signature that clusters with hepatocellular carcinoma datasets, indicating that chronic viral infection drives tumor-associated transcriptional programs.[121]

Organoid systems incorporating immune components further enable functional interrogation of antiviral responses. In a microfluidic coculture platform, HLA-matched $CD8^+$ T cells specifically recognize viral antigens and induce targeted killing of infected liver organoids.[122] In parallel, perturbation of antigen presentation through tapasin knockdown in dendritic cells suppresses $CD8^+$ T cell proliferation and impairs antiviral cytotoxic responses in HBV-infected organoid systems, demonstrating the role of host immune regulation in viral clearance.[123] Beyond the liver, multilineage iPSC-derived organoids reveal the tissue tropism of HEV infection, with productive viral replication observed in liver, intestinal, and brain organoids, accompanied by tissue-specific functional impairment and inflammatory responses.[124] Collectively, viral and immune-integrated liver organoid models extend the field beyond passive infection systems toward experimentally tractable platforms for studying human-specific host-pathogen interactions in precision hepatology.

Primary liver cancer

Primary liver cancers, including HCC and cholangiocarcinoma (CCA), exhibit marked interpatient and intratumoral heterogeneity.[125] Broutier *et al.* established organoids derived from patient samples of primary liver cancer subtypes and demonstrated that these cultures faithfully preserve the histological architecture, gene expression programs, and genomic landscape of the parental tumors, providing a robust platform to interrogate tumor-intrinsic regulatory programs.[126] Nuciforo *et al.* further showed that organoids derived from tumor needle biopsies retain interpatient heterogeneity, enabling the study of patient-specific tumor states even in advanced disease settings where surgical specimens are not available.[127] Human primary liver cancer organoid lines from spatially distinct regions have revealed profound functional heterogeneity uncoupled from genomic variation, suggesting that therapeutic sensitivity is governed more by cellular state than mutational profiles.[128] In addition, genome-wide CRISPR screening in patient-derived HCC organoids has enabled systematic identification of survival-essential genes, revealing spliceosome components such as SF3B4 as key regulators of tumor progression, thereby providing a functional framework to dissect gene dependencies and therapeutic vulnerabilities in liver cancer.[129]

Engineered models provide additional systems to dissect early oncogenic events. In human liver organoids, CRISPR/Cas9-mediated BAP1 loss disrupts chromatin accessibility and downregulates junctional and cytoskeletal programs, leading to loss of epithelial identity and increased cellular motility; in a background carrying TP53, PTEN, SMAD4, and NF1 mutations, BAP1 loss further induces malignant transformation upon xenotransplantation.[130] In a separate system, directly reprogrammed human hepatocyte organoids with TP53 and RB inactivation acquire tumorigenic potential upon c-MYC overexpression, where enhanced mitochondria-endoplasmic reticulum coupling promotes hepatocellular carcinoma initiation, and RAS-driven lineage conversion toward cholangiocarcinoma can be suppressed by combined inhibition of Notch and JAK-STAT signaling.[131] Complementary mouse-derived organoids generated from choline-deficient, amino acid-defined, high-fat diet (CDAHFD)-induced MASH-HCC models recapitulate drug response dynamics, where repeated lenvatinib treatment induces resistance, and transcriptomic analysis of resistant organoids identifies activation of KRAS signaling, inflammatory pathways, and epithelial-mesenchymal transition programs as key features associated with resistance.[132] Together, liver cancer organoid models serve as mechanistic platforms for studying tumor metabolism, regulatory heterogeneity, and therapeutic response in precision hepatology.

TRANSLATIONAL IMPLEMENTATION OF LIVER ORGANOIDS IN PRECISION HEPATOLOGY

From a translational perspective, liver organoids are emerging not only as improved disease models, but also as experimentally tractable human systems that enable functional interrogation of regulatory states and clinically relevant phenotypes. In this context, three application scenarios are particularly crucial for the development of precise hepatology: patient stratification, clinical trial drug screening, and the development of companion diagnostics.

For patient stratification, the most immediate opportunity is to use organoids to identify patient subgroups with differential drug responsiveness prior to treatment allocation. In metabolic liver disease, organoids derived from damaged MASH livers retain patient-specific inflammatory, fibrosis-associated, and tumor-related transcriptional programs,[133] indicating that they preserve clinically relevant inter-patient heterogeneity. In parallel, engineered human hepatocyte organoids have captured genotype- and mechanism-specific steatotic states, including the PNPLA3 I148M risk variant and APOB/MTTP-driven lipid retention,[109] revealing distinct response patterns to metabolic interventions across these subtypes. Consistent with clinical observations, the therapeutic response to MASH is highly heterogeneous. For example, in a phase III trial, the thyroid hormone receptor-β agonist resmetirom achieved MASH resolution in only approximately 25-30% of treated patients,[134] indicating that a substantial proportion of patients do not respond to therapy. These findings suggest that patient-derived organoid-based drug screening may help identify responder and non-responder subgroups prior to clinical treatment.

For clinical trial drug screening, the strongest evidence comes from primary liver cancer. Early studies by Broutier *et al.* and Nuciforo *et al.* established that patient-derived liver cancer organoids faithfully preserve tumor architecture and molecular features while supporting patient-specific drug response testing, including sorafenib sensitivity.[126,127] Li *et al.* then expanded this concept by screening 129 anticancer drugs across 27 liver cancer organoid lines, revealing marked intra-tumor and inter-patient functional heterogeneity and showing that most drugs were either ineffective or active only in selected organoid lines.[128] Together, these studies establish primary liver cancer organoids as an early translational example of clinically relevant drug screening, while also pointing toward the future development of analogous organoid platforms for other liver diseases.

For companion diagnostics, liver organoids can be viewed as a potential functional platform to directly assess drug sensitivity in a patient-specific context. Studies using patient-derived liver cancer organoids have demonstrated variable responses to clinical drugs such as sorafenib,[127] supporting the feasibility of organoid-based drug sensitivity testing in a patient-specific

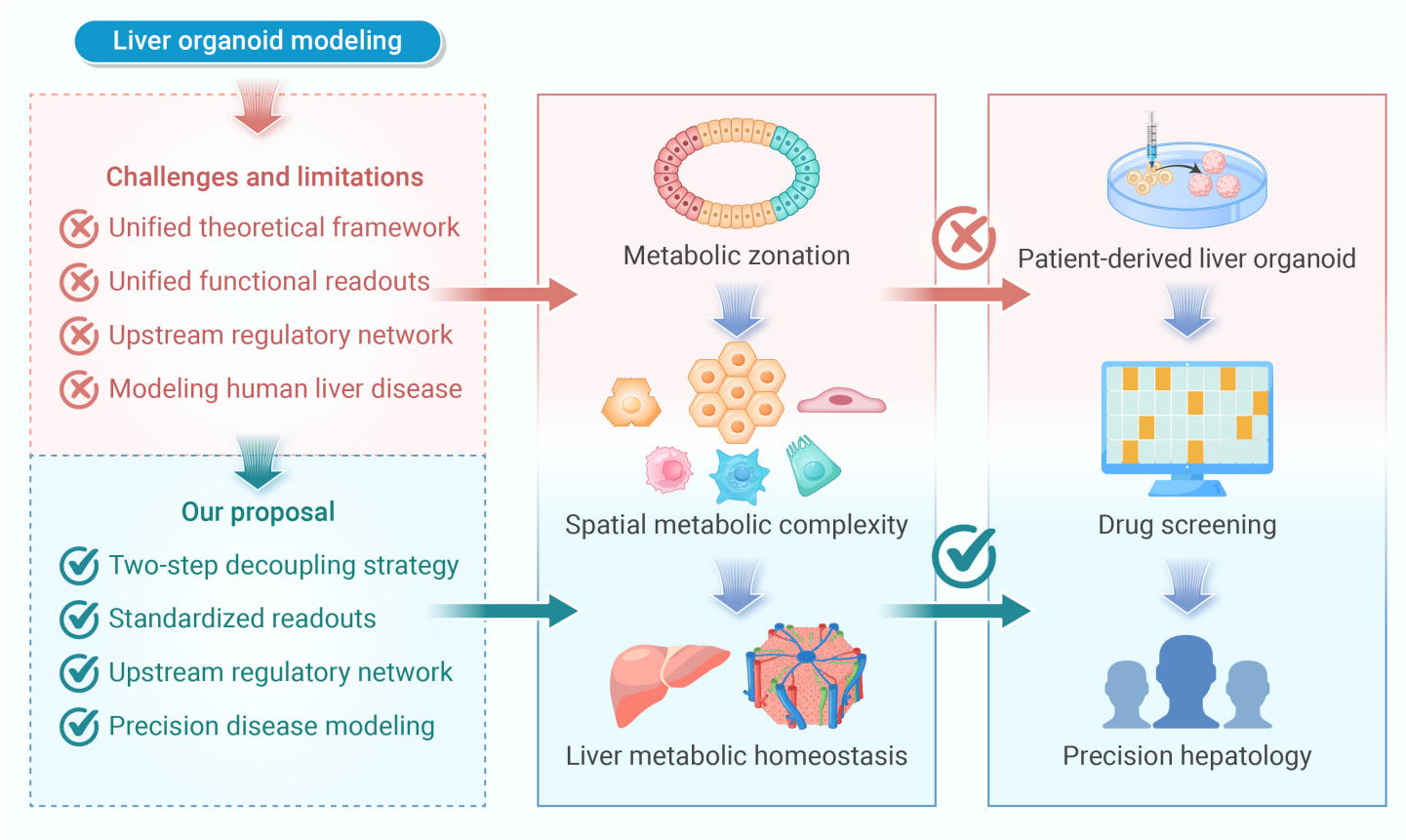

Figure 5. A unified framework for decoding spatial metabolism toward precision hepatology This schematic illustrates how key limitations in current liver organoid modeling (left) hinder reconstruction of spatial metabolic organization (center) and downstream translational applications (right). In conventional systems, the lack of a unified theoretical framework, unified and standardized functional readouts, and integration of upstream regulatory networks restricts the ability to recapitulate spatial metabolic complexity, including metabolic zonation and multicellular interactions, ultimately limiting the reconstruction of liver metabolic homeostasis (center). At the same time, these limitations compromise the reliability of patient-derived organoids for drug screening and their translation into precision hepatology applications (right). To address these challenges, we propose a unified framework integrating a two-step expansion-differentiation decoupling strategy, standardized readouts, and reconstruction of upstream regulatory networks. This integrated approach restores both biological fidelity and analytical consistency, thereby enabling more accurate modeling of spatial metabolic organization and improving the predictive performance of patient-derived organoid platforms for drug screening and precision hepatology.

context. Although sufficient evidence for companion diagnostics in liver organoids remains limited, studies in other organoid systems have provided proof of concept for this application. For example, Tiriac *et al.* established patient-derived pancreatic cancer organoids and showed that *in vitro* responses to standard chemotherapeutic regimens closely matched the corresponding clinical treatment outcomes.[135] Future studies are needed to further develop and validate organoid-based companion diagnostic strategies in liver diseases.

Compared with conventional 2D cultures and animal models, the translational advantage of liver organoids lies in their ability to preserve human-specific regulatory architecture while remaining experimentally tractable. For example, organoid-based HBV infection models have enabled the study of virus-host interactions in a human-specific context, revealing transcriptional programs associated with viral persistence and early oncogenic transformation that are not recapitulated in conventional 2D systems or murine models.[121] In addition, human hepatic organoids have been used to functionally characterize disease-causing JAG1 mutations in Alagille syndrome, revealing how disrupted Notch signaling drives liver pathology in a human-specific context.[136] Therefore, organoids occupy a crucial intermediate position, combining the human relevance that animal models lack, while also possessing the multi-dimensional mechanism analysis capabilities that cannot be achieved in standard two-dimensional systems.

Taken together, available evidence suggests a stepwise translational path for liver organoids, moving from mechanism discovery in human disease-relevant tissues to patient-specific *in vitro* phenotyping, co-clinical drug testing, and ultimately clinical decision support. The field has already reached proof-of-concept for biopsy-based organoid generation, patient-specific drug testing, and biomarker discovery across multiple liver disease contexts.[11,137] A key remaining challenge is to establish standardized and reproducible workflows through assay harmonization, prospective multicenter validation, and integration with histological, genomic, and clinical readouts.

INTEGRATING HIGH-THROUGHPUT SCREENING AND ARTIFICIAL INTELLIGENCE FOR MECHANISTIC DISCOVERY

The convergence of high-throughput screening (HTS) with AI-based analysis has the potential to expand the application of liver organoid platforms in functional screening and phenotype analysis.[138-140] Compared with conventional 2D hepatocyte systems that rapidly lose hepatic functions,[141] liver organoids better preserve hepatic architecture and metabolic activity, but their broader implementation in large-scale screening remains limited by the complexity of 3D culture handling, imaging burden, and organoid heterogeneity.[142,143] Integration of HTS workflows with AI-assisted image analysis may help address some of these limitations by enabling automated phenotypic profiling and scalable analysis of complex organoid datasets.[144]

A recent study illustrates this potential, Tan *et al.* analyzed brightfield and z-stack images of human liver organoids treated with 30 FDA DILI Rank 2.0 compounds using a BEiT-V2 vision transformer.[145] The AI model classified DILI categories with 82.3% accuracy, suggesting that 3D organoid morphology captures features associated with hepatocellular injury and metabolic stress.[145] In parallel, deep-learning approaches have been applied to organoid image segmentation and morphometric characterization, supporting automated analysis of complex three-dimensional phenotypes and scalable data extraction from HTS-compatible workflows.[146,147] Together, AI-assisted analysis can improve scalability and phenotypic characterization in liver organoid systems.

However, several major challenges remain. Organoid heterogeneity and

batch variation compromise phenotypic reproducibility and cross-study comparability.[138] In addition, morphological phenotypes often cannot be directly linked to the underlying regulatory or metabolic mechanisms,[140] limiting mechanistic interpretability and causal inference. Future progress will therefore require standardized organoid platforms integrated with mechanism-based molecular readouts.[148,149] Together with standardized datasets and computational analysis frameworks,[150] these approaches may improve mechanistic interpretation and cross-platform comparability in future liver organoid studies.

CONCLUSIONS AND FUTURE PERSPECTIVES

Decoding the spatial organization of hepatic metabolism remains a central unmet challenge for precision hepatology and liver-targeted drug discovery. Although recent organoid advances have improved cellular complexity and hepatic function, most platforms are still primarily evaluated by downstream metabolic phenotypes,[26-29] leaving unresolved whether the upstream regulatory architecture governing metabolic identity and zonation has been faithfully reconstructed. In this Review, we synthesize a two-step expansion-differentiation decoupling strategy as a unifying theoretical framework for liver organoid modeling. By temporally separating scalable expansion from functional maturation, this framework provides an experimental basis for reconstructing and interrogating upstream regulatory programs governing hepatocyte identity, metabolic specialization, and spatial organization. More broadly, this regulatory-centric perspective shifts liver organoids from descriptive phenotypic models toward mechanistically informed platforms for studying disease-specific metabolic regulation and therapeutic response in precision hepatology (Figure 5).

A major challenge lies in the incomplete decoding of how metabolic programs are spatially specified, coordinated, and remodeled in the liver. Hepatic metabolism is governed by tightly coupled regulatory layers, including cell fate specification regulators, lineage identity stabilization regulators, and metabolic sensing and functional specialization regulators, which collectively establish zonal identities and dynamic metabolic states.[75] Most organoids capture only fragments of this architecture and rely on endpoint functional readouts, limiting insight into causal links between upstream regulation and disease-specific metabolic outputs.[9,10] As a result, comparisons across platforms and disease contexts remain difficult, and the mechanistic basis of metabolic heterogeneity in liver disease remains poorly defined.

Future progress requires organoids to evolve from descriptive systems to platforms that actively probe metabolic regulation. A key direction is reconstructing and validating upstream networks that encode spatial metabolism, paired with standardized, mechanism-based readouts reflecting regulatory state rather than isolated functions. Such approaches are essential to distinguish causality from correlation and to define reproducible metabolic states across health and disease.

Another challenge is balancing system complexity with mechanistic interpretability. While incorporation of vascular, stromal, and immune components is necessary to approximate *in vivo* physiology, excessive complexity risks obscuring regulatory principles.[70] Rational model design must therefore balance physiological realism with analytical tractability, selecting levels of complexity appropriate to the specific biological or translational question. AI-assisted analysis may offer new opportunities to extract latent spatial and regulatory patterns from high-dimensional organoid datasets, particularly when applied to patient-derived organoids to link disease-specific phenotypes with their underlying regulatory mechanisms; however, its utility will critically depend on the availability of standardized and mechanistically grounded inputs. Emerging biosensor- and metabolic sensor-based strategies, combined with genome-wide CRISPR-Cas9 functional screening,[151] may enable dynamic interrogation of regulatory states and metabolic responsiveness in organoid systems.

In this context, organoid-on-chip platforms represent a promising direction to further advance liver organoid systems. By integrating liver organoids with microfluidic devices, these systems enable control of perfusion, oxygen gradients, and biomechanical cues, thereby supporting the reconstruction of the spatial and dynamic microenvironment of the liver.[49] Meanwhile, increasing organoid-on-chip system complexity needs to be approached with caution, as excessive layering may compromise mechanistic interpretability. One practical way forward is to adopt a more modular and parameter-defined design, where key variables such as flow conditions and oxygen gradients can be independently tuned.[152] Establishing consistency in these parameters will be important for improving reproducibility and facilitating comparison across different studies.

Addressing these challenges will expand the utility of liver organoids in precision hepatology. By decoding spatial metabolic regulation, next-generation organoid platforms may provide a foundation for predictive disease modeling, target identification, and patient-tailored therapeutic discovery. Answers to these questions are expected to bridge current translational gaps and to position liver organoids as integral tools in the development of future liver-directed therapies.

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AUTHOR CONTRIBUTIONS

X.X. drafted the manuscript and created the figures. Y.L. and Y.Z. provided critical revision and expert input. C.Z. and Y.F. assisted in figure creation and table organization. T.Y., F.J.G., and H.H. conceived the study, revised the manuscript, and supervised the project. All authors contributed to the manuscript and approved the final version.

DECLARATION OF INTERESTS

Haiping Hao is an Editorial Board member of The Innovation Drug Discovery and was blinded from reviewing or making final decisions on the manuscript. Peer review was handled independently of this member and their research group. The other authors declare no conflicts of interest.

DATA AND CODE AVAILABILITY

No new data were created or analyzed in this study.

SUPPLEMENTAL INFORMATION

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