

Etiology-Driven Mouse Models of Hepatocellular Carcinoma: Paving the Way for Precision Oncology

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Background: Hepatocellular carcinoma (HCC) is biologically heterogeneous, and its genomic alterations, inflammatory context, and tumor immune microenvironment are strongly shaped by the underlying etiology, including chronic hepatitis B virus (HBV) infection, metabolic dysfunction-associated steatotic liver disease (MASLD), and alcohol exposure. This etiological diversity complicates the selection and interpretation of preclinical models. Genetically engineered mouse models (GEMMs), particularly when combined with dietary, chemical, viral, or alcohol-related insults, provide useful systems for dissecting how defined genetic drivers interact with disease-specific liver environments in an immunocompetent host.

Main Body: This review summarizes etiology-aligned GEMMs and related mouse models for HCC. In HBV-related models, we discuss how viral antigen exposure, HBV-associated genomic instability, *Tert* activation, and *Trp53* loss support a multi-driver framework rather than a single-lesion model of carcinogenesis. In MASLD-associated HCC, we examine models involving Wnt/ β -catenin activation, *Acvr2a* loss, *Pten* deficiency, and *MUP-uPA* driven steatohepatitis, emphasizing their value for studying immune exclusion, lactate-rich immunosuppression, IgA+ plasma-cell accumulation, and altered responses to immune checkpoint blockade. In alcohol-associated HCC, we review models centered on *Aldh2* deficiency, ER stress-lysosomal lipid remodeling through the ATF4/LPLA2 axis, NF- κ B-related inflammatory regulation, and neutrophil-driven tumor promotion. Across these etiologies, we compare model strengths and limitations, including tumor latency, penetrance, reproducibility, lack of cirrhotic remodeling, sex-dependent variability, microbiome-related environmental effects, and incomplete modeling of tumor-stroma co-evolution.

Short Conclusion: Etiology-aligned GEMMs can help match biological questions to appropriate preclinical platforms and generate testable hypotheses about therapy response. However, etiology alone should not be treated as a substitute for molecular profiling or clinical validation. Future models should integrate precise genetic engineering with fibrotic or cirrhotic backgrounds, microbiome-aware environmental modulation, and complementary human-relevant systems to better capture the complex evolution of human HCC.

Keywords: hepatocellular carcinoma, mouse models, HBV, MASLD, alcohol-associated hcc, precision medicine

Introduction

Primary liver cancer represents a major global health burden. According to GLOBOCAN statistics, it ranks as the sixth most common malignancy worldwide and the third leading cause of cancer-related mortality.¹ Histologically, hepatocellular carcinoma (HCC) accounts for approximately 75% to 85% of all primary liver cancer cases.² Although molecularly targeted therapies and immunotherapies have expanded the therapeutic landscape of HCC, the initiation and progression of this disease remain governed by complex interactions among genetic alterations, chronic liver injury, inflammation, fibrosis, and immune remodeling.³ A central feature of HCC is etiological heterogeneity: beyond traditional hepatitis B virus (HBV) and hepatitis C virus (HCV) infections, metabolic dysfunction-associated steatotic liver disease (MASLD),

formerly grouped under NAFLD) is rapidly emerging as a major contributor to HCC burden, while alcohol-associated HCC continues to rise globally.^{4,5}

Animal models are indispensable for dissecting HCC pathogenesis and testing therapeutic hypotheses, but each model class has intrinsic limitations. Chemically induced models can reproduce selected injury and inflammatory processes but often lack genetic precision. Xenograft models are useful for tumor growth and drug-response studies but usually fail to recapitulate an intact immune system and the chronic liver-disease background in which most human HCCs arise. In contrast, genetically engineered mouse models (GEMMs), using systems such as Cre-loxP and CRISPR/Cas9, allow controlled manipulation of defined oncogenic drivers or tumor suppressors in an immunocompetent host.^{6,7} However, no single GEMM fully reproduces the complete inflammation–fibrosis–cirrhosis–HCC sequence observed in humans. Their interpretability depends on model-selection parameters such as tumor latency, penetrance, strain background, sex, environmental injury, microbiome status, immune competence, and the degree of fibrosis or cirrhosis achieved before tumor development.

Given the biological differences among HBV-related, MASLD-associated, and alcohol-associated HCC, etiology-aligned GEMMs provide a useful framework for matching specific mechanistic questions to appropriate preclinical platforms. For example, HBV-related models are useful for studying viral antigen exposure, HBx-mediated host-gene disruption, genomic instability, and cooperation with host drivers such as TERT activation or TP53 loss. MASLD-associated models can be used to interrogate Wnt/ β -catenin activation, ACVR2A loss, metabolic stress, and immune exclusion. Alcohol-associated models provide insight into acetaldehyde metabolism, ER stress, lysosomal lipid remodeling, and inflammation-driven tumor promotion. Nevertheless, etiology should be regarded as a model-selection context rather than a validated surrogate for molecular profiling or clinical treatment selection.

This review summarizes the current landscape of etiology-aligned GEMMs and related mouse models for HCC, focusing on HBV-related, MASLD-associated, and alcohol-associated disease. HCV-related models are not treated as a central category because direct-acting antivirals have substantially reduced HCV-driven disease burden, but the residual HCC risk after sustained virologic response, particularly in patients with advanced fibrosis or cirrhosis, is addressed in the discussion of translational limitations. We emphasize model construction strategies, mechanistic insights, tumor latency and penetrance, reproducibility, fibrosis/cirrhosis status, and major limitations, with the goal of providing a critical framework for selecting appropriate preclinical systems rather than presenting GEMMs as direct substitutes for human molecular stratification.

HBV-Related HCC Models

Despite substantial progress in antiviral therapies, chronic Hepatitis B Virus (HBV) infection remains the primary driver of HCC globally, imposing a heavy clinical burden that is difficult to eradicate.^{8,9} In recent years, GEMMs based on the full HBV genome or specific viral proteins (eg., HBsAg, HBx) have successfully simulated the state of chronic viral antigen exposure in hosts. These models have played a key role in deciphering carcinogenic mechanisms, particularly confirming that the cytotoxicity of HBsAg and the transcriptional regulatory functions of HBx drive malignant transformation by inducing persistent hepatic inflammation, remodeling host gene expression profiles, and disrupting genomic stability.^{10–12}

Beyond these mechanisms, HBV DNA integration into the host genome and the consequent genomic instability represent another core mechanism driving hepatocarcinogenesis.¹³ Whole-genome sequencing analyses have revealed characteristic high-frequency somatic mutations in HBV-related HCC, predominantly involving key genes such as *TERT* and *TP53*.^{14,15} To precisely mimic the synergistic oncogenic effects of viral proteins and host gene mutations, researchers frequently employ composite models combining HBx transgenic mice with gene recombination technologies. A paradigmatic example involves using the Cre-loxP system to specifically activate *Kras*G12D mutations in the livers of HBx transgenic mice, thereby confirming the synergistic effect of HBx and *Kras* mutations in accelerating hepatocarcinogenesis.¹⁶ Among the affected genes, Telomerase Reverse Transcriptase (*TERT*) holds special pathological significance: it is not only the most common target for HBV genomic integration,¹⁴ but its promoter mutations are also the most specific and highly prevalent genetic alterations in HBV-related HCC.¹⁷ Currently, intervention strategies

targeting *TERT* and its downstream products—ranging from traditional inhibitors to emerging base-editing therapies for mutant promoters—have become a major direction in HCC therapeutic development.¹⁸

Regarding model construction, previous studies often utilized *Tert* knockout mice to analyze the roles of telomere shortening and genomic instability in liver cancer evolution.¹⁹ However, existing *Tert* overexpression models have largely focused on anti-aging and tissue regeneration. Authoritative studies indicate that in mice with normal tumor suppressor function (eg., wild-type *p53*), *Tert* overexpression alone primarily delays aging and extends lifespan, but is insufficient to induce spontaneous malignancies, particularly HCC.^{20,21} This limitation was further corroborated by recent findings from Mishima et al (2024). Using Cre-*loxP* technology to construct liver-specific *Tert* overexpression mice, they discovered that even under the backdrop of chemically induced chronic inflammation, *TERT* upregulation alone failed to cross the threshold for carcinogenesis; significant HCC development occurred only when combined with *Trp53* deletion—a “second hit”.²² This suggests that the oncogenic process of *Tert* is strictly environment-dependent, often requiring synergy with genomic instability or specific oncogenic signals.

Although chronic HBV infection provides an environment of chronic inflammation and genomic damage, there is a lack of double transgenic models in the literature that combine HBV transgenes with *Tert* overexpression. In HBV-associated HCC, *TP53* is a key driver gene with a mutation frequency second only to the *TERT* promoter.²³ As a core tumor suppressor, *p53* function is often directly inhibited by the HBV X protein (HBx), which blocks *p53*’s sequence-specific DNA binding and transcriptional activity, thereby deregulating the cell cycle and apoptosis.²⁴ However, experiments have shown that *Trp53* knockout alone in the context of HBV infection has low oncogenic efficiency and a long latency period, limiting its feasibility as a high-efficiency experimental model. Consequently, multi-gene editing strategies have been widely adopted. Literature confirms that simultaneous knockout of *Trp53* and *Pten* in HBV transgenic mice using CRISPR/Cas9 significantly accelerates HCC onset and progression compared to *Pten* knockout alone.²⁵ Importantly, the tumors induced by this model are not only pathologically confirmed as HCC but also mirror the molecular features of human HCC at the transcriptomic level.²⁶ Thus, whether it is *TERT* activation or *TP53* inactivation, HBV-driven carcinogenesis often requires a specific synergistic milieu—namely, virus-induced chronic inflammation and genomic instability. The findings by Mishima et al and the success of the *Trp53/Pten* dual-knockout model strongly validate the “Multi-hit” hypothesis in liver cancer modeling. Therefore, constructing dual/multi-driver models capable of simultaneously simulating the HBV persistent infection environment and key genetic variations (such as *Tert* or *Trp53*) represents a critical breakthrough for overcoming current limitations and precisely recapitulating the evolutionary laws of human HBV-HCC (Figure 1).

Metabolic-Associated HCC Models

Non-alcoholic fatty liver disease (NAFLD), recently redefined by international consensus as Metabolic Dysfunction-Associated Steatotic Liver Disease (MASLD), is currently the fastest-growing chronic liver disease globally. As the primary driver for the surge in HCC incidence,²⁷ MASLD-associated HCC shows a trend toward younger patients and is projected to surpass viral hepatitis as the leading cause of liver cancer worldwide within the next decade.⁵ Furthermore, MASLD often acts as a critical comorbidity, synergizing with HBV, HCV, or alcohol consumption to accelerate malignant progression.^{28,29}

At the molecular pathology level, metabolic-associated HCC exhibits a distinct landscape compared to viral HCC. While HBV-related HCC is enriched in *TP53* mutations, metabolic HCC is driven by a unique mutational spectrum. Genomic analyses confirm that aberrant activation of the Wnt/ β -catenin pathway (represented by *CTNNB1* mutations) and inactivation of the TGF- β pathway (characterized by *ACVR2A* mutations) are the most prominent and characteristic molecular driver events in metabolic HCC.³⁰ These unique features provide a clear genetic basis for utilizing gene-engineering technologies (eg., targeting *Ctnnb1* or *Acvr2a*) to construct etiology-informed metabolic HCC mouse models.

CTNNB1 (β -catenin) mutations are the most characteristic molecular event in Metabolic Dysfunction-Associated Steatohepatitis (MASH)-HCC, occurring in approximately 20%–39% of cases, whereas they are rarer in HBV-related HCC.³¹ Mechanistically, mutations in Exon 3 of *CTNNB1* are the most common. This region encodes serine/threonine residues crucial for β -catenin phosphorylation; mutations here prevent phosphorylation and ubiquitination, thereby

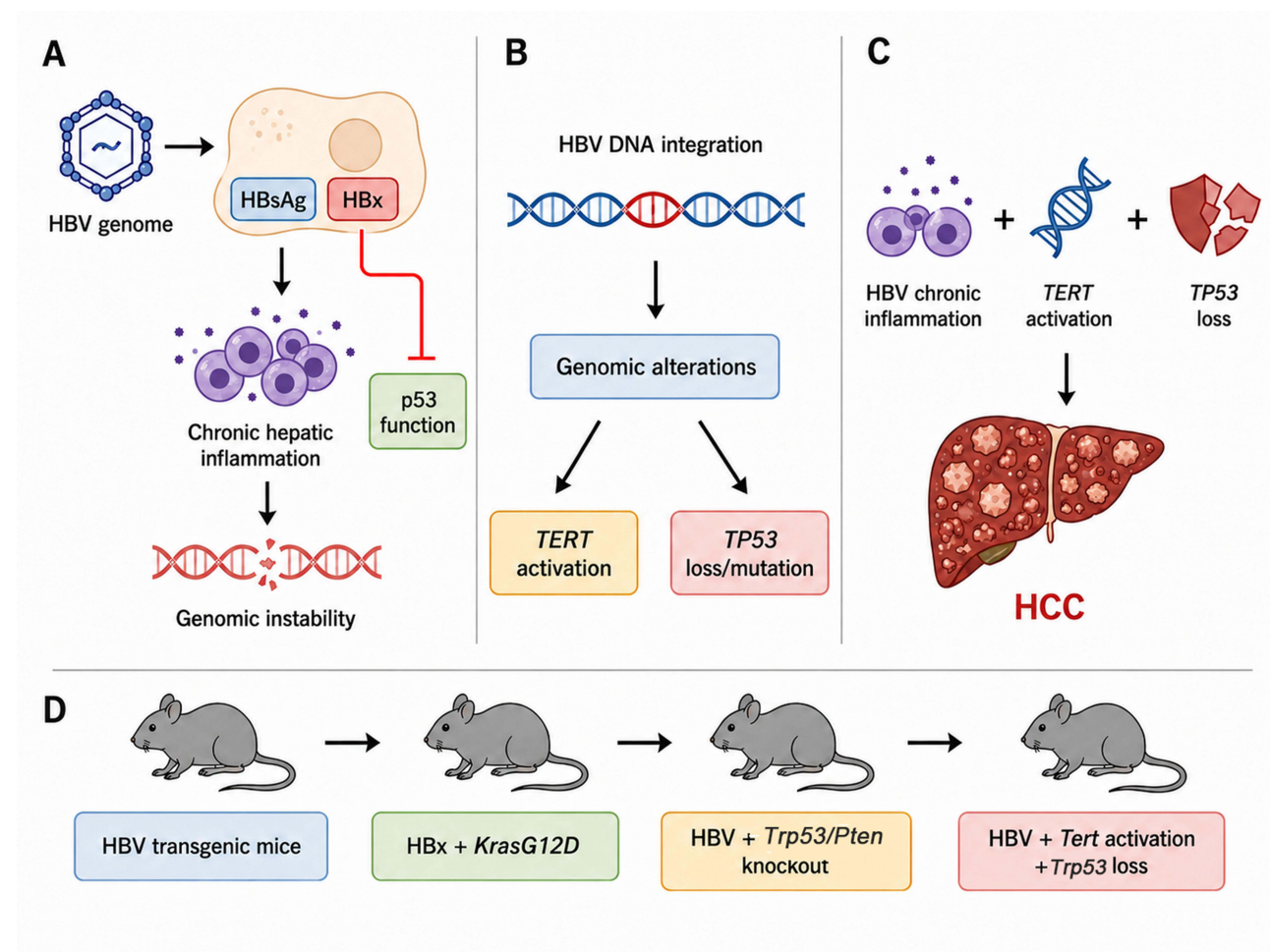


Figure 1 Mechanisms of HBV-associated hepatocarcinogenesis and the evolution of genetically engineered mouse models (GEMMs). **(A)** Chronic HBV antigen exposure promotes hepatocarcinogenesis through the expression of viral proteins such as HBsAg and HBx, which contribute to persistent hepatic inflammation, disruption of p53-related tumor-suppressive signaling, and genomic instability. **(B)** HBV DNA integration into the host genome generates genomic alterations that frequently converge on key driver events, including TERT activation and TP53 loss or mutation. **(C)** HBV-related HCC is better represented by an environment-dependent multi-hit model rather than by a single-driver mechanism. Chronic inflammatory injury, TERT activation, and TP53 inactivation may cooperate to cross the threshold for malignant transformation. **(D)** The conceptual evolution of HBV-related HCC mouse models progresses from simple HBV transgenic mice modeling chronic viral antigen exposure to composite models combining viral factors with oncogenic drivers (e.g., HBx plus *Kras*^{G12D}). High-efficiency models utilize CRISPR-mediated *Trp53/Pten* disruption in HBV transgenic backgrounds, and future multi-driver models aim to integrate HBV-associated injury with *Tert* activation and *Trp53* loss.

allowing the protein to escape proteasomal degradation.^{32,33} This “stabilizing mutation” leads to cytoplasmic accumulation and nuclear translocation of β -catenin, constitutively activating Wnt signaling to drive cell proliferation, metabolic reprogramming, immune evasion, and metastasis.^{31,32} Early attempts to model this using Albumin (Alb) promoter-driven Cre recombinase to delete Exon 3 of *Ctnnb1* were problematic; Harada et al found that this strategy caused severe hepatomegaly and lethality in mice within 20–30 days post-birth, without inducing overt liver tumors, suggesting that constitutive β -catenin activation alone during early development may be lethal or insufficient for independent carcinogenesis.³⁴

To address this, researchers optimized modeling strategies. Professor Fangming Liu’s team utilized tail vein injection of Adenovirus-Cre to achieve spatiotemporally specific activation of *Ctnnb1* in adult mouse livers, establishing a model that captures selected pathological and molecular features. Using this model, they further employed Cre-*loxP* technology to co-delete *Trp53* or crossbreed with HBV transgenic mice, confirming that multi-gene synergy significantly accelerates tumor progression. They also revealed that oncogenic β -catenin-stimulated AKT2-CAD-mediated pyrimidine synthesis is a druggable vulnerability in liver cancer.³⁵ Additionally, a team from Zhejiang University used in situ genome editing to systematically construct composite models combining *CTNNB1* with the overexpression of oncogenes like *MYC*, *RAS*,

AKT, or *EGFR*. These combined models achieved high tumor induction in selected combinations and modeled aspects of molecular heterogeneity.³⁶ In the realm of immunotherapy, given that *CTNNB1* mutations are often associated with T-cell exclusion (“immune-cold” tumors), recent studies using activated *Cttnb1* models have explored strategies to improve immune efficacy. It was reported that targeting Matrix Metalloproteinase 9 (MMP9) effectively restores the anti-tumor immune activity of CD8+ T cells in *Cttnb1*-mutated HCC, thereby significantly enhancing the efficacy of anti-PD-1 therapy.³⁷

ACVR2A, a key type II transmembrane serine/threonine kinase receptor in the TGF- β superfamily, plays a vital role in negative immune regulation and growth inhibition in maintaining liver homeostasis.³⁸ Under physiological conditions, Activin signaling acts as a “molecular brake,” limiting excessive hepatocyte proliferation and inducing apoptosis in abnormal cells.³⁹ However, loss of *ACVR2A* function leads to uncontrolled downstream signaling, relieving transcriptional repression of key glycolytic enzymes and resulting in significant upregulation of LDHA and MCT4, thus driving the Warburg effect in tumor cells. This metabolic reprogramming not only meets the energy demands of rapid tumor proliferation but, crucially, constructs a lactate-rich immunosuppressive microenvironment. High lactate concentrations specifically recruit regulatory T cells (Tregs) while directly inhibiting the cytotoxic function of CD8+ T cells, leading to immune escape.^{40,41} Large-scale genomic profiling confirms that *ACVR2A* mutations are indeed etiologically characteristic of MASH-HCC.³¹ Given its specificity and profound impact on the immune microenvironment, recent studies have primarily employed CRISPR-Cas9-edited syngeneic transplant models to elucidate its function. Yasukawa et al (2025) successfully replicated the high lactate metabolism and Treg infiltration features observed in human MASH-HCC by implanting *Acvr2a*-knockout mouse liver cancer cell lines into immunocompetent mice.⁴⁰ Furthermore, liver-specific knockout mice based on the *Acvr2a^{fllox/flox}* and Alb-Cre system provide genetic tools to further explore early molecular events in the progression from MASH to HCC.

Beyond these, *Pten* liver-specific knockout models and *MUP-uPA* transgenic models are typical GEMMs for simulating the pathological evolution of metabolic liver cancer. *PTEN* is a widely expressed tumor suppressor. Upon liver-specific *Pten* deletion via the Alb-Cre system, mice develop a steatosis–steatohepatitis–fibrosis–HCC sequence of “Steatosis $\rightarrow$ MASH $\rightarrow$ Fibrosis $\rightarrow$ HCC,” typically developing macroscopic tumor nodules by 12–15 months of age, with pathological morphology resembling selected features of human MASH-HCC.^{41,42} This model is widely used not only for studying HCC mechanisms but also for exploring MASH pathophysiology and drug interventions.^{43–45} The *MUP-uPA* mouse model is constructed by integrating the urokinase-type plasminogen activator (*uPA*) gene driven by the Major Urinary Protein (*MUP*) promoter into the genome. This model induces hepatocyte Endoplasmic Reticulum (ER) stress via uPA overexpression; when combined with a High-Fat Diet (HFD), mice rapidly progress to MASH within 10–18 weeks and develop tumors by 26–34 weeks.⁴⁶

Using this model, Shalapour et al deeply analyzed the immune microenvironment of MASH-HCC, discovering that MASH-induced chronic inflammation promotes the accumulation of IgA+ plasma cells. These cells directly inhibit CD8+ T cell cytotoxicity by expressing PD-L1 and IL-10, creating an immunosuppressive landscape that shares features with human MASH-HCC.⁴⁷ Building on this, a *Nature* study by Pfister et al further revealed that the MASH environment causes CD8+ T cells to adopt an “exhausted and auto-aggressive” state. These aberrant T cells lose their tumor surveillance function and instead promote HCC progression by exacerbating tissue damage, explaining why immune checkpoint inhibitors have limited efficacy in MASH-HCC.⁴⁸ Regarding metabolic mechanisms, a recent *Nature* study identified Fructose-1,6-bisphosphatase 1 (FBP1), a key gluconeogenic enzyme, as having important tumor-suppressive functions. The aberrant loss of FBP1 was found to be a critical “molecular switch” driving the progression from MASH to HCC. Researchers utilized Cre-*loxP* technology to construct *MUP-uPA/Fbp1* double-mutant and *Trp53/Fbp1* double-mutant mice, confirming that *Fbp1* knockout significantly accelerates MASH-HCC pathology and shortens tumor latency.⁴⁹

Regarding clinical application, a review by Fornari summarized the efficacy evaluation of various immune and metabolic drugs in MASH-related HCC models but noted a lack of model trials targeting hepatocyte-specific delivery for precise intervention.⁵⁰ In summary, unlike HBV-related HCC, which is primarily controlled by viral integration and *TP53* mutations, MASH-HCC presents a unique genetic landscape characterized by *CTNNB1* activation and *ACVR2A* inactivation. This essential difference dictates that research paradigms for viral liver cancer cannot simply be applied here. The

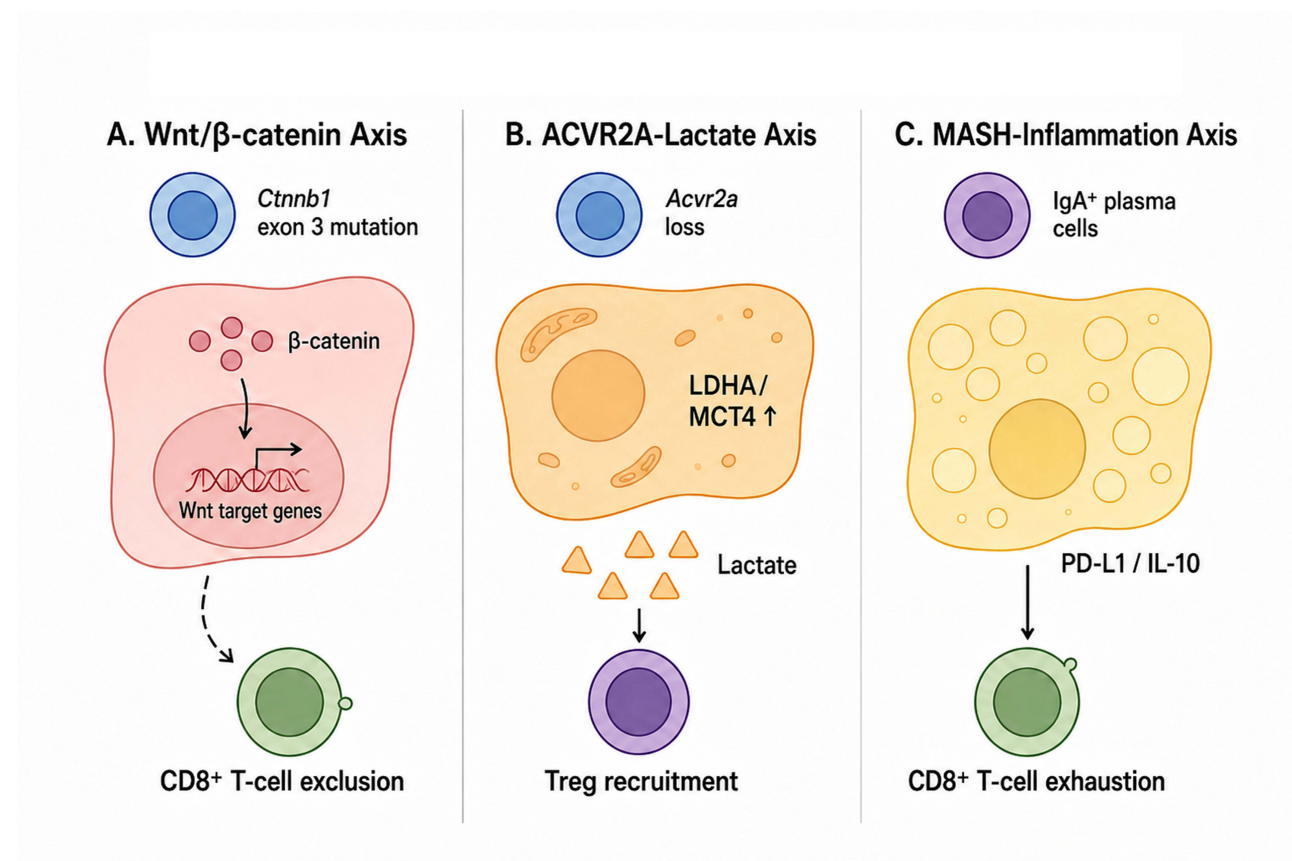


Figure 2 Distinct metabolic drivers and immune microenvironment remodeling in MASLD-associated HCC. **(A)** The Wnt/ β -catenin axis represents a characteristic molecular pathway in metabolic HCC. Models with hepatocyte β -catenin activation, including *Ctnnb1* exon 3 mutations or related genome-editing strategies, activate Wnt target-gene transcription and are associated with CD8⁺ T-cell exclusion, providing a mechanistic basis for immune-cold HCC phenotypes. **(B)** The *Acvr2a*-lactate axis illustrates how loss of Activin receptor signaling promotes metabolic reprogramming. *Acvr2a* deficiency increases LDHA/MCT4 expression and lactate production, generating a lactate-rich immunosuppressive microenvironment that favors regulatory T-cell (Treg) recruitment. **(C)** The MASH-inflammation axis highlights immune remodeling in steatohepatitis-associated HCC models. In models such as MUP-uPA combined with a high-fat diet, chronic metabolic inflammation promotes the accumulation of IgA⁺ plasma cells expressing PD-L1 and IL-10. This microenvironment promotes CD8⁺ T-cell exhaustion, suppresses cytotoxicity, and contributes to impaired antitumor immune surveillance and reduced responsiveness to immune checkpoint blockade.

“metabolic-immune” microenvironments replicated by models like *Pten* knockout or *MUP-uPA* confirm the existence of specific immune evasion mechanisms (eg., auto-aggressive CD8⁺ T cells) in metabolic HCC. Deepening the application of these specialized models will help us precisely address the growing challenge of metabolic HCC in the “post-viral hepatitis era” and provide theoretical support for developing combined metabolic-immune therapies distinct from traditional chemotherapy (Figure 2).

A critical appraisal of established MASLD-HCC models is therefore essential. Liver-specific *Pten* deletion recapitulates selected aspects of steatohepatitis-associated hepatocarcinogenesis, but its long latency, pathway-specific genetic background, and variable penetrance limit its generalizability as a universal MASLD-HCC model.^{41–45} Similarly, *MUP-uPA* combined with high-fat diet accelerates steatohepatitis and tumor development, but the model is strongly shaped by uPA-induced endoplasmic reticulum stress and dietary injury, which may introduce metabolic and inflammatory confounders.^{46,47,49} These models should therefore be selected according to the specific biological question, with latency, penetrance, strain background, sex, fibrosis stage, and reproducibility reported whenever available.

Alcohol-Associated HCC Models

Epidemiological Background and Current Models

Recent epidemiological data indicate a continuous upward trend in global alcohol exposure, with approximately 30% of new liver cancer cases annually attributable to long-term alcohol consumption.⁵¹ The pathological evolution of Alcohol-

Associated HCC (AL-HCC) spans the entire spectrum from Alcoholic Fatty Liver (AFL) and Alcoholic Steatohepatitis (ASH) to cirrhosis and HCC. This malignant transformation involves multidimensional molecular remodeling, in which ALDH2 (alcohol metabolism), LPLA2 (lipid remodeling and stress), NF- κ B1 (inflammatory regulation), and c-MYC (oncogene activation) exhibit distinct etiology-dependent functions in the pathogenic pathway of alcohol.^{52,53} Traditional AL-HCC mouse models mainly rely on combinatorial chemical induction (eg., Ethanol combined with CCl₄ or Lieber-DeCarli diet).⁵⁴ To overcome the issues of low tumorigenicity and long cycles in traditional models, Alarcón-Sánchez et al (2024) developed a novel chemical model based on “Multiple Hepatotoxics.” By combining ethanol, Lipopolysaccharide (LPS), sucrose, and low-dose Diethylnitrosamine (DEN), they successfully reproduced the full spectrum of human ALD pathology—from steatosis and inflammatory fibrosis to eventual HCC—in mice.⁵⁵ Although such improved chemical models have made breakthroughs in simulating multifactorial synergy, their complex inducing factors make it difficult to precisely dissect the specific contributions of single genes or pathways in an alcohol context. Therefore, GEMMs based on specific targets remain irreplaceable for elucidating AL-HCC molecular mechanisms and screening targeted drugs.

Key GEMMs and Mechanisms

ALDH2 Deficiency Model: A Specific Target for Metabolic-Immune Interaction

Mitochondrial Aldehyde Dehydrogenase 2 (ALDH2) is the key enzyme for clearing acetaldehyde and exhibits high etiological specificity. *ALDH2* mutations are rare in non-alcoholic HCC (<5%) but significantly promote carcinogenesis under alcohol exposure via the accumulation of acetaldehyde-DNA adducts; notably, this mutation is highly prevalent in East Asian populations.^{56,57} To simulate the high-risk clinical scenario of “cirrhosis background + alcohol exposure,” Seo et al (2019) constructed a liver-specific *Aldh2* knockout mouse model. This model achieved a tumorigenesis rate of 80%-90% at 28 weeks, effectively addressing the deficiencies of traditional models.⁵⁸ Recent studies further revealed that acetate accumulation resulting from ALDH2 deficiency serves as “metabolic fuel” for tumor-associated macrophages, driving HCC metastasis, thus establishing it as a critical target for metabolic-immune therapy.⁵⁹

ER Stress and Lysosomal Lipid Remodeling Model (ATF4/LPLA2 Axis)

Beyond direct acetaldehyde toxicity, alcohol-induced organelle dysfunction is another core mechanism of AL-HCC. A recent study by Zhou et al (2025) revealed a novel oncogenic axis linking Endoplasmic Reticulum (ER) stress to lysosomal lipid remodeling.⁶⁰ The study found that chronic alcohol exposure-induced ER stress upregulates the transcription factor ATF4, which directly binds to and activates the promoter of *PLA2G15* (*LPLA2*). Abnormally high expression of LPLA2 leads to the significant accumulation of BMP (bis(monoacylglycero)phosphate), a lipid unique to lysosomes. This aberrant lipid microenvironment further activates the MAPK/ERK signaling pathway, driving tumor proliferation. Notably, this mechanism was validated in an HBx transgenic mouse (HBx-Tg) model fed with alcohol: alcohol significantly accelerated HBx-mediated hepatocarcinogenesis via the ATF4/LPLA2 axis. This suggests that constructing liver-specific *Pla2g15* overexpression mice or *Pla2g15/Cln5* gene-modified mice holds promise as an ideal new tool for studying the synergistic carcinogenesis of alcohol-induced metabolic remodeling and viral infection.⁶⁰

NF- κ B1 Deficiency Model: Neutrophil-Driven Inflammatory Mechanisms

The NF- κ B pathway permeates the entire process of alcohol-induced chronic inflammation initiation, malignant transformation, and drug resistance.^{61,62} Research by Wilson et al confirmed that *Nfkb1* is a key tumor suppressor gene inhibiting neutrophil-driven HCC. *Nfkb1* knockout mice constructed via ES cell homologous recombination exhibit chronic liver disease characteristics, with approximately 50% progressing spontaneously to HCC by 20 months of age, and show increased sensitivity to chemical carcinogens.⁶³ Furthermore, the regulatory effect of NF- κ B1 on PD-L1 transcription and subsequent tumor immune evasion—achieved through direct binding to the PD-L1 promoter—has been validated in general hepatocellular carcinoma models (not restricted to alcohol-associated disease).⁶⁴ This regulatory mechanism is equally applicable to alcohol-driven hepatocarcinogenesis. A current limitation is that this model typically involves whole-body knockout; there is an urgent need to develop liver-specific NF- κ B pathway modification models to exclude the interference of systemic inflammation.⁶⁵

P16 (CDKN2A)-Related Mechanisms: Epigenetic Remodeling and Dual Roles

P16 is a classic tumor suppressor, and its inactivation in AL-HCC exhibits unique metabolic dependence. Recent clinical research (Gut, 2025) revealed the dynamic dual role of P16 in alcoholic liver disease: in the early stages, alcohol-induced DNA damage activates P16 expression, inducing cellular senescence and the secretion of SASP factors, thereby exacerbating liver inflammation;⁶⁶ in advanced stages, long-term alcohol metabolism disrupts the SAME (methyl donor) cycle, leading to hypermethylation of the *CDKN2A* promoter and expression silencing, ultimately promoting the malignant transformation from cirrhosis to HCC.⁶⁷ Although p16 editing has been explored in large-animal platforms such as Oncopigs, no established mouse model currently reproduces the dynamic sequence of early *CDKN2A* activation followed by late epigenetic silencing during alcohol-associated hepatocarcinogenesis.⁶⁸ Therefore, *CDKN2A* should be viewed as a clinically relevant epigenetic mechanism and a defined modeling gap, rather than as an established AL-HCC mouse model. Future work will require mouse systems capable of separating transient senescence-associated *Cdkn2a* activation from stable promoter methylation-mediated silencing in advanced alcohol-associated liver disease.

Myc Overexpression Model: Etiology-Oriented Molecular Subtyping

The c-Myc protein is a core molecule maintaining stem cell pluripotency,⁶⁹ and the *MYC* gene is highly expressed in up to 70% of patients with HBV- and alcohol-associated hepatocellular carcinoma.⁷⁰ Genomic analysis shows that the tumor transcriptomic profile of *Myc* overexpression mice most closely matches human alcohol-associated liver cancer, embodying the “Etiology-Oriented” feature of the model.⁷¹ Liver-specific *Myc* overexpression mice constructed using Cre-*loxP* technology progress rapidly, with male mice exhibiting early lesions at 47 days and advancing to late-stage liver cancer within 3 months.⁷² As this model not only reproduces the aggressive phenotype of AL-HCC but also presents specific immune barriers, it is now widely used to evaluate the efficacy of immunotherapies.⁷³

In summary, existing GEMMs have evolved from single oncogene validation to deep mechanistic dissection of alcohol-specific metabolic toxicity (eg., ALDH2, PLA2G15 pathways) and microenvironmental remodeling (eg., NF- κ B, P16). The latest findings by Zhou et al (2025) regarding the ER stress-lipid metabolism axis offer a completely new perspective for understanding the synergistic carcinogenic mechanisms of alcohol and viruses (HBV). However, single gene modifications still struggle to fully replicate the complex etiological features of human AL-HCC. Future modeling strategies will trend toward “multi-gene modification combined with environmental induction” to better simulate the tumor immune microenvironment of clinical patients, thereby providing a more reliable preclinical platform for screening precision therapeutics for alcoholic liver cancer (Figure 3).

Beyond intrinsic cellular signaling and classical environmental insults, the gut-liver axis is an important modifier of etiology-associated hepatocarcinogenesis. Microbiota-derived metabolites can reshape hepatic inflammation, bile acid signaling, metabolic stress, and tumor immunity in both MASLD- and alcohol-associated liver diseases.⁷⁴ Future GEMM strategies should therefore incorporate microbiome-aware approaches, including co-housing, antibiotic depletion, germ-free or gnotobiotic housing, fecal microbiota transplantation, defined dietary protocols, or colonization with patient-derived microbial communities. These approaches may help distinguish hepatocyte-intrinsic genetic effects from microbiome-dependent inflammatory signals and improve the reproducibility of etiology-aligned preclinical models.

Conclusion and Perspectives

This article has systematically reviewed key advances in Genetically Engineered Mouse Models (GEMMs) for recapitulating Hepatocellular Carcinoma (HCC) induced by diverse etiologies. From simulating HBV integration-driven carcinogenesis (eg., *Tert* promoter mutations,¹⁷ HBx and *Trp53* synergy^{25,26}), to elucidating immune microenvironment remodeling in metabolic HCC (eg., the discovery of IgA+ plasma cells in MUP-uPA models⁴⁷ and the critical role of *Fbp1* loss⁴⁹), and exploring metabolic defects in alcohol-driven carcinogenesis (*Aldh2* mutations⁵⁸ and NF- κ B pathways⁶³), GEMMs have successfully established an etiology-based research atlas covering multiple molecular subtypes (Table 1).

Therefore, etiology-based GEMM research may help prioritize biologically relevant model systems and generate testable hypotheses regarding therapeutic vulnerability across HCC subtypes.^{3,71} However, etiology alone should not be treated as a validated surrogate for molecular profiling or as a current basis for clinical treatment selection. Its predictive

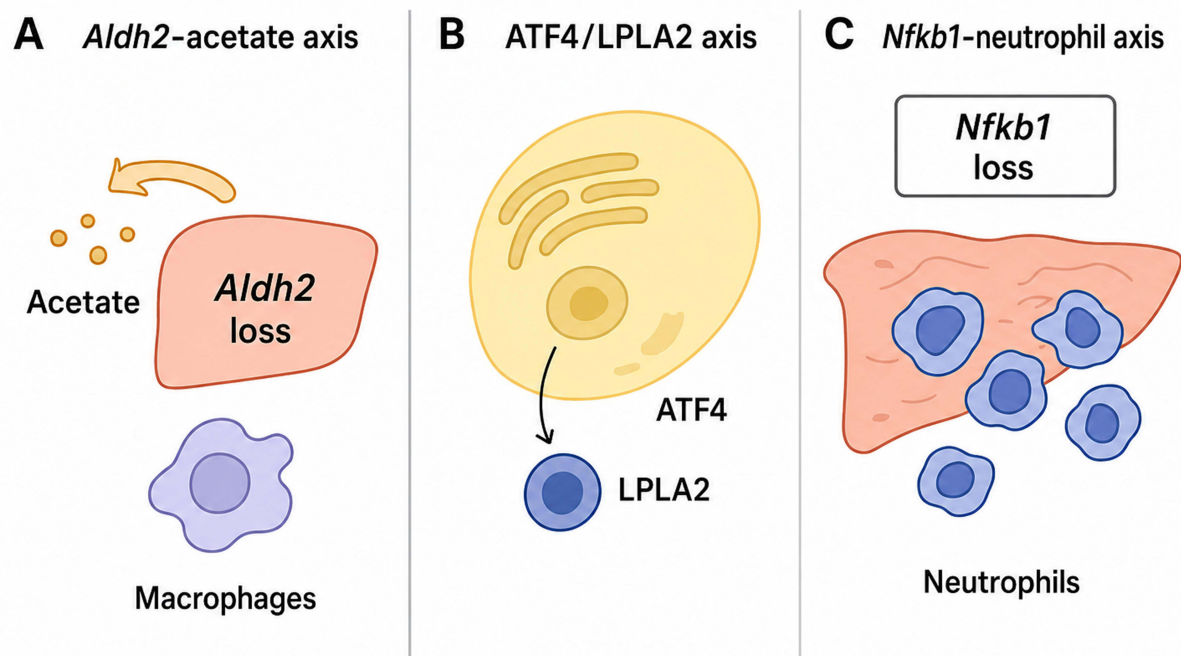


Figure 3 Unique metabolic and inflammatory mechanisms in Alcohol-Associated HCC models **(A)** The *Aldh2*–acetate axis illustrates how impaired aldehyde metabolism can promote alcohol-associated hepatocarcinogenesis. Liver-specific *Aldh2* deficiency enhances tumor development under alcohol exposure, while acetate accumulation may provide metabolic support for tumor-associated macrophages (TAMs) and facilitate metastatic progression. **(B)** The ATF4/LPLA2 axis links alcohol-induced endoplasmic reticulum (ER) stress to lysosomal lipid remodeling. Chronic alcohol exposure activates ATF4, which upregulates LPLA2/PLA2G15 and promotes the accumulation of lysosome-enriched BMP lipids, thereby enhancing oncogenic signaling and hepatocarcinogenesis. **(C)** The *Nfkb1*–neutrophil axis represents an inflammation-driven mechanism of liver tumor promotion. *Nfkb1* deficiency enhances chronic hepatic inflammation and neutrophil-associated tumor development. However, because established *Nfkb1* models commonly involve systemic gene disruption, future liver- or cell-type-specific models are needed to separate hepatocyte-intrinsic effects from systemic inflammatory contributions.

value for therapy response requires rigorous validation in human cohorts and prospective translational studies. In addition, although HCV-related models are not the central focus of this review because direct-acting antivirals have substantially reduced HCV-driven disease burden,^{75,76} sustained virologic response does not completely eliminate HCC risk, particularly in patients with pre-existing advanced fibrosis or cirrhosis. HCV-associated models therefore remain relevant for selected questions, including post-SVR carcinogenesis, persistent epigenetic or inflammatory alterations, fibrotic niche remodeling, and surveillance strategies in post-cure populations.⁷⁷

Despite promising prospects, current GEMMs still suffer from two fatal shortcomings:

First, the lack of a cirrhotic background. Clinical HCC predominantly arises on a foundation of cirrhosis, whereas existing models (especially *Alb-Cre* mediated specific gene knockouts) often induce tumors directly, skipping the classic “inflammation-fibrosis-cirrhosis” evolutionary stage (Table 2). Mechanistically, the absence of cirrhosis is not merely a histological limitation. Cirrhosis alters extracellular matrix stiffness, sinusoidal architecture, hypoxia, vascular remodeling, immune-cell localization, hepatocyte regenerative pressure, liver functional reserve, and drug metabolism. As a result, non-cirrhotic GEMMs may overestimate therapeutic efficacy or fail to capture toxicity and immune-response patterns that emerge in cirrhotic human HCC.^{6,50} Sex should also be incorporated as a biological variable in GEMM-based HCC research. Human HCC shows marked male predominance, and several mouse models exhibit sex-dependent differences in tumor latency, penetrance, inflammatory signaling, and hormone-regulated immune responses. Future

Table 1 Key Genetically Engineered Mouse Models (GEMMs) for Etiology-Specific Hepatocellular Carcinoma

Etiology	Target Gene/ Model	Construction Strategy	Key Pathological & Molecular Features	Translational Implications & Ref.
HBV-Related	HBx-Tg + <i>Kras</i>^{G12D}	<i>Alb-Cre</i> mediated activation of <i>Kras</i> ^{G12D} in HBx transgenic mice	Accelerated hepatocarcinogenesis; confirms synergistic effects of viral protein and oncogene.	Validating synergistic drivers in viral HCC. ¹⁶
	HBV-Tg + <i>Trp53</i>/ <i>Pten</i> KO	CRISPR/Cas9-mediated dual knockout in HBV transgenic mice	High tumorigenic efficiency; transcriptomic profile highly mirrors human HCC.	Ideal for testing drugs targeting genomic instability. ^{25,26}
Metabolic (MASLD)	<i>Ctnnb1</i> (β-catenin) Activation	Adenovirus-Cre or in situ editing to delete <i>Ctnnb1</i> Exon 3	Wnt/β-catenin pathway activation; "Immune-cold" phenotype (T-cell exclusion).	Testing immune modulators (eg., MMP9 inhibition) to restore T-cell activity. ^{35–37}
	<i>Acy2a</i> Deficiency	CRISPR-Cas9 edited syngeneic transplantation	Hyperglycolysis (Warburg effect); High lactate microenvironment recruits Tregs.	Targeting metabolic-immune axes (Lactate/ Tregs). ⁴⁰
	<i>Pten</i> Deficiency	<i>Alb-Cre</i> mediated liver-specific knockout	Spontaneous "Steatosis → NASH → Fibrosis → HCC" sequence; mimics human NASH pathology.	Studying the complete NASH-HCC pathological evolution. ^{41,42}
	MUP-uPA/<i>Fbp1</i> Loss	uPA overexpression (ER stress) + HFD; <i>Fbp1</i> knockout	IgA+ plasma cell accumulation; "Auto-aggressive" CD8+ T cells; <i>Fbp1</i> loss accelerates MASH-to-HCC transition.	Evaluating immunotherapy resistance and metabolic "switches" in NASH. ^{47–49}
Alcohol-Associated	<i>Aldh2</i> Deficiency	Liver-specific <i>Aldh2</i> knockout + Alcohol feeding	Accumulation of acetaldehyde/acetate; acetate fuels macrophages to drive metastasis.	Targeting metabolic cross-talk in AL-HCC metastasis. ^{58,59}
	<i>Nfkb1</i> Deficiency	Whole-body <i>Nfkb1</i> knockout	Chronic liver disease progressing to HCC; Neutrophil-driven inflammation.	Understanding inflammation-driven carcinogenesis. ⁶³
	<i>Myc</i> Overexpression	<i>Alb-Cre</i> or Transgenic <i>Myc</i>	Rapid progression; aggressive phenotype; specific immune barrier remodeling.	Modeling aggressive AL-HCC subtypes for immunotherapy screening. ^{71–73}

Abbreviations: GEMMs, Genetically Engineered Mouse Models; HCC, Hepatocellular Carcinoma; HBV, Hepatitis B Virus; HBx-Tg, Hepatitis B virus X protein transgenic; KO, Knockout; MASLD, Metabolic Dysfunction-Associated Steatotic Liver Disease; MASH, Metabolic Dysfunction-Associated Steatohepatitis; HFD, High-Fat Diet; AL-HCC, Alcohol-Associated Hepatocellular Carcinoma. Bold text is utilized to highlight the specific genetic alterations (eg., *Kras*^{G12D}) and the core immunological or metabolic microenvironment characteristics unique to each etiology (eg., "Immune-cold" phenotype). Murine genes are denoted with initial capital *italics* (eg., *Trp53*).

Table 2 Critical Appraisal of Quantitative Parameters and Limitations in Etiology-Specific HCC GEMMs

Etiology	Target Gene/Model	Reported Tumor Latency	Reported Tumor Penetrance	Latency Variability/Key Modifiers	Reproducibility Across Genetic Backgrounds	Fibrosis/Cirrhosis Status	Major Limitation	Ref
HBV-Related	HBx-Tg + <i>Kras</i> ^{G12D}	~ 4–6 months	High (near 100%)	Strongly dependent on HBx transgene expression levels and the exact timing of Cre induction.	Moderate; random transgenic integration sites can cause line-to-line variation.	Typically absent (unless chronically paired with chemical fibrogens).	<i>KRAS</i> is rarely mutated in human HBV-HCC; lacks native chronic fibrotic remodeling.	[16]
	HBV-Tg + <i>Trp53</i> / <i>Pten</i> KO	~ 4–6 months	~ 100%	Low intra-batch variability; primarily modulated by the delivery efficiency and titer of CRISPR/AAV vectors.	High when utilizing standardized CRISPR protocols, but sensitive to viral vector quality.	Non-cirrhotic at baseline.	Acute multiplex gene knockout fails to mimic decades of gradual mutation accumulation and immune editing.	[25,26]
Metabolic (MASLD)	<i>Ctnnb1</i> (β-catenin) Activation	> 10 months (alone); 4–6 wks (combined)	Low (alone); 100% (combined)	Extremely high alone; drastically shortened and stabilized by cooperating oncogenic signals (eg., <i>Myc</i> , <i>Ras</i> , <i>Akt</i>).	High for conditional alleles across multiple standard genetic backgrounds.	Absent	Constitutive activation alone is often insufficient or early-developmentally lethal; requires a non-physiological secondary hit.	[35–37]
	<i>Acrv2a</i> Deficiency	2–4 weeks (Syngeneic)	100% (Syngeneic)	Low variability; determined by initial inoculation cell numbers, edited cell line aggressiveness, and host immunity.	High within standardized and immunocompetent C57BL/6 syngeneic platforms.	Absent (unless host mice are pre-treated with fibrogenic diets).	Syngeneic transplantation models completely bypass the spontaneous, multi-step MASH-to-HCC transition.	[40]
	<i>Pten</i> Deficiency	10–15+ months	Variable (40%–100%)	High variability; strongly accelerated by high-fat/high-fructose diets, housing temperatures, and baseline cohort age.	Poor to variable ; highly sensitive to specific murine strains (eg., C57BL/6 vs. mixed or outbred backgrounds).	Progressive steatohepatitis accompanied by prominent pericellular and bridging fibrosis.	Prolonged and unpredictable experimental timeline; potential interference from systemic metabolic phenotypes.	[41,42]
	MUP-uPA/ <i>Fbp1</i> Loss	24–34 weeks	~ 80%–100% (on HFD)	Modest variability; strictly dictated by dietary composition (HFD vs. CDAHFD) and the status of metabolic switches like FBPI.	Requires careful backcrossing; the uPA transgene can face epigenetic silencing over generations.	Advanced bridging fibrosis and early cirrhotic remodeling are frequently present.	Relies on severe, potentially non-physiological endoplasmic reticulum (ER) stress induced by massive uPA overexpression.	[47–49]
Alcohol-Associated	<i>Aldh2</i> Deficiency	~ 28 weeks	~ 80%–90%	Moderately variable; dependent on the duration/dosage of ethanol exposure and optional co-administration of DEN.	High when employing tightly controlled and standardized chronic alcohol-feeding protocols.	Chronic lobular inflammation and marked perisinusoidal fibrosis are present.	Spontaneous progression is slow; often necessitates chemical co-induction (eg., CCl ₄) to achieve high HCC incidence efficiently	[58,59]
	<i>Nfkb1</i> Deficiency	~ 20 months	~ 50%	Exceptionally high variability; strongly modulated by baseline age, housing conditions, and gut microbiome composition.	Modest; spontaneous tumor development rate is highly variable across different animal facilities.	Chronic low-grade inflammation, architectural distortion, and progressive fibrosis.	Widespread systemic (whole-body) knockout limits the precise isolation of hepatocyte-intrinsic mechanisms.	[63]
	<i>Myc</i> Overexpression	4–12 weeks	~ 100%	Low variability; exhibits profound sex dimorphism where male mice progress significantly faster than female mice.	Very high and consistent across multiple genetic strain backgrounds.	Completely absent.	Massive oncogenic drive rapidly forces malignant transformation, completely bypassing pre-neoplastic chronic injury stages.	

Notes: This table summarizes the tumor latency, penetrance, latency variability, reproducibility, and histological characteristics of key preclinical platforms. Notably, tumor latency and penetrance are not static parameters but are highly variable, governed by key genetic or environmental modifiers (eg., dietary formulations, alcohol exposure duration, and viral titers). Acknowledging these variations, strain-dependent reproducibility, and the widespread historical absence of a cirrhotic microenvironment is essential for selecting appropriate models and avoiding translational misinterpretations.

studies should report animal sex, analyze male and female mice separately when feasible, and avoid extrapolating male-only data to all biological contexts.⁷⁸

Second, insufficient tissue specificity and challenges of cellular heterogeneity. The liver is a complex organ composed of highly heterogeneous cell populations; beyond hepatocytes responsible for metabolic functions, it contains various non-parenchymal cells such as Kupffer cells, stellate cells, and endothelial cells. However, some existing models (such as those involving systemic gene modifications of NF- κ B or *Cdkn2a*) lack strict targeting of hepatocytes, making it difficult to completely rule out interference from systemic inflammation or extrahepatic organs.^{10,63} Given the distinct functions of different cell subpopulations in tumorigenesis, future model construction must emphasize “Hepatocyte-specific Targeting” to precisely distinguish intrinsic oncogenic driver events in hepatocytes from confounding effects of non-parenchymal cells or systemic backgrounds, thereby avoiding mechanistic misinterpretations caused by cellular heterogeneity.^{35,36}

To overcome these limitations, future GEMM designs will advance toward multi-gene orthogonal editing systems based on Cre-*loxP*, Flp-*FRT*, and Dre-*rox*. Current liver cancer GEMMs rely heavily on a single Cre-*loxP* system for hepatocyte-specific modification (eg., *Alb-Cre* mediated *Ctnnb1* or *Pten* knockout), making it difficult to dissect dynamic interactions between tumor cells and the Tumor Microenvironment (TME). By integrating three non-interfering recombinase systems—Cre-*loxP*, Flp-*FRT*, and Dre-*rox*—researchers can achieve precise, “divide-and-conquer” regulation within the same mouse. For instance, one could use the Cre system to specifically induce oncogenic mutations (eg., *Trp53* loss) in hepatocytes, while simultaneously using the Dre system to manipulate immune checkpoints or fibrotic genes in Kupffer cells or stellate cells, and the Flp system to control the temporal window of gene expression. This strategy not only ensures absolute specificity of gene editing in specific cell subpopulations, avoiding off-target effects, but also authentically simulates the co-evolutionary mechanisms of tumor parenchyma and stroma in HCC development, providing the most sophisticated genetic tools for dissecting the complex tumor microenvironment.

To make orthogonal recombinase systems more actionable, they should be integrated with controlled environmental injury models rather than discussed only as abstract genetic tools. For example, hepatocyte-specific oncogenic events induced by Cre-*loxP* could be combined with Flp-*FRT*- or Dre-*rox*-based manipulation of hepatic stellate cells, Kupffer cells, endothelial cells, or immune compartments. These genetic systems may be further paired with fibrogenic or metabolic injury protocols, such as CCl₄, thioacetamide, CDAHFD, ethanol-containing regimens, or carefully titrated multiple-hepatotoxic protocols.⁵⁵ However, models containing low-dose DEN require cautious interpretation because DEN introduces mutagenic pressure that may obscure driver-specific mechanisms. Such hybrid strategies should therefore be validated by lineage tracing, cell-type-resolved transcriptomics, fibrosis staging, and standardized reporting of tumor latency and penetrance.

Recent studies on RNA epigenetic regulation and three-dimensional MASLD modeling further highlight the need to integrate GEMMs with complementary molecular and human-relevant systems. For example, NAT10-mediated ac4C modification has been implicated in SMAD3 mRNA stability and HCC progression,⁷⁹ while three-dimensional dynamic coculture systems have been developed to model progressive stages of MASLD.⁸⁰ These platforms do not replace GEMMs, but they may help validate candidate mechanisms and improve cross-platform translational inference.

Data Sharing Statement

Not applicable. Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

Ethics Approval and Consent to Participate

Not applicable. This manuscript is a review article and does not involve primary research on humans or animals conducted by the authors. All data discussed are from previously published studies.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically

reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

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