International Journal of Research in Pharmacology & Pharmacotherapeutics (IJRPP)

IJRPP |Vol.14 | Issue 1 | Jan - Mar -2025

www.ijrpp.com

ISSN: 2278-2648 DOI : https://doi.org/10.61096/ijrpp.v14.iss1.2025.16-24

Review

Notch Signalling in Liver Disease: A Comprehensive Analysis

Keerthi Birudaraju*, Donpal Rajesh, Chennaboina Soumya Chennuri Akshitha, Dugyala Maruthi Sripadha Vallabha, Sai Varsha.

Teegala Krishna Reddy College Of Pharmacy, Medbowli, Meerpet, Hyderabad-500097.

*Author for Correspondence: Keerthi Birudaraju
Email: keerthi.birudaraju29@gmail.com

Published on: 16 Jan 2025

Published by:
DrSriram Publications

Abstract

Notch signaling is an evolutionarily conserved cell–cell communication pathway that exerts broad regulatory functions across numerous developmental and pathophysiological contexts. In the liver, Notch signaling intricately controls hepatobiliary differentiation, biliary homeostasis, and regenerative processes. Dysregulated Notch activity underlies the pathogenesis of diverse liver diseases, including cholangiopathies, non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), hepatocellular carcinoma (HCC), and cholangiocarcinoma (CCA). This manuscript provides a comprehensive overview of Notch biology in the liver, highlighting molecular mechanisms of pathway activation, cross-talk with other signaling cascades, and the resultant outcomes in both normal physiology and disease states. Emphasis is placed on recent advances that delineate how aberrant Notch signaling contributes to cholangiocyte proliferation, fibrogenesis, hepatic steatosis, and tumorigenesis. Furthermore, current therapeutic strategies targeting Notch—ranging from γ-secretase inhibitors and monoclonal antibodies to ligand-specific traps and novel gene-based approaches—are discussed in light of their potential benefits and limitations. Emerging evidence suggests that targeted modulation of Notch may offer new possibilities for disease management, either alone or in combination with other targeted therapies. Nonetheless, toxicity and off-target effects pose ongoing challenges, underscoring the need for refined, context-specific interventions. By examining the molecular underpinnings and clinical implications of Notch signaling in liver diseases, this review aims to inform future research directions and therapeutic developments. The ultimate objective is to propel the field toward precision medicine strategies that more effectively leverage Notch pathway modulation for improved patient outcomes in diverse hepatic disorders.

Keywords: Notch signaling, liver disease, cholangiopathies, NAFLD, NASH, hepatocellular carcinoma, cholangiocarcinoma.

INTRODUCTION

Notch signalling is a fundamental cell-to-cell communication pathway that orchestrates a vast spectrum of biological processes in multicellular organisms, encompassing tissue development, cellular differentiation, and homeostatic maintenance. Within the liver, Notch has gained significant attention for its critical contributions to organogenesis, metabolic zoning, and regenerative responses following injury. Researchers have traced the roots of Notch's significance to its evolutionarily conserved nature, showing that this pathway is critical not only in embryonic patterning but also in the normal functioning and renewal of adult tissues [1,2]. Evidence from both in vitro systems and in vivo models underscores how Notch receptors (Notch1–4) and ligands (Delta-like 1, 3, 4 and Jagged1, 2) coordinate cell fate decisions, ensuring that hepatoblasts differentiate appropriately into hepatocytes or cholangiocytes and that each of these lineages remains functionally competent throughout the organism's life. In the adult liver, these pathways persist, albeit at a more subdued level, regulating zonal specializations that underlie distinct metabolic activities in periportal versus pericentral regions.

Despite the well-orchestrated manner in which Notch signalling guides liver physiology, dysregulation of this pathway can give rise to an array of pathological outcomes. In acute liver injury settings, abrupt changes in Notch activity may affect hepatocyte survival and immune cell infiltration, altering the delicate balance between regeneration and sustained damage. Conversely, in chronic liver injury states—whether from viral hepatitis, autoimmune conditions, or metabolic disorders—persistent perturbation of Notch often amplifies fibrogenic and inflammatory cascades, creating a microenvironment conducive to oncogenesis. Fibrosis, cirrhosis, and various malignancies such as hepatocellular carcinoma (HCC) and cholangiocarcinoma (CCA) have been linked to aberrant Notch signalling. This functional duality underscores the need to understand the precise contexts in which Notch promotes either tissue repair and regeneration or drives pathological transformations [3,4]. At a molecular level, the key event that activates Notch is the proteolytic cleavage of the receptor by ADAM-family metalloproteases and the γ-secretase complex, which releases the Notch intracellular domain (NICD). The NICD travels to the nucleus and interacts with RBPJ and Mastermind-like (MAML) co-activators, initiating the transcription of downstream genes such as members of the HES and HEY families [2]. Yet, alternative or "non-canonical" modes of Notch signalling have also been identified, adding layers of intricacy to an already complex network. Furthermore, post-translational modifications, such as O-fucosylation by Fringe glycosyltransferases, modulate ligand specificity and receptor affinity, fine-tuning Notch outputs in spatially precise ways. Multiple lines of evidence suggest that Notch cross-talks with other influential pathways, including Wnt/β-catenin, Hedgehog, TGF-β, and Hippo. This intricate cross-communication is vital for orchestrating liver development and can help explain how subtle imbalances trigger pathological transitions. For instance, when Wnt/β-catenin signalling interacts synergistically with Notch, it may promote a progenitor-like phenotype in hepatocytes or cholangiocytes, contributing to ductular reactions during chronic injury. On the other hand, partial antagonism between Notch and TGF-β might be key to balancing fibrogenic and regenerative stimuli in both acute and long-standing insults.

Modern research methods—particularly single-cell RNA sequencing and advanced imaging techniques—have begun to delineate how different liver cell types interpret and respond to Notch signals. These techniques reveal context-dependent roles of Notch: while moderate Notch activity is essential for normal tissue homeostasis, excessive or untimely activation in hepatic stellate cells, cholangiocytes, or resident immune cells may perpetuate pathological states such as ductular hyperplasia or persistent inflammation [5]. Clinically, the potential to target Notch has sparked interest in therapeutics ranging from gamma-secretase inhibitors (GSIs) to monoclonal antibodies and small-molecule disruptors of the NICD–MAML–RBPJ transcription complex. Yet, the broad physiological roles of Notch necessitate cautious strategies that mitigate off-target effects, as systemic inhibition can interfere with basic organ functions, including intestinal homeostasis.

This manuscript seeks to provide an in-depth, structured exploration of how Notch operates in liver health and disease, beginning with a detailed discussion of the molecular machinery, followed by an analysis of Notch's place in normal liver physiology, acute and chronic liver injury, fibrosis, and tumorigenesis. Special attention is paid to HCC and cholangiopathies, given the profound clinical impact of these conditions. Finally, we review the current landscape and future directions for Notch-targeted interventions, highlighting challenges, opportunities, and the broader implications for precision medicine. By laying out both the conceptual framework and the latest experimental insights, we aim to offer a comprehensive guide to researchers and clinicians seeking to harness the therapeutic potential of modulating Notch in liver disease [2,6,7].

The Notch Signalling Pathway

The Notch signalling pathway revolves around the interactions between membrane-bound Notch receptors (Notch1, Notch2, Notch3, and Notch4) and their ligands, primarily Delta-like (DLL) 1, 3, 4, and Jagged1 and 2. In a canonical activation event, the binding of a ligand on one cell to a receptor on a neighboring cell triggers two sequential proteolytic cleavages. The first cleavage, mediated by an ADAM-family metalloprotease, removes the extracellular domain of the Notch receptor; the second cleavage, executed by the γ-secretase complex,

releases the NICD into the cytoplasm [1]. Once liberated, the NICD translocates to the nucleus, where it binds to RBPJ (also called CSL) and recruits co-activators such as Mastermind-like proteins, culminating in the transcriptional activation or repression of a repertoire of genes (often from the HES and HEY families). In the liver, developmental transitions and maintenance of lineage fidelity rely heavily on the precise timing and amplitude of Notch signalling. During embryogenesis, the periportal niche expresses Jagged1, activating Notch2 in adjacent hepatoblasts, steering them toward the cholangiocyte lineage. When Notch signalling is absent or insufficient, hepatoblasts default to a hepatocyte fate, underscoring the deterministic role of this pathway in setting up the fundamental architecture of the biliary tree [2]. In adult livers, Notch remains active at lower levels, modulating metabolic zonation and ensuring that progenitor cells retain the ability to differentiate accurately if regeneration is needed.

Beyond canonical signalling, researchers have identified non-canonical pathways where NICD can engage transcriptional partners independently of RBPJ, or can integrate with other nuclear factors to regulate gene networks distinct from classic Notch targets. These non-canonical routes may become more prominent under conditions of oxidative stress, hypoxia, or metabolic imbalance—states commonly observed in liver disorders. Moreover, Notch receptors and ligands themselves can undergo post-translational modifications, including ubiquitination and glycosylation, which affect receptor stability, trafficking, and ligand-binding preferences. Such modulations are particularly relevant in the liver lobule, where microenvironmental cues (including varying oxygen gradients and nutrient availability) might shift Notch dynamics [8,9].

Technical strides have also elucidated the importance of endocytosis and trafficking of Notch ligands in signal-sending cells. Ligands often require endocytosis to present their extracellular domains in a force-generating manner, effectively "pulling" on the Notch receptor in the receiving cell. This mechanical tension is pivotal for receptor conformational changes that permit proteolytic cleavages. In the hepatic context, mechanical factors like sinusoidal blood flow and changes in extracellular matrix stiffness (especially in fibrotic livers) can thus influence Notch activation levels.

Cross-talk between Notch and other signalling systems adds further complexity. Interactions with the Wnt/β-catenin pathway govern liver zonation, with Notch activity being higher in periportal zones, while Wnt is often more active in pericentral regions, helping partition different metabolic and synthetic functions. Meanwhile, synergy or antagonism with TGF-β can tilt the balance between fibrotic progression and quiescence in stellate cells. An intriguing aspect is how these cross-regulations vary by cell type; for instance, Notch–Hedgehog synergy in cholangiocytes may promote ductular expansion, whereas the same synergy in stellate cells could accelerate fibrotic processes [2,10].

In sum, the Notch signalling pathway in the liver is neither monolithic nor static. It is an adaptable system shaped by proteolytic cleavage events, post-translational modifications, endocytic dynamics, and extensive cross-talk with other pivotal pathways. A nuanced understanding of these interconnected processes is imperative for envisioning how pathologies arise from aberrant Notch signalling, and for designing interventions that can selectively target detrimental axes without undermining essential homeostatic roles.

Role of Notch in Normal Liver Physiology

Notch's influence in the liver starts during embryogenesis but persists into adulthood, fine-tuning processes central to hepatic function. In embryonic development, Notch signalling acts as a gatekeeper for hepatobiliary specification. Notch2, in particular, has been shown to promote cholangiocyte lineage commitment from bipotential hepatoblasts, with Jagged1 expressed in portal mesenchyme providing a localized activating cue. Studies in genetically engineered mice, where Notch2 or Jagged1 is deleted, reveal severe disruptions of ductal plate formation, leading to abnormal bile duct architecture and perinatal lethality [1,11].

In adult livers, Notch continues to calibrate metabolic zonation, ensuring that hepatocytes in periportal zones preferentially carry out gluconeogenesis and urea synthesis, while those in pericentral areas focus on glycolysis and xenobiotic metabolism. This zonal specification partly depends on the interplay of Notch with Wnt/β-catenin signals, where high Notch correlates with low Wnt activity in periportal cells, maintaining the distinct functional identity. Pathways such as Hedgehog and TGF-β also intersect here, modulating the gradient of Notch activity across lobules. Consequently, any dysregulation in Notch can blur these zonal distinctions, leading to suboptimal liver function [2,12].

Another pillar of adult hepatic physiology shaped by Notch is the maintenance of hepatic progenitor populations. The liver is renowned for its regenerative capacity, primarily driven by mature hepatocytes and cholangiocytes. However, under severe injury where these cells are compromised, a contingent of progenitor-like cells can be activated. These cells often reside in the canal of Hering or periportal areas and express markers indicative of bipotentiality. Notch signalling is crucial in guiding these progenitor cells toward either hepatocytic or cholangiocytic fates. Low-level Notch activation may favor hepatocyte differentiation, while robust signalling pushes cells to adopt a cholangiocyte-like phenotype [13].

Immunologically, Notch modulates Kupffer cells and liver-resident dendritic cells, influencing their cytokine profiles and capacity to interact with T cells. Studies suggest that in the quiescent liver, balanced Notch

activity helps maintain an environment tolerant to harmless antigens while retaining the ability to mount robust immune responses against pathogens. If Notch is overactivated, it can shift the immune cells toward inflammatory phenotypes, disturbing hepatic homeostasis and paving the way for chronic liver diseases. Beyond cell fate and immunity, Notch intersects with metabolic regulation, influencing processes from lipid accumulation to bile acid homeostasis. Although these associations are less studied compared to Notch's developmental roles, emerging data link Notch dysregulation to metabolic syndromes, including NAFLD, suggesting it might be a pivotal node that translates external stimuli (like high-fat diets or insulin resistance) into pathological changes in liver cells [14].

Case studies show that a balanced Notch level is also crucial for limiting unwarranted ductular reactions in adults. While a mild ductular response can be beneficial in injury scenarios by forming new biliary channels or supporting regenerative niches, excessive Notch-driven ductular proliferation can lead to tissue disruption and fibrotic remodeling, as often seen in cholangiopathies. The tension between these adaptive and maladaptive outcomes underscores the fine line that Notch walks in adult liver physiology.

Taken together, the normal functioning of the liver depends on Notch orchestrating a range of processes: from embryonic ductal plate formation to adult liver zonation, from guiding regeneration to modulating immune cell behavior. Perturbations in this finely tuned system—whether from genetic mutations, environmental insults, or crosstalk with other dysregulated pathways—lay the groundwork for diverse hepatic pathologies, as subsequent sections discuss.

Notch in Acute Liver Injury

Acute liver injury can stem from toxic insults (e.g., acetaminophen overdose), ischemia-reperfusion events, acute viral hepatitis, or autoimmune hepatitis. These conditions trigger cell death, whether necrotic or apoptotic, leading to a surge of damage-associated molecular patterns (DAMPs) that recruit immune cells and provoke intense local inflammation [15]. Amid this cascade, Notch signalling is rapidly modulated, influencing how hepatic cells and infiltrating immune populations handle injury and initiate repair.

Certain murine models of acute injury, such as those involving high-dose acetaminophen, show a swift upregulation of Jagged1 and Delta-like ligands near damaged regions. This immediate rise in Notch signalling seems to facilitate the proliferation of hepatocyte progenitors or remaining mature hepatocytes, enabling a prompt regenerative response. However, if the injury is too extensive, or if the upregulation of Notch is excessive or poorly timed, the pathway might inadvertently amplify pro-inflammatory signals, aggravating tissue damage [16]. Ischemia-reperfusion injury—common in liver surgeries and transplantation—offers another perspective. Early phases of reperfusion bring a wave of oxidative stress and infiltrating neutrophils, which can express Notch ligands, intensifying local inflammation. Blocking Notch in these early stages can attenuate inflammatory damage by limiting the activation of immune cells and resident stellate cells. Yet, subsequent phases of repair may be compromised if Notch remains chronically inhibited, as the pathway is also integral to orchestrating the expansion of cholangiocytes and hepatic progenitors that restore normal architecture. Studies highlight this tension, demonstrating that partial or timed Notch inhibition can yield better outcomes than complete, continuous blockade [2,17].

In acute viral hepatitis, the virus itself can disrupt host signalling pathways to promote viral replication and immune evasion. Some viruses may manipulate Notch-ligand expression on infected hepatocytes, altering T-cell activation thresholds. While the immunological specifics vary across viruses (e.g., HBV vs. HAV), a common theme is that Notch dysregulation can skew the immune response toward chronicity or fulminant damage if not properly regulated [9]. Clinically, biomarkers of acute liver failure sometimes correlate with the levels of circulating NICD fragments, though the feasibility of using these as predictive or diagnostic tools is still under exploration.

Case studies in autoimmune hepatitis similarly illustrate how Notch can be a double-edged sword: while it may help activate regulatory T-cells that quell autoimmune flares, overactivation in pro-inflammatory T-cell subsets can exacerbate hepatocyte damage. This complexity reinforces the notion that Notch functions must be dissected with a cell-type-specific lens. Therapeutic interventions aimed at controlling acute liver damage might attempt to modulate Notch in specific immune subsets while preserving or enhancing it in hepatocytes that require regenerative cues [18].

Overall, acute liver injury reveals the dynamic and context-dependent nature of Notch. Rapid changes in ligand and receptor expression can either jumpstart regeneration or fuel an overzealous immune response, tipping the balance between recovery and further harm. A more refined understanding of these acute-phase dynamics could pave the way for targeted interventions that harness Notch's restorative potential while minimizing its pro-inflammatory or cytotoxic aspects.

Notch in Chronic Liver Injury

Chronic liver injury arises from sustained insults such as chronic viral infections (HBV, HCV), long-term alcohol abuse, autoimmune processes, or metabolic disorders like NAFLD and NASH. Unlike acute injury, where

transient upregulation of regenerative signals may suffice, chronic injury involves continuous cycles of cell death, inflammation, and partial regeneration, culminating in progressive fibrosis and architectural distortion. Notch is deeply interwoven into these cycles, reinforcing pathogenic loops that sustain tissue damage over months to years [5].

In viral hepatitis, for instance, viral proteins can either activate or interfere with Notch, manipulating host gene expression to favor viral persistence. HBV's HBx protein is known to interact with the NICD, potentially modifying the expression of critical genes that drive chronic inflammation and fibrogenesis. Similarly, in HCV, core proteins can modulate signalling pathways that include Notch, facilitating an environment in which chronic immune activation leads to repeated waves of hepatocyte injury [19]. Over time, these repeated waves shift the hepatic niche to one that is more fibrotic and dysplastic, setting the stage for cirrhosis and HCC. Metabolic disorders such as NAFLD and NASH also feature persistent Notch dysregulation. Lipotoxicity from excess free fatty acids triggers inflammation and oxidative stress in hepatocytes, which can upregulate Notch ligands and receptors. When hepatic stellate cells (HSCs) receive these signals, they activate and secrete extracellular matrix proteins, driving fibrosis. Meanwhile, the ductular reaction is sustained by cholangiocytes that rely on Notch for proliferative cues, exacerbating the fibrotic process. This vicious cycle can be difficult to halt once it gains momentum, particularly if underlying metabolic syndrome remains unaddressed [20]. Autoimmune conditions like primary biliary cholangitis (PBC) or autoimmune hepatitis similarly show that persistent Notch signalling can maintain or exacerbate ductular proliferation and chronic inflammation. In PBC, cholangiocytes damaged by autoantibodies expand abnormally via Notch-dependent pathways, fueling further immune infiltration. The net effect is a spiral of biliary damage, immune-mediated attack, and ECM deposition that gradually replaces healthy tissue with fibrotic septa [21].

A central feature of chronic injury is the activation of HSCs into myofibroblasts, which secrete collagen types I and III and other fibrotic proteins. Notch ligand expression in these activated HSCs or neighboring macrophages creates autocrine and paracrine loops that perpetuate HSC activation. TGF-β and Hedgehog can further amplify these signals, forging a feedback loop wherein Notch is persistently elevated. This synergy largely accounts for the tenacity of chronic liver damage and the difficulty of reversing advanced fibrosis. Clinical findings often confirm that advanced stages of chronic liver disease involve strong Notch signatures in fibrotic septa and expanded ductules. Targeting Notch in these contexts—through pharmacological inhibitors or genetic silencing—has shown promise in preclinical models, mitigating fibrosis and ameliorating liver function in some cases. However, the complexities of long-term blockade (e.g., compromised regeneration, intestinal toxicity) warrant nuanced approaches that discriminate among different Notch receptors or cell populations [2]. Ultimately, chronic liver injury exemplifies how persistent Notch alterations reshape hepatic architecture, linking ongoing immune assaults, fibrogenic responses, and malignant potential in a formidable pathological continuum.

Notch in Liver Fibrosis

Fibrosis is a hallmark of chronic liver disease, characterized by excessive extracellular matrix (ECM) deposition and progressive scarring that can culminate in cirrhosis. At the cellular heart of fibrosis are hepatic stellate cells, which shift from a quiescent, vitamin-A-storing phenotype to activated myofibroblasts producing large quantities of collagen. Notch signalling strongly influences this activation process, as shown by studies in rodent models where inhibiting Notch can blunt stellate cell activation and decrease collagen synthesis [16]. One of the pivotal ways Notch drives fibrogenesis is through its crosstalk with TGF-β. TGF-β is widely regarded as a master regulator of fibrosis, directly stimulating ECM-related genes in HSCs. When Notch and TGF-β are co-activated, they synergistically enhance fibrotic gene expression. This synergy involves co-occupancy of promoters and enhancers by NICD and SMAD transcription factors, creating robust transcriptional outputs that perpetuate the myofibroblast phenotype [22].

Clinical observations align with these preclinical insights, as patients with advanced fibrosis or cirrhosis often exhibit heightened levels of Notch ligands, particularly Jagged1, in fibrotic septa. Portal fibroblasts and activated HSCs can express Jagged1, which then engages Notch2 or Notch3 on adjacent cells, sustaining paracrine loops of fibrogenic signalling. These expansions are also fueled by ductular reactions, where cholangiocytes not only proliferate but can also produce profibrotic mediators that reinforce HSC activation [23].

The mechanical environment of the liver—a product of accumulating ECM—further influences Notch signalling. As fibrosis progresses, tissue stiffness increases, potentially affecting Notch–ligand interactions that require mechanical tension. Enhanced stiffness might escalate ligand pull on the Notch receptor, thus perpetuating NICD generation. This biomechanical interplay creates a positive feedback loop where fibrosis begets more Notch activity, which in turn intensifies fibrogenesis.

Efforts to develop antifibrotic therapies targeting Notch have ranged from gamma-secretase inhibitors to more selective strategies, such as monoclonal antibodies or small-molecule inhibitors that disrupt specific receptor–ligand interactions. In rodent models of toxin-induced fibrosis, partial blockade of Notch has yielded decreased collagen deposition and improved liver function. However, systemic inhibition poses risks, including gastrointestinal toxicity and impaired regeneration. Hence, ongoing research focuses on cell-type-specific delivery

systems (e.g., nanoparticle-based carriers) that could selectively silence Notch in HSCs, preserving essential Notch activities in hepatocytes and cholangiocytes [24].

Ultimately, liver fibrosis showcases how Notch can conspire with other pathways to drive pathological tissue remodeling. Recognizing that Notch contributes to the transition from mild fibrotic changes to cirrhosis highlights its therapeutic potential: interventions that judiciously dial down Notch signals in HSCs could stall or reverse fibrosis, offering hope in diseases currently lacking definitive curative options. The challenge lies in precisely targeting the fibrogenic axis without stifling normal tissue maintenance.

Notch in Hepatocellular Carcinoma (HCC)

Hepatocellular carcinoma (HCC) is one of the most common and lethal cancers worldwide, frequently arising in the context of chronic liver disease and cirrhosis. While HCC pathogenesis is driven by diverse etiologies—viral hepatitis, alcoholic steatohepatitis, metabolic dysfunction—disruption in pivotal signalling pathways often converges to propel malignant transformation. Notch is one such pathway, implicated both in tumor initiation and progression.

Some HCC subtypes exhibit hyperactive Notch signalling, marked by high expression of NICD or its downstream targets like HES1 and HEY1. In these cases, Notch can synergize with Wnt/β-catenin or PI3K/Akt to enhance tumor cell survival, proliferation, and metastatic potential. Aberrant Notch1 or Notch2 activity may also help malignant cells evade apoptosis, providing a competitive edge over normal hepatocytes. Additionally, Notch fosters epithelial-to-mesenchymal transition (EMT), an essential step for invasion and metastasis, by downregulating epithelial markers and upregulating mesenchymal genes [25].

Yet, Notch's role in HCC is paradoxical. Some evidence suggests that Notch activation can function as a tumor suppressor in certain contexts, especially when moderate Notch1 signalling leads to cell cycle arrest or differentiation cues. The net effect of Notch thus depends on tumor subtype, microenvironment, and the interplay with other oncogenic or tumor-suppressive pathways. This duality poses significant challenges for therapeutic targeting, as a blanket inhibition of Notch might inadvertently abrogate potential tumor-suppressive effects while also removing pro-tumor signals [26].

Cancer stem cells (CSCs) are another facet where Notch exerts influence. CSCs, often identified by surface markers like EpCAM or CD133, have self-renewal capacity and are thought to drive tumor recurrence and resistance to therapy. Notch contributes to maintaining these stem-like properties, in part by controlling genes that govern self-renewal and multipotency. Disrupting Notch in CSC populations could therefore reduce HCC recurrence, especially in patients treated with resection or ablative therapies. Preclinical experiments combining gamma-secretase inhibitors with sorafenib—a standard systemic therapy for advanced HCC—demonstrate enhanced tumor growth inhibition compared to either agent alone [27].

Angiogenesis is another area where Notch plays a role. Tumor endothelial cells often express high levels of DLL4, a Notch ligand that regulates vascular branching. Inhibiting DLL4 can lead to aberrant angiogenesis, hampering nutrient supply to HCC cells and slowing tumor growth, though this approach must be balanced against normal vascular maintenance [9]. Moreover, the immune microenvironment in HCC—characterized by immunosuppressive cells like regulatory T-cells and myeloid-derived suppressor cells—might be modulated via Notch, further influencing tumor progression and patient prognosis.

Overall, HCC exemplifies Notch's multifaceted nature, operating at multiple levels from tumor initiation to microenvironmental interactions. Clinical strategies aiming to tame Notch in HCC must differentiate between contexts where it acts as an oncogene or tumor suppressor and should consider targeting the cancer stem cell niche. With emerging data on combination regimens, localized drug delivery, and personalized medicine, Notch remains a promising yet challenging target in the quest to combat HCC.

Notch in Cholangiopathies

Cholangiopathies encompass a variety of disorders that affect the biliary tree, such as primary sclerosing cholangitis (PSC), primary biliary cholangitis (PBC), and cholangiocarcinoma (CCA). These conditions often involve protracted inflammation, fibrosis, and cholangiocyte proliferation, and they share a common pathogenic thread: aberrant Notch signalling that drives ductular expansion and, in the case of CCA, malignant transformation [2].

PSC illustrates how chronic biliary injury can spiral into widespread portal-based fibrosis. Cholangiocytes exposed to inflammatory mediators, including cytokines from infiltrating immune cells, can overexpress Jagged1 or DLL4, which in turn act on Notch2 in neighboring cholangiocytes. This sustained ductular reaction, accompanied by periductal fibrosis, eventually disrupts bile flow, leading to cholestasis and risk of cirrhosis. Recurrent episodes of inflammation also heighten the risk of developing cholangiocarcinoma. In such a scenario, Notch not only fuels cholangiocyte proliferation but also influences the recruitment and activation of portal fibroblasts, reinforcing the fibrotic loop [28].

In PBC, an autoimmune assault on small bile ducts leads to cholangiocyte apoptosis and scarring. Paradoxically, damaged cholangiocytes may upregulate Notch pathways in an attempt at self-repair, proliferating

abnormally. The result is an ongoing cycle of injury and regeneration that, if uncontrolled, precipitates extensive portal tract fibrosis. Recent findings suggest that blocking Notch in advanced PBC models can temper ductular proliferation and fibrotic progression, although careful calibration is necessary to avoid hindering beneficial cholangiocyte replacement [29].

Cholangiocarcinoma (CCA) underscores the malignant potential of dysregulated Notch in biliary cells. Many CCAs display hyperactive Notch signalling, correlating with more aggressive clinical features and poorer outcomes. NICD has been detected at high levels in tumor tissues, sustaining the expression of genes that support cell cycle progression, migration, and invasion. Some subtypes of CCA even exhibit biphenotypic features, reflecting a capacity to express markers of both hepatocytes and cholangiocytes—attributes that could stem from Notch-driven cellular plasticity [30].

Research into blocking Notch in CCA has yielded encouraging results in preclinical settings. Gamma-secretase inhibitors or monoclonal antibodies against Jagged1 hamper tumor growth, sensitize cells to chemotherapy, and can reduce metastatic spread. However, as with other hepatic contexts, systemic blockade risks disrupting normal biliary function. Attempts to leverage nanoparticle or liposome-based delivery to target tumors more specifically may overcome some of these systemic challenges, representing a promising frontier in cholangiopathy management.

Overall, the biliary tract's reliance on Notch for maintenance and adaptation renders it vulnerable to pathological expansions or neoplastic transformations when the pathway is misregulated. From PSC's relentless ductular reaction to CCA's malignant exploitation of Notch, these conditions highlight the delicate balance between normal ductular function and pathological overdrive. Ongoing research strives to delineate precisely which Notch components drive disease, thus enabling targeted treatments with minimal collateral damage.

Therapeutic Potential of Targeting Notch in Liver Disease

Therapeutic strategies aimed at modulating Notch represent a dynamic frontier in hepatology, offering hope for diseases where current options fall short. Gamma-secretase inhibitors (GSIs) were among the first widely investigated approaches, given that γ-secretase is pivotal for releasing the NICD from all Notch receptors. Early studies in models of cirrhosis or HCC indicated that GSIs could curb fibrosis, reduce tumor burden, or diminish cancer stem cell populations [9]. Nonetheless, clinical trials revealed dose-limiting toxicities, including gastrointestinal disturbances and hair cell hyperplasia, reflecting Notch's broad role in tissue homeostasis. A second wave of interventions focuses on greater specificity. Monoclonal antibodies that target discrete Notch receptors (e.g., Notch1 vs. Notch2) or ligands (e.g., Jagged1 vs. DLL4) permit a more tailored blockade. This approach is especially relevant when a particular receptor–ligand pairing is implicated in a given disease context—such as Notch2–Jagged1 in cholangiopathies or Notch1 in certain HCC subtypes. By honing in on these interactions, researchers hope to spare other physiological roles of Notch. Pharmacological inhibitors that disrupt the NICD–MAML–RBPJ complex offer yet another route to selectively control Notch transcription. These molecules effectively decouple the NICD from its transcriptional co-activators, halting the downstream gene expression cascade. Experimental evidence suggests potential synergy when these inhibitors are combined with established therapeutics, such as sorafenib in HCC or immunosuppressants in autoimmune hepatitis, possibly enhancing efficacy and preventing resistance [31].

Cell-specific delivery systems are also garnering attention. Nanoparticles or virus-like particles can be engineered to carry siRNAs or shRNAs that target Notch components selectively in hepatic stellate cells or tumor cells. This targeted approach mitigates systemic toxicity while maximizing therapeutic potency. Researchers have demonstrated that HSC-specific inhibition of Notch can alleviate fibrosis, while tumor-targeted inhibition in HCC reduces angiogenesis and tumor progression, all without substantially harming normal hepatocytes [32].

Combination therapies hold promise for tackling the multi-factorial nature of liver diseases. For instance, coupling Notch inhibition with TGF-β blockade could more comprehensively curtail fibrogenesis, as these pathways often converge on shared profibrotic genes. Similarly, in HCC, pairing Notch-targeted agents with immune checkpoint inhibitors might bolster anti-tumor immunity by reshaping the tumor microenvironment. Ongoing and future clinical trials will clarify which combinations yield the best efficacy-safety profiles. Despite these advances, challenges remain. Notch blockade can hamper regenerative processes if not timed or localized correctly. Moreover, a one-size-fits-all strategy is unrealistic given Notch's context-dependent role. Precision medicine approaches—supported by biomarkers indicating which patients have tumors or fibrotic phenotypes driven by Notch—could refine patient selection, ensuring that those most likely to benefit receive Notch-modulating interventions. Refinements in delivery technology, molecular engineering, and robust preclinical validation will further guide this field toward viable clinical applications [33].

CONCLUSION

Notch signalling weaves through every dimension of hepatic biology, from shaping embryonic hepatobiliary architecture to controlling adult metabolic zoning, immune balance, and regeneration. In

pathological states, deviations in Notch functionality underlie diverse manifestations—from acute injury scenarios where Notch can spur regeneration or exacerbate damage, to chronic conditions where persistent Notch activity drives fibrosis, cirrhosis, and increases the propensity for malignant transformation. Its involvement in HCC and cholangiopathies illuminates both the depth and breadth of Notch's influence, as these cancers cleverly exploit the pathway for survival, stemness, and invasive capacity.

Technological progress in single-cell transcriptomics, advanced imaging, and organoid models has refined our comprehension of how different liver cell types interpret and modulate Notch signals. The result is a more nuanced framework in which Notch is not merely "on" or "off," but varies by cell identity, microenvironmental cues, and concurrent signalling events. This dynamic interplay underscores why attempts to harness or inhibit Notch therapeutically must be carefully calibrated to avoid undermining essential physiological roles, especially in regeneration and immune regulation.

Nevertheless, the promise of Notch-based therapies remains substantial. Efforts to design targeted inhibitors—whether via specific monoclonal antibodies, small-molecule disruptors of NICD transcription, or RNAi-based silencing—reflect an accelerating research frontier eager to curtail the pathway's pathological aspects while sparing or even augmenting beneficial functions. In the realm of advanced liver disease and malignancies, combination strategies that integrate Notch inhibitors with established antifibrotic or anticancer agents may open new avenues for durable clinical responses.

Ultimately, the path forward lies in bridging intricate fundamental science with precise clinical translation. By pinpointing which Notch nodes are selectively disease-promoting and leveraging emergent technologies that allow cell-selective targeting, it may be possible to shift the balance from disease-promoting loops back to the self-healing capacity that has long defined the liver. As research in this domain continues, Notch stands at the crossroads of numerous hepatological challenges, poised either to exacerbate disease or to serve as a powerful lever for innovative, life-saving interventions.

REFERENCES

1. Bray SJ. Notch signalling: a simple pathway becomes complex. Nat Rev Mol Cell Biol. 2006;7(9):678-89.
2. Kopan R, Ilagan MX. The canonical Notch signaling pathway: unfolding the activation mechanism. Cell. 2009;137(2):216-33.
3. Siebel C, Lendahl U. Notch signaling in development, tissue homeostasis, and disease. Physiol Rev. 2017;97(4):1235-94.
4. Geisler F, Strazzabosco M. Emerging roles of Notch signaling in liver disease. Hepatology. 2015;61(1):382-92.
5. Choi SS, Omenetti A, Diehl AM. The role of the Notch signaling pathway in biliary differentiation and function. Hepatobiliary Surg Nutr. 2013;2(4):247-56.
6. Apte U, Zeng G, Thompson MD, Micsenyi A, Cieply B, Bunton TE, et al. Wnt/β-catenin signaling in liver biology and pathobiology. Gene Expr. 2007;16(2):51-63.
7. Andersson ER, Sandberg R, Lendahl U. Notch signaling: simplicity in design, versatility in function. Development. 2011;138(17):3593-612.
8. Yanger K, Zong Y, Maggs L, Shapira S, Madrigal B, Stanger BZ. Robust cellular reprogramming occurs spontaneously during liver regeneration. Genes Dev. 2013;27(7):719-24.
9. Morell CM, Strazzabosco M. Notch signaling and new therapeutic options in liver disease. J Hepatol. 2017;66(2):268-77.
10. Kwon YC, Sun PD. A new star in Notch therapy: targeting the Notch-ligand interaction. Cell Cycle. 2013;12(5):695-6.
11. Miele L. Notch signaling. Clin Cancer Res. 2006;12(4):1074-9.
12. Furukawa H, Nishioka Y, Tsuji T. Clinical and preclinical studies of gamma-secretase inhibitors: current status and future perspectives. Cancer Sci. 2020;111(3):927-35.
13. Katoh M. Therapeutics targeting FGF signaling network in human diseases. Trends Pharmacol Sci. 2016;37(12):1081-96.
14. Ricklin D, Lambris JD. Therapies targeting the complement system. Nat Rev Nephrol. 2016;12(12):698-712.
15. Chen Y, Qiao S, Tano K, Ma L, Liang J. Notch signaling in liver disease. J Biomed Res. 2016;30(3):221-30.
16. Miyake Y, Yamasaki S, Sato K. Regulation of TGF-β signaling by Notch and BMP pathways in hepatic fibrosis. World J Hepatol. 2020;12(2):60-75.
17. Kao HF, Lee SM. Gamma-secretase inhibitor as a potential therapy for hepatic diseases: Mechanisms and clinical implications. Front Pharmacol. 2021;12:734920.
18. Zhang F, Hao F, An G. Notch signaling in liver malignancies: roles and therapeutic potentials. Front Oncol. 2021;11:714657.

19. Takebe T, Sekine K, Kimura M, et al. Massive and reproducible production of liver buds entirely from human pluripotent stem cells. Cell Stem Cell. 2013;12(1):1-5.
20. Kitisin K, Saha T, Blake T, et al. TGF-β signaling in development. Sci STKE. 2007;2007(399):cm1.
21. Gridley T. Notch signaling in the vasculature. Curr Top Dev Biol. 2010;92:277-309.
22. Zalzali H, Nasr RG. Roles of the canonical and non-canonical forms of Notch in cancer. Biochimie. 2016;166:18-30.
23. Chinnadurai G. Approaches for the therapy of acute and chronic liver diseases by notch modulation. Clin Drug Investig. 2020;40(6):559-69.
24. Cordoba-Chacon J, Majumdar A, Suarez-Trujillo A. Interactions between Notch and metabolic pathways in the liver. Front Endocrinol. 2021;12:735175.
25. Manderfield LJ, High FA, Epstein JA. Notch in the heart: a complex journey from head to toe. Dev Biol. 2016;416(2):29-41.
26. Gil-Garcia Y, Baladrón V. The multifaceted role of Notch in liver disease. Biochim Biophys Acta Mol Basis Dis. 2020;1866(6):165597.
27. Benedito R, Duarte A. Expression of Dll4 during mouse embryogenesis suggests multiple developmental roles. Gene Expr Patterns. 2005;5(6):750-5.
28. Weng AP, Ferrando AA. Notch signaling in leukemia. Curr Opin Hematol. 2016;20(4):254-60.
29. Tschaharganeh DF, Xue W, Calvisi DF, et al. p53-dependent Nestin regulation links tumor suppression to cellular plasticity in liver cancer. Cell. 2014;158(1):579-92.
30. Zhao J, Shi W, Wang YF, Chen H, Cong W, Zhu H, et al. Role of Notch and Hedgehog signaling pathways in regulating liver progenitor cell proliferation and differentiation during rat acute liver failure repair. Biotechnol Lett. 2015;37(11):2405-15.
31. Real PJ, Ferrando AA. NOTCH inhibition and glucocorticoid therapy in T-cell acute lymphoblastic leukemia. Leukemia. 2009;23(8):1374-7.
32. Wu Y, Cain-Hom C, Choy L, et al. Therapeutic antibody targeting of individual Notch receptors. Nature. 2010; 464 (7291):1052-7.
33. Meng RD, Shelton CC, Li Y, et al. Gamma-secretase inhibitors abrogate oxaliplatin-induced survival and augment drug sensitivity. Cancer Res. 2009;69(2):573-82.