Characteristics and Unmet Clinical Needs Related to Hepatocellular Carcinoma

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Abstract

Advances in biomedical research require animal models that accurately recapitulate human disease. Without such models, progress against human diseases such as cancer is significantly hindered. Here, we present the current landscape on available and emerging hepatocellular carcinoma (HCC) animal models. HCC is the second leading cause of cancer death worldwide, with an annual death toll exceeding 745,000. Stunningly, only 15% of HCC patients are candidates for curative therapy, leading 85% of patients to seek palliative therapeutic options. The VX2 rabbit model is considered the most relevant and widely used HCC model; however, more reliable HCC models are critically needed. In general, animal models for biomedical research should (1) mimic the human disease on a molecular basis, (2) derive from a relevant cell line that lends itself to in vitro study, (3) be reliable and predictable, (4) manifest survival differences, (5) allow for accurate treatment assessment, (6) be readily imaged, and (7) occur in similar background settings as the human disease. Over the past decades, numerous small animal models have been utilized for HCC studies; however, the development of new large animal models as qualified alternatives to murine models represents a key technology to advance research into human clinical trials.

Keywords
- hepatocellular carcinoma
- animal models
- human
- porcine model
- translational research

Liver cancer is the fifth most prevalent cancer in the world, and second leading cause of cancer death worldwide.[1,2] Most of the death toll is attributed to hepatocellular carcinoma (HCC), the most common form of primary liver cancer.[3] HCC is also one of the few cancers increasing in incidence and death rate in the United States with more than 39,000 new HCC diagnoses and 27,000 deaths anticipated in 2016. The overall 5-year survival rate of HCC is 17.2%, with more than half of patients diagnosed at regionally advanced, distant (metastatic), or unknown stages, respectively, carrying relative survival rates of 10.7, 3.1, and 6.1%.[4] Unfortunately, due to tumor and patient factors, the vast majority of HCC patients are not candidates for surgery with curative intent, therefore leaving at least 85% of patients to consider alternative palliative therapies.[4] Such therapies include interventional radiology (IR) locoregional therapies (LRTs), defined as minimally invasive image-guided embolic or ablative therapies, which are recommended by the National Comprehensive Cancer Network (NCCN) guidelines. Sorafenib (Nexavar, Bayer Pharmaceuticals, Whippany, NJ), a tyrosine kinase inhibitor, is the only Food and Drug Administration (FDA)-approved systemic therapy for advanced HCC and provides, on average, only 12 weeks of additional survival benefit with no differences in the median time to symptomatic progression.[5] Thus, there is an urgent need for novel and more effective treatment strategies.

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At the molecular level, HCC is caused by mutations in pivotal tumor suppressor and oncogenes, resulting in altered cellular signaling and the promotion of cellular growth and tumor development. This increased proliferation is further enabled by external microenvironment signals that promote tumor development. Together these internal and microenvironmental factors, referred to as the hallmarks of HCC, represent essential features necessary for malignancy. These hallmarks of HCC have significant impacts on tumor biology underlying differences in treatment responses, providing valuable insights for development of treatment strategies. This information can also help improve early detection methods critical to improving HCC survival rates, as fewer than half of HCC diagnoses are made at the local stage. Animal models represent a cornerstone for advancing medicine; however, current cancer models have significant limitations in terms of their ability to mimic human biology. The lack of competent preclinical in vivo HCC models impedes the development of safe and effective treatment strategies, resulting in the current reliance on highly toxic drugs for HCC treatment, many of which outweigh the therapeutic benefits. Advances in animal models mimicking key features of human HCC tumor biology and comorbid conditions are required to overcome these challenges.

Review of Liver Anatomy and Function

The human liver is the largest internal organ in the body and is divided into a larger right lobe and a smaller left lobe (►**Fig. 1A**).[6,7] A unique feature of the liver is its dual origin blood supply; 75 to 80% of the liver's blood is supplied by the portal vein, while the hepatic artery supplies the other 20 to 25%.[8] The right and left lobes are further divided into eight independent segments based on their dual vascular inflow,

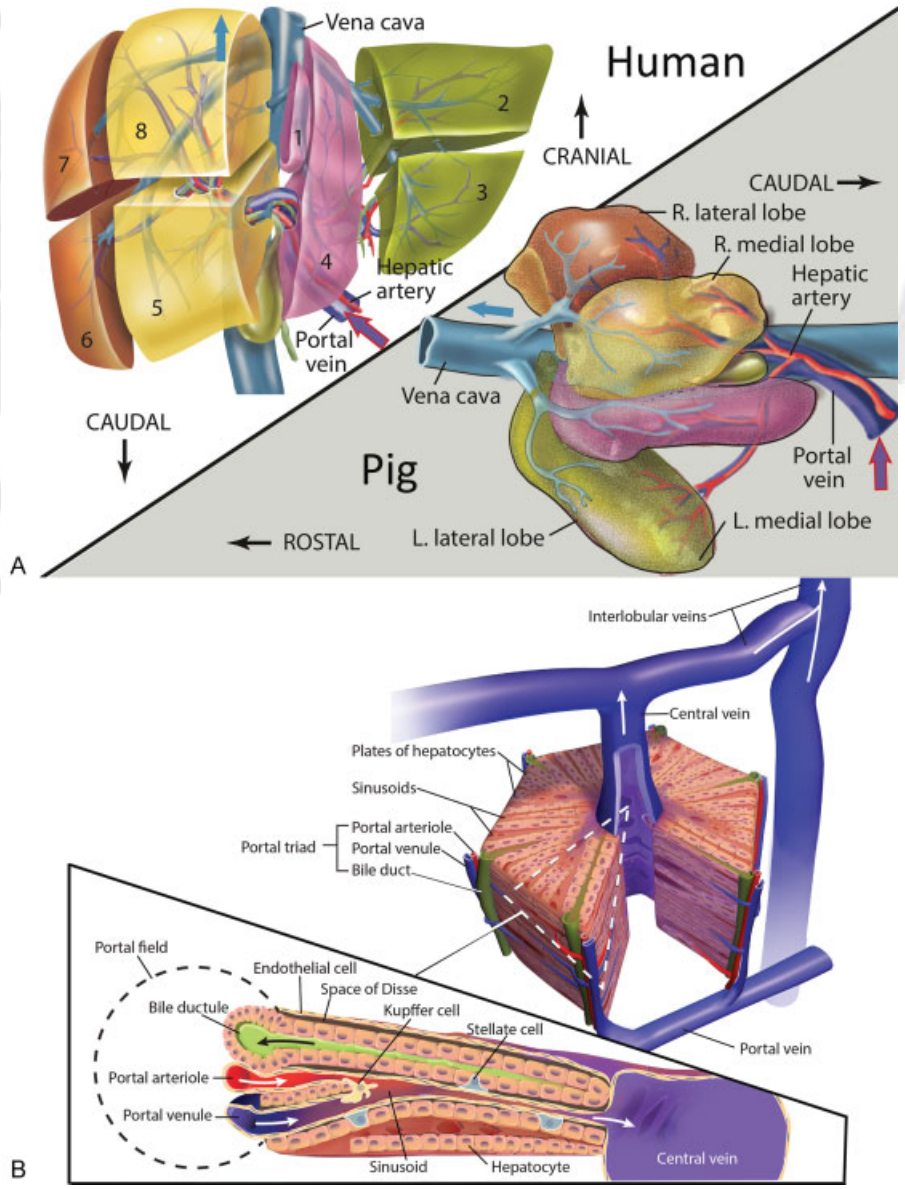

Fig. 1 Anatomy of the human and porcine liver. (**A**) Depiction of the human and porcine liver showing macroscopic anatomical similarities between species. Colors represent the same anatomical lobes in both species. Numbers indicate segments. (**B**) Microscopic anatomy of the hepatic lobes and liver sinusoids.

biliary drainage, and lymphatic drainage (►**Fig. 1A**). The presence of self-contained liver segments allows for surgical resection of segments without inflicting damage to the surrounding segments, allowing the liver to remain viable. Key hepatic functions include maintaining blood glucose levels, controlling lipid metabolism, metabolizing crucial proteins, creating fat for storage, creating bile, storing vitamin A, and filtering toxins from the blood.[6] These functions are made possible by the microscopic liver anatomy and the presence of specialized cell types working in conjunction to perform the above-mentioned functions.

Each liver segment is composed of hexagonal hepatic lobules consisting of hepatocyte plates that extend out from the central vein (►**Fig. 1B**). Hepatocytes, which undergo malignant transformation leading to HCC, are the major cell type located in the liver and constituting 75% of the liver cells.[7] Their main function is to break down and store amino acids, carbohydrates, and lipids, in addition to detoxifying blood and producing bile. A structure consisting of a hepatic artery branch, a hepatic portal vein branch, and a bile duct, referred to as the portal triad, is located at the corners of each hepatic lobule (►**Fig. 1B**). Large capillaries that drain blood from the portal triads to the central vein, referred to as liver sinusoids, are located between the hepatocyte plates (►**Fig. 1B**). Other cell types present in the liver sinusoids include Kupffer cells, which are star-shaped macrophages protecting the liver from foreign substances such as harmful bacteria. The stellate (Ito) cells, which have the ability to store vitamin A and participate in wound-healing processes (►**Fig. 1B**),[7] are also located in the liver sinusoids. During fibrosis, Ito cells transform into highly prolific cells and become the main source of collagen, creating a matrix barrier.[7] Liver sinusoidal endothelial cells line the outside of the sinuses and function as a selective membrane for extraction of proteins from the blood.[7] Liver progenitor cells function to repair the liver after chronic damage has occurred.[7] Finally, pericytes are adhered to endothelial cells thus stabilizing blood vessels.[1,7]

The liver possesses the unique ability to regenerate itself through development of all mature resident liver cells following various forms of injury.[9,10] From as little as 25% of functional cells, the liver has the capacity to regenerate an entire liver organ.[11] This process utilizes myofibroblasts that replace injured hepatic tissue with a matrix of collagen that acts as a protective barrier or scar.[12] The combination of segmental anatomy with the regenerative capability has been utilized to cure patients with resectable liver tumors. Although beneficial, this regenerative capacity also provides an ideal microenvironment for cirrhosis and HCC development.[13]

Liver Cancer: The Impact of HCC

Owing to its high mortality rate, liver cancer is a significant public health concern. In 2011, the global incidence of liver cancer neared 748,000.[14] In the United States, HCC accounts for the vast majority (75%) of new liver cancer cases and related deaths,[15,16] with an estimated 39,000 new cases expected in the United States in 2016.[16] In addition, an estimated 27,000 HCC-related deaths are expected in the United States in 2016,[16] resulting in a mortality to incidence ratio close to 1. HCC arises from transformation of hepatocytes, and commonly develops in the presence of comorbidities including hepatitis B virus (HBV), hepatitis C virus (HCV), alcoholic cirrhosis, hemochromatosis, nonalcoholic fatty liver disease (NAFLD), and nonalcoholic steatohepatitis (NASH).[17,18] In the United States, cirrhosis from alcohol abuse and HCV infection are the leading comorbid factors of HCC, with incidence rates expected to increase due to the increasing prevalence of NAFLD; for example, NASH in rising U.S. and global obese populations. With recent estimates of prevalence in adults above 25% worldwide,[19] NAFLD has become a global epidemic.

Other factors driving HCC development are the accumulation of driver mutations altering signaling pathway regulation resulting in tumorigenesis. Although difficult to define, HCC is often differentiated into two main subtypes. The proliferation class is highlighted by an increase in cell proliferation signals and an aggressive phenotype with a higher rate of recurrence, while the nonproliferation class tends to maintain molecular features similar to normal hepatocytes and is associated with a less aggressive phenotype (►**Table 1**).[20] Tumors in the proliferation class are also more commonly associated with HBV infection, while the nonproliferation class is commonly associated with HCV.[20] Still, little is known about the molecular basis of HCC subtypes. Although limited by pathologic and clinical staging, treatment options for HCC include chemotherapy, surgical resection, liver transplant, and LRTs such as ablation, chemoembolization, or radioembolization with yttrium-90 (^{90}Y).[16,21] However, the low 5-year HCC survival rate highlights the need for a better understanding of the HCC characteristics underlying differential treatment responses.

HCC Driver Genes and Pathways

Mutations in key oncogenes, tumor-suppressor genes, and signaling pathways controlling cell growth and the cell cycle are the main drivers for malignant transformation of primary hepatocytes into a HCC tumor.[22] Although information regarding the presence of key genetic mutations is available, HCC treatment strategies rarely take genetic makeup into account. This is partially due to the complexity of cellular signaling, making it difficult to decipher which alterations are the cause and which are the results of tumorigenesis. However, given the recent advances in cancer treatment techniques aimed at

Table 1 Characteristics of HCC subtypes

Proliferation class	Nonproliferation class
Clinical features	
• HBV • High AFP levels • Poor differentiation • High vascular invasion • Poor prognosis (recurrence/survival)	• HCV/Alcohol • Low AFP levels • Well differentiation • Low vascular invasion • Less aggressive

Abbreviations: AFP, alpha-fetoprotein; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus.

targeting specific molecular pathways, this area of research is critical for reducing the high HCC mortality rate. Genes commonly mutated in HCC include *TP53*, *CTNNB1*, *WNT*, *JAK*, *STAT*, *TERT*, and *HNF1A*.[22–25] These mutations typically fall into two categories: inactivating mutations, which lead to reduced or complete loss of protein function, and activating mutations, which can result in a stronger or even altered effect of the protein. Mutations in genes that regulate cellular signaling are often oncogenic drivers, that is to say they are promoters of tumorigenesis. These mutations often lead to altered cellular signaling, defined as increased signaling of pathways typically repressed in nontransformed cells, or vice versa.

TP53 is an important tumor-suppressor gene mutated in a wide variety of human cancer types and is considered the "guardian of the genome."[26] It regulates several functions through the *TP53* signaling pathway, including DNA repair, cell cycle progression, and apoptosis signaling (►**Fig. 2A**).[24,26] The suppressive abilities of *TP53* arise from its ability to inhibit cell proliferation through the activation of *CDKN1A* and commence apoptosis in malignant cells.[24] Inactivation of *TP53* through inactivating mutations or epigenetic silencing frequently leads to tumorigenesis.[24] Of HCC patients, 18 to 35% harbor inactivating *TP53* mutations.[22] In addition, *TP53* mutations are more common in patients with HBV than with HCV, suggesting a link between HBV infection and altered *TP53* signaling.[25] Another commonly mutated tumor-suppressor involved in *TP53* signaling is *RB*.[20,24] *RB* halts cell cycle progression by preventing cells from progressing past the G1/S checkpoint through inhibition of the *E2F* transcription factor (►**Fig. 2A**).[24] In healthy cells, progression into the S phase is achieved through inactivation of *RB* via phosphorylation. However, *RB* inactivating mutations are observed in 28% of HCC cases, resulting in progression through the G1/S checkpoint and uncontrolled cellular proliferation.[24]

In addition to altered *TP53* signaling, Wnt signaling activation has recently been identified to play an important role in tumorigenesis, with increased signaling observed in 62.5% of HCC cases.[22] The Wnt signaling pathway is an important developmental pathway that regulates several processes including cell migration, polarity, neural patterning, proliferation, survival, stemness, and organogenesis. *CTNNB1* encodes for β-catenin, a crucial transcriptional activator that plays a key role in the activation of Wnt signaling through the *N*-dependent pathway (►**Fig. 2B**).[27] Activating mutations in *CTNNB1* are observed in a wide range of cancer types, including 10 to 32% of HCC tumors.[22] In addition, *CTNNB1* activating mutations are associated with HCV as opposed to HBV infection in HCC patients.[25] Several tumor-suppressor genes, including *AXIN1*, *APC*, and *GSK3β*, make up an inhibitory complex that reduces the ability of *CTNNB1* to activate Wnt signaling.[20,25] Inactivating mutations in this complex of genes are also commonly observed in HCC tumors, with *AXIN1* mutations occurring in 15% of cases,[20,22] highlighting the importance and multimechanistic nature of Wnt signaling activation in HCC. Another signaling pathway commonly altered in HCC is the *JAK/STAT* pathway, which is altered in 45.5% of cases and plays an important role in regulating cellular immunity, growth, and differentiation.[22,23]

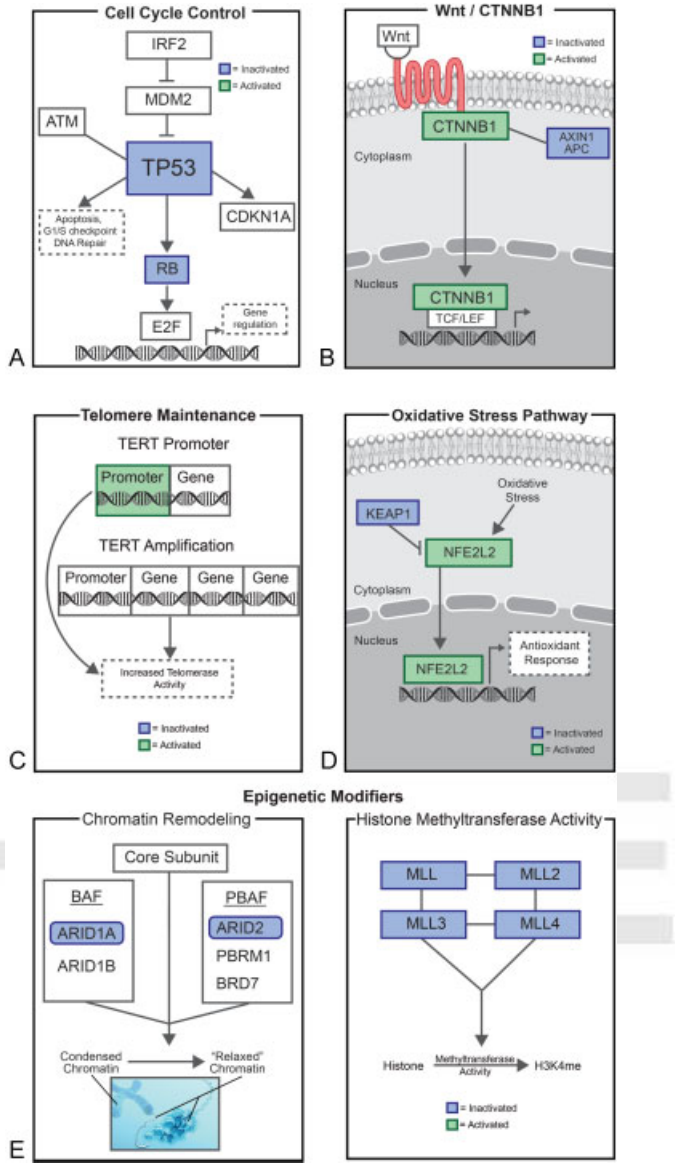

Fig. 2 Key hepatocellular carcinoma (HCC) transcriptional alterations. Diagrams of key signaling pathways and epigenetic mechanisms altered in human HCC, including (**A**) cell cycle regulation, (**B**) Wnt/CTNNB1 signaling, (**C**) telomere maintenance, (**D**) oxidative stress, and (**E**) epigenetic modifications. (Adapted from Zucman-Rossi et al.[21])

Together, these two pathways are considered to be the major oncogenic drivers of HCC.[22] The most commonly mutated gene in the *JAK/STAT* pathway is *JAK1*, with activating mutations leading to growth factor and cytokine-induced *STAT* signaling activation observed in 9% of HCC cases.[22,23]

TERT encodes for an RNA polymerase that allows for limitless replicative potential through the addition of telomere repeat sequences to chromosomal ends. Although *TERT* is silenced in somatic cells,[28] it is reactivated in more than 90% of HCC cases through a combination of promoter mutations and gene duplications (►**Fig. 2C**).[20] This makes *TERT* reactivation one of the most common transcriptional hallmarks of HCC, allowing the cell to bypass senescence and continue to divide without any degradation of telomeres and important genes.[29]

The oxidative stress pathway is altered in up to 15% of HCC cases through activating mutations in *NFE2L2* and inactivating mutations in *KEAP1* (►**Fig. 2D**).[30,31] These genes control the expression of genes involved in detoxification and removal of reactive oxygen species through enhancement of cellular antioxidant responses. Constitutive activation of this pathway has been identified as a driver event in tumor progression, acting as a protective mechanism preventing death of HCC tumor cells following exposure to reactive oxygen species.[32]

In addition to direct signaling pathway alterations, epigenetic modifications responsible for genome-wide transcriptional disruptions are consistently observed in HCC.[33] Inactivating *ARID1A* and *ARID2* mutations, genes involved in the SNF/SWI chromatin remodeling complex required for transcriptional activation of genes normally repressed by chromatin (►**Fig. 2E**), are observed in up to 18% of HCC cases.[20] Further alterations in chromatin remodeling are observed through inactivation of MLL genes involved in H3K4 methylation (►**Fig. 2E**).[33–35] H3K4 methylation is an important histone mark, having been identified to bind to promoter regions of actively transcribed genes throughout the genome.[36] Together, these transcriptional hallmarks provide key insights into the molecular mechanisms underlying differences in tumor biology among HCC subtypes.

Impact of Tumor Microenvironment on HCC

The tumor microenvironment, defined as the immediate microscopic environment surrounding a tumor, is composed of various cell types and cellular communication signals that activate HCC tumorigenesis, progression, metastasis, and drug resistance. The HCC microenvironment is a critical factor driving HCC development, and translational HCC animal models will need to properly mimic this microenvironment to accurately portray the human disease. For example, upregulation of growth factors (i.e., increased levels of growth factors) is observed in tumor adjacent cells compared with normal cells. HCC tumors and their microenvironment are composed of cancerous cells, noncancerous cells, and assisting stromal cells. The main HCC microenvironment cell type is the carcinoma-associated fibroblast, which stages the microenvironment by producing extensive extracellular matrix, including collagens to create the tumor capsule.[37,38] Macrophages, cancer stem cells, dendritic cells, cancer-associated fibroblasts, and pericytes are all found within this tumor capsule.[37] Cancer stem cells are self-renewing cells that produce cytokines and chemokines, which contribute to the heterogeneity of cells in the tumor.[39] In addition, hepatic stellate cells that reside in the space of Disse (►**Fig. 1B**) are recruited to invade the sinusoidal spaces of the tumor.[37]

Stellate cells promote migration of other stellate cells and production of proangiogenic factors such as *VEGFA*, resulting in tumor hypervascularization. Endothelial cells are responsible for recruiting cells from adjacent tissues to aid in blood vessel formation through excretion of vascular endothelial and platelet growth factors.[37] As angiogenesis occurs, endothelial cell platelet-derived growth factors are released to recruit pericytes. These pericytes promote vessel stability by making loose physical contact with the blood vessel, as it provides nutrients and oxygen to the tumor.[37] Tumor-associated macrophages also promote angiogenesis in hypoxic conditions, as well as regulate tumor growth through the release of growth factors, cytokines, enzymes, and chemokines.[37] Chemokine secretion by macrophages and fibroblasts also results in recruitment of immune cells, including lymphocytes, dendritic cells, and leukocytes that aid in immunosuppression.[37]

Macroenvironment: Risk Factors for HCC

In addition to the impact of the microenvironment and genetic factors on HCC development, environmental and behavioral factors also contribute to HCC development (►**Fig. 3**). Cirrhosis is the most common liver disease worldwide, and involves histological development of regenerative nodules that form due to chronic liver injury. This process results in portal hypertension, end-stage liver disease, and HCC development through the interference of hepatocyte regeneration.[13,17] HBV and HCV infections precede 70 to 85% of all HCC cases due to their ability to contribute to cirrhosis progression.[17,40] The estimated 170 million people infected with HCV in Japan and Egypt,[17,40] the high prevalence of HBV in China and developing countries,[17] and chronic HBV and HCV infection represent significant HCC risk factors worldwide. HCV and HBV infect hepatocytes,[40,41] resulting in cirrhosis development in 15 to 30% of HCV-infected patients[42] and 20 to 30% of HBV-infected patients.[43] Before the 1990s, the main method of HCV infection was through blood transfusions due to a lack of appropriate screening methods. Other common HCV transmission routes include fetal blood sharing systems, needle sharing, and unprotected sex.[40]

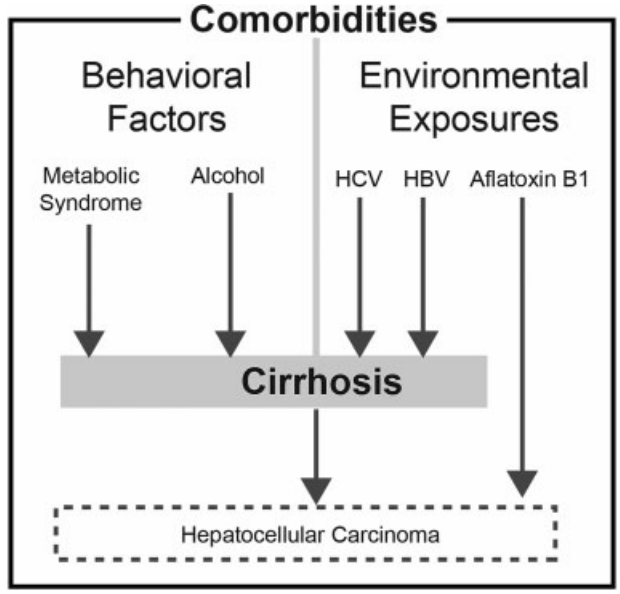

Fig. 3 Environmental and behavioral factors contributing to hepatocellular carcinoma (HCC) development. Depiction of key behavioral and environmental factors contributing to cirrhosis induction and HCC development.

In developing countries, the most frequent cause of cirrhosis is HBV.[13] However, due to availability of HBV vaccines in developed countries, the prevailing cause of cirrhosis in these countries is HCV infection, alcohol abuse, and metabolic syndrome resulting in NASH.[13] Males and individuals older than 50 years with chronic HCV infections are at increased risk of developing cirrhosis.[13] In addition, those suffering from NASH, obesity, type 2 diabetes, hypertension, or hyperlipidemia are also more likely to develop cirrhosis.[13] Cirrhosis diagnosis and severity are typically determined through imaging such as computed tomography (CT) scan and magnetic resonance imaging (MRI).[13]

Liver biopsies are currently the gold standard for diagnosing cirrhosis, despite known issues regarding sampling error biases and potential seeding of malignant cells into normal surrounding liver parenchyma.[13] While there is currently no cure for cirrhosis, progression can be slowed through elimination of the earlier-mentioned risk factors, thereby reducing the chance of developing HCC.[13] Lamivudine treatment has yielded positive results for a portion of patients with HBV and cirrhosis, although a tolerance to the drug is usually built up over time.[13] Patients with HCV and cirrhosis tend to see positive treatment responses to interferon-based antiviral treatments.[13] In addition, the combination ledipasvir/sofosbuvir treatment (Harvoni, Gilead Sciences, Foster City, CA), which consists of viral NS5A (ledipasvir) and viral RNA polymerase (sofosbuvir) inhibitors, cures HCV infections in 96% of patients.[44]

NAFLD and NASH affect approximately 30% of people worldwide, with type 2 diabetes and obesity being the highest risk factors.[45] The disease results from the accumulation of fatty acids and insulin resistance.[46] Diabetes mellitus type 2 is an insulin-resistant form of diabetes that leads to coagulation of proteins within hepatocytes.[46] Low insulin levels signal hepatocytes to store fatty acids rather than secrete them.[46] The accumulation of fatty acids blocks oxygen flow to the cells, resulting in hypoxic-like conditions.[46] Oxidative stress is largely the driver for transitioning NAFLD into NASH.[46] When the liver is affected by NASH, scar tissue accumulates in disease causing sinusoidal capillarization, interrupting normal physiological liver functions.[46] Scar tissue blocks the interaction of the liver sinusoidal endothelial cells, preventing the exchange of proteins and other molecules between the blood and hepatocytes.[12,47] Management of NAFLD and NASH for obese patients mainly involves weight loss; however, it may also lead to fibrosis and worsen necrofibrosis.[46]

HCC Diagnosis and Treatment

HCC is rarely diagnosed early enough to prevent progression. It is primarily identified in later stages when treatment options are few and the risk of death is high. Therefore, increased in early detection of HCC would have a significant effect on treatment outcomes and prognosis. In most cases, the aforementioned preexisting liver diseases lead to cirrhosis and HCC development. To diagnose HCC, high-resolution imaging techniques and biopsies are used.[48] Surveillance of alpha-fetoprotein (AFP) serum levels in high-risk populations is the primary screening technique. However, as this technique successfully detects HCC in only 50% of cases, it lacks the sensitivity and specificity to serve as a reliable early detection biomarker.[48] Similar difficulties arise when attempting to discern between HCC tumors and high-grade dysplastic nodes using liver biopsies, resulting in a 30% false-negative rate.[48] Ultrasounds can be useful tools for identifying differences between malignant and benign tumors when performing screenings at 6-month intervals.[48] However, more advanced imaging methods are needed for further characterization of HCC tumor progression. In this regard, four-phase CT and MRI techniques are commonly used for diagnosing cirrhosis and HCC features useful for determining optimal treatment strategies.[48]

HCC progression is assessed using the Barcelona Clinic Liver Cancer (BCLC) staging system, a widely accepted system that uses classifications to estimate variability in patient prognosis and treatment.[49] Treatment options are categorized as curative or palliative, and are prescribed depending on the disease stage. Early-stage HCC curative therapies include surgical resection and liver transplantation. Patients without cirrhosis and a tumor of less than 3 cm in diameter are typically candidates for surgical resection with a 5-year survival rate of 60 to 75%. If cirrhosis is present following resection, a second resection is typically performed.[48] In addition to resections, liver transplants are commonly performed, carrying a high 4-year nonrecurrence rate (90%) and survival rate (80%).[48] However, once tumors reach a certain size, as indicated by the BCLC criteria, surgery is no longer an option. Instead, LRTs are utilized to slow down tumor growth and attempt to make patients eligible for surgery.[48]

IR procedures directly treat tumors using ablation methods—chemical and thermal—in a locoregional setting and can also deliver chemotherapy. Chemical ablation consists of injection of ethanol or acetic acid to dehydrate and necrose the tumor.[48] The most frequently used ablation technique is radiofrequency ablation, as this has resulted in the highest success rates to date.[48] However, radiofrequency ablation still carries a high recurrence and low survival rate compared with surgery.[48] Transarterial chemoembolization (TACE) directly delivers chemotherapy to the tumor, embolizing the blood vessels, causing ischemia and thereby cutting off nutrient flow to the tumor.[48] Although TACE is currently approved as standard-of-care therapy for HCC, fewer than half of patients see a 25% reduction in tumor size,[48] highlighting the need for further research into HCC treatment.

In addition to LRTs, HCC patients can also be placed on a systemic treatment regimen. Currently, the only systemic treatment approved for treatment of advanced stage HCC is sorafenib (Nexavar, Bayer Pharmaceuticals, Whippany, NJ).[48] Sorafenib is an orally administered tyrosine kinase inhibitor that competes with ligands for receptors in many of the aforementioned tumor promoting pathways. The resulting binding promotes antiangiogenic and anti–cell proliferation responses, effectively blocking the tumor's access to the bloodstream and reducing growth potential.[50] However, sorafenib is highly toxic, and only results in an average 12-week increase in survival.[48]

The Unmet Needs of Existing Animal Models

Animal models are instrumental to understand HCC development and metastasis, and facilitate discovery of new therapeutics.[51,52] They are commonly used when the target population is costly, difficult, and unethical to use (humans).[53] The choice of model has traditionally come down to available housing space and affordability.[53] However, to be effective, animal models must recapitulate specific attributes present in the target population.[53] Specifically, an ideal animal model of human cancer should (1) mimic the human disease on a molecular basis; (2) derive from a relevant cell line that lends itself to propagation, characterization, storage, and study in vitro; (3) be reliable and predictable in tumor generation and growth kinetics; (4) manifest survival differences; (5) allow for accurate assessment of treatment effects including no spontaneous tumor necrosis; (6) be readily imaged; and (7) occur in similar background settings as the human disease.

Owing to their availability, cost, and short-generation intervals, HCC rodent models tend to be favored over larger animals.[51] However, rodent models produce tumors that are pathologically different from human tumors, or require immunosuppression of the model animal, eliminating the ability to test immunotherapeutic regimens. The two main strategies used to induce tumor growth in these models are implanting and insulting.[51] The implantation method utilizes a xenograft cell line or tissue fragment inoculation for tumor development.[51] This method allows for shorter disease initiation and progression to treatable size, in addition to previous characterization of the cell line/tumor for genomic and functional genomic variation.[51] The second method is the insulting method, which involves injection of carcinogens resulting in tumors histologically similar to human tumors, but with the negative effect of slower tumor development rates.[51]

The VX2 rabbit model is considered to be the most relevant and widely used model to study HCC, particularly for investigation of IR procedures and techniques. However, this model has significant drawbacks, including (1) squamous cell origin (dissimilar to HCC), (2) unknown tumor biology, (3) intrinsic internal necrosis, (4) only peripheral vascularization, (5) varying tumor kinetics, and (6) unknown genome organization.[54] Other small animal models include the woodchuck HBV model, which produces an imitation of human HCC[51,52] but is limited to modeling a specific comorbidity. Each of these models has drawbacks that prevent researchers from better understanding and treating the disease. None of the current models focus on the genetic makeup of the tumor, even though this can be critical when choosing treatment strategies. Another major drawback for current HCC models is the inability to model cirrhosis,[52] as well as their small size in relation to humans. As research performed in small animal HCC models requires compact instruments, utilization of the same equipment and techniques employed in the clinical realm is not feasible.[51,55] Together, these drawbacks highlight the need for new HCC models that mimic humans in terms of anatomy, physiology, size, metabolism, genetics, and epigenetics.

Porcine HCC Models

In an effort to address unmet clinical needs for HCC, researchers all over the world are working to produce porcine HCC models. Due to the many parallels between human and porcine biology, there are significant advantages associated with using pigs compared with existing small animal HCC models. First, pigs are similar in size to humans compared with other commonly used small animal models, allowing for the utilization of clinically relevant tools, as well as treatment and imaging techniques.[56] As a result, minimal further training is required for clinical researchers already familiar with the tools/devices used in the clinical realm. In addition, the size and anatomy of the pig liver is highly similar to that of humans (►**Fig. 1A**). This is particularly important for IRs performing LRTs and investigating new targeted and combinatorial treatment strategies for HCC. In addition, the pig's similar size to humans also provides the added benefit of acting as a training tool for medical students.

Another advantage is the similarity in drug metabolism between pigs and humans. With currently available small animal models, it can be difficult to translate drug testing to clinical trials due to differential drug metabolism mechanisms. Pigs, on the other hand, have similar drug metabolism pathways to humans, which allows the model to accurately represent the effects of different drugs on humans.[56] For example, the xenosensor pregnane X receptor, which regulates *CYP3A* expression and is responsible for the metabolism of the majority of prescription drugs, as well as its basal metabolic rate is very similar to humans.[57–59] Once again, the similar size of pigs and humans is an added benefit, allowing for easier translation of drug dosages to clinical settings. Finally, with the increasing interest and success of immunotherapy as a treatment for several cancer types, the need for an HCC model with a functioning immune system is required. Since mouse models require immunosuppression, they have limited utility to test these new forms of treatment. However, pig models produced to date allow for tumor formation without immunosuppression. This allows researchers to test immunotherapies in pig models in the form of pre- and co-clinical trials with significantly higher potential to lead to successful translation to clinical practice than those performed in currently available small animal models.

Chemically Induced Porcine Models

One of the strategies employed to produce porcine HCC models is chemical induction of cirrhosis and HCC tumor formation. Intraperitoneal injection of *N*-nitrosodiethylamine (DENA) over a period of 3 months followed by a 10-month period without chemical treatment has been utilized to produce HCC tumors of a clinically relevant size in a background of cirrhosis in pigs.[60] CT scans revealed multiple HCC tumors with human HCC characteristics including hyperattenuation in the arterial phase, hypervascularity, and retention of lipiodol in all pigs in a time frame of 13 to 15 months. The HCC tumors are also surrounded by cirrhotic regions and express AFP. However, this model has several drawbacks, including differential cirrhosis induction mechanism than observed in humans; inability to

control tumor biology including number of tumors, location, and genetics; and the long time (>1 year) required to produce HCC tumors. Additional groups have utilized peritoneal diethylnitrosamine (DEN) in combination with partial liver embolization (PLE) in an attempt to reduce the time to tumor onset in a minipig HCC model.[61] Although this model closely mimics progressive disease stages and molecular marker expression observed in humans, PLE increased the time to adenoma but not HCC development.

Oncopig HCC Model: The Next Generation

In addition to chemically induced models, researchers have also created a transgenic porcine cancer model (Oncopig).[56] The Oncopig Cancer Model (OCM) was specifically designed with mutations commonly found in more than 50% of human cancers, $KRAS^{G12D}$ and $TP53^{R167H}$. Cre recombinase exposure results in expression of the mutant transgenes, and this inducible nature allows for utilization of this model for a broad range of human cancers.[56] Using the OCM, researchers have produced histologic HCC via liver resection, hepatocyte isolation, malignant transformation of hepatocytes via exposure to Cre recombinase, and subsequent percutaneous injection back into the host animal.[62] The Oncopig HCC cell lines display transcriptional hallmarks of human HCC, including *TERT* reactivation, apoptosis evasion, angiogenesis activation, altered cell cycle regulation, Wnt signaling activation, and conservation of master regulators of gene expression. Autologous injection of Oncopig HCC cells subcutaneously results in hypervascular tumors histologically blindly characterized as Edmondson Steiner grade 2 HCC with trabeculae formation, pseudoacinar patterning, and well-vascularized stroma. This model allows for control of the number and location of HCC tumors with known genetic background, although tumor formation in the liver still needs to be demonstrated. Together, the Oncopig and other porcine HCC models will be instrumental in improving detection, treatment, and biomarker discovery, as well as translation of novel discoveries in small animal models to clinical practice, contributing significantly to early detection and treatment strategies for this deadly disease.

Conclusion

Liver cancer, as the second leading cause of cancer death worldwide, demands a qualified alternative large animal model in order to achieve clinically relevant progress against the substantial annual death toll. As the largest internal organ, the liver exhibits several unique characteristics, including dual blood supply and ability to regenerate. HCC, which originates from malignant transformation of hepatocyte, generally develops on the background of viral hepatitis, alcoholic cirrhosis, or NASH cirrhosis. Treatment for HCC is stratified between curative therapy (for 15% of patients who are surgical candidates) and either LRT (including ablation, TACE, or ^{90}Y radioembolization) or targeted systemic therapy (sorafenib). Although research into the driver genes and pathways has evolved over recent years, treatments do not yet incorporate this information. Genes and pathways commonly mutated include *TP53*, *CTNNB1*, *WNT*, *JAK*, *STAT*, *TERT*, and *HNF1A*. Until now, the animal models for HCC have exhibited numerous flaws. Rodent and rabbit models are commonly utilized in the investigation of HCC; however, their smaller size precludes the translation of devices from animal to human clinical practice. Furthermore, the VX2 rabbit model produces tumors of squamous origin and is prone to spontaneous tumor necrosis, thereby limiting its impact on therapeutic investigation. The woodchuck model produces HBV, which imitates HCC but is limited to modeling one specific comorbidity. Finally, on the horizon are chemically induced and transgenic porcine HCC models. These pigs exhibit similar size, metabolism, and physiology to humans and can be readily imaged and easily scaled to perform co-clinical trials investigating new therapeutic approaches. They represent qualified alternatives to traditionally used small animal HCC models for HCC, and have the potential to significantly enhance detection technologies, drug and device therapeutic interventions, and biomarker discovery against human HCC.

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