ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 18, Issue 5, 2025

Online - 2455-3891
Print - 0974-2441

Review Article

EXPERIMENTAL RODENT MODELS FOR HEPATOCELLULAR CARCINOMA AND CHOLANGIOCARCINOMA: A PRE-CLINICAL FRAMEWORK FOR LIVER CANCER RESEARCH

LOKESHVAR RAVIKUMAR, RAMAIYAN VELMURUGAN*, YOKESH S, MAHA SWETHA K

Department of Pharmacology, Saveetha College of Pharmacy, Saveetha Institute of Medical and Technical Sciences, Chennai, Tamil Nadu, India.
***Corresponding author: Ramaiyan Velmurugan; Email: velmuruganr.scop@gmail.com**

Received: **25 February 2025,** ***Revised*** **and** ***Accepted:*** **08 April 2025**

ABSTRACT

Every year, more people die from primary liver malignancies such as cholangiocarcinoma (CCA) and hepatocellular carcinoma (HCC). For physicians, this is a major issue. Numerous in vivo models of malignant malignancies have lately been established, yielding vital new insights into their development. These models are especially important for pre-clinical testing of novel therapeutic agents to evaluate newly developed compounds as potential treatments for primary liver malignancies. To correctly analyze study results and plan future research, the technical components of each model must be carefully considered, as they are an essential aspect of the research process. The primary goal of this review is to provide a thorough description of the technical and experimental features of the most important rodent models, with an emphasis on highlighting the similarities and differences between these models and the corresponding human diseases. This will aid in the development of novel therapeutic strategies. This review is divided into two main sections. The first section investigates HCC models developed by a range of strategies, including genetic modification, nutritional manipulation, and the use of toxic chemicals. To provide a comprehensive overview of the various methods used to study these liver cancers, the second section will go into detail about CCA models established in rodents, such as rats and mice, using a variety of techniques, including xenograft and syngeneic models, bile duct cannulation, genetic manipulation, toxin administration, and surgical interventions.

Keywords: Liver carcinogenesis, Hepatocellular carcinoma, Cholangiocarcinoma, Rodent models, Experimental oncology.

 DOI: http://dx.doi.org/10.22159/ajpcr.2025v18i5.54026. Journal homepage: https://innovareacademics.in/journals/index.php/ajpcr

INTRODUCTION

Primary liver cancers, such as hepatocellular carcinoma (HCC) and cholangiocarcinoma (CCA), are becoming a significant issue for doctors due to rising morbidity and mortality rates. Notably, HCC is the sixth most common cancer worldwide, with an estimated 630,000 new cases diagnosed each year [1]. HCC is a common liver malignancy caused not only by the persistent hepatitis C virus (HCV) and by hepatitis B virus (HBV) viral infection but also by other factors such as heavy alcohol intake, aflatoxin-contaminated foods, smoking, obesity, and type 2 diabetes [2]. As part of the disease's clinical course, approximately 80% of people with HCC develop fibrosis, cirrhosis, and, eventually, malignancy. Malignant tumors are the second leading cause of death, and hepatocellular carcinoma is one of the world's third deadliest cancers, with very high morbidity and mortality rates and poor prognosis [3]. Although there are various variables that contribute to the development of HCC, the most common are alcohol-induced liver damage and HBV and C virus infections. Although less prevalent, certain immunological and metabolic disorders, such as non-alcoholic fatty liver disease and non-alcoholic steatohepatitis, might contribute to the development of HCC. Overall, the increased incidence of HCC highlights the importance of better understanding and treatment of this complex and diverse disease.

Aflatoxin B1 (AFB) is another, though less common, component that contributes to liver cancer, particularly in Asian and African populations [4]. Research is ongoing into the complex interactions between multiple pathways and chemicals involved in the mechanisms by which various etiological factors, such as aflatoxin B1 (AFB), contribute to the development of hepatocellular carcinoma (HCC). Cholangiocytes, which are epithelial cells that line the intra- and extra-hepatic biliary epithelium, can convert into CCA when they become malignant. CCA, a highly aggressive disease, has become increasingly prevalent in Western countries [5]. According to recent estimates, around 6,000 new cases of CCA are identified each year in the United States alone [6], emphasizing the importance of better understanding and treatment of this devastating illness. To identify new therapy targets and improve patient outcomes, researchers are intensively investigating the distinct genetic and cellular processes that underpin CCA development and progression.

CCA is typically identified after the disease has progressed to severe stages, resulting in delays in diagnosis. Patients have a poor prognosis because of the scarcity of available therapeutic alternatives, both pharmacological and surgical. Unfortunately, most patients die from the condition soon after being identified, typically within a few months [5,7]. Although various risk factors, such as cirrhosis, liver fluke infections, hepatolithiasis, chronic HCV, primary sclerosing cholangitis, and toxin exposure, have been associated with the development of CCA, the disease's fundamental pathophysiology remains unknown [5,7]. These risk factors are similar in that they both promote inflammation and chronic cholestasis in the liver and biliary tissues. Animal models must be developed to better understand the etiology of these aggressive cancers. Several *in vivo* models of HCC and CCA have recently been established, providing vital new insights into the etiology of primary liver malignancies. These models are also valuable tools for evaluating newly produced compounds as potential therapeutic agents in pre-clinical settings, which will eventually contribute to the development of more effective therapies for these severe diseases. Our understanding of the intricate mechanisms behind HCC and CCA has grown significantly as a result of this research, which has also cleared avenues for the development of novel therapeutic options.

Rodents are currently widely employed in cancer research due to their high reproductive capacity and short lifetime, particularly when examining CCA and HCC. The genesis and evolution of these liver tumors have been extensively investigated using mice (Mus musculus) and rats (Rattus norvegicus) as models. Mice, in particular, have been

extensively employed to explore the impact of genetic change in the development of HCC and CCA due to their ease of handling and manipulation utilizing knock-out or transgenic models. The goal of this study is to provide a complete examination of the experimental and technical aspects of the most prominent rodent models of HCC and CCA, with a focus on the similarities and differences between these models and the corresponding human diseases. Each animal model has been given a specific name to assist clarity and reader comprehension. This makes it easier to understand the intricate relationships between these models and the human conditions they are intended to simulate. Researchers can gain a better understanding of the biology underlying HCC and CCA by evaluating the benefits and drawbacks of these rodent models, which will eventually help them develop more effective treatment approaches.

HCC-EXPERIMENTAL MODELS

Chemotoxic substances

Numerous compounds have been identified as hepatotoxic agents, which means they can injure the liver and promote the growth and spread of cancer (Table 1). Based on current scientific understanding, carcinogenic substances are broadly categorized into two groups: (i) genotoxic agents, which directly induce tumor formation, and (ii) promoting agents, which, when combined with genotoxic agents, increase tumor development [8]. When tumor-promoting medications are provided, pre-neoplastic cells can replicate clonally, accelerating tumor growth and aggressiveness. One of the primary advantages of chemically created models is that they replicate the injury-fibrosis-malignancy cycle found in humans, making them an invaluable resource for understanding the complex processes that underpin the development of liver cancer. Scientists can use these models to understand more about the molecular and cellular processes that cause tumor genesis, growth, and dissemination. This insight will enable them to develop more effective prevention and therapeutic strategies for liver cancer. The use of chemically induced models allows for the investigation of the synergistic effects of genotoxic and promoting chemicals, which can give critical insights into the fundamental mechanisms of liver carcinogenesis. Overall, research on chemically created liver cancer models has substantially enhanced our understanding of the disease and may help uncover novel therapeutic targets and solutions.

MODEL "N-NITROSODIETHYLAMINE"

N-nitrosodiethylamine (DEN) is utilized to develop a well-known model of HCC in mice [9,10]. The carcinogenic effects of DEN are caused by two distinct mechanisms: (i) alkylation of deoxyribonucleic acid (DNA) structures, which causes DNA damage and cell degeneration, and (ii) activation of cytochrome P450 in hepatocytes, which induces the creation of reactive oxygen species (ROS) [11,12]. The DEN model's notable features include (i) dose-dependent efficacy, (ii) time of administration, (iii) efficacy heterogeneity by age, gender, and mouse strain, and (iv) potential synergy with co-administered boosting medications (Table 1) [13-17]. When given to 15-day-old mice, a single dose of DEN causes tumor development in around 80% of cases, but long-term DEN treatment results in a 100% success rate in triggering tumor formation [18,19]. The molecular and cellular mechanisms underlying the development of HCC have been extensively researched using the DEN model, and its characteristics make it an ideal tool for investigating the impact of various genetic and environmental variables on the course of liver cancer. Because DEN is so successful at inducing HCC in mice, it is also an excellent model for pre-clinical testing of potential treatment agents. This allows researchers to test the safety and efficacy of new medicines in a suitable and reliable animal model.

Phenobarbital (PB) is a well-studied promoting factor associated with DEN-initiated hepatocarcinogenesis. The effects of PB on DEN-initiated mice differ substantially depending on strain, age, and sex. The date of DEN introduction is a crucial aspect in determining the encouraging effects of PB. For example, when adult male B6C3F1 mice are given DEN at 6–10 weeks of age and then exposed to PB in their drinking water for 36 weeks, PB acts as a potent tumor-promoting agent [20,21]. The Solt-Farber technique [22], which begins with a hepatocarcinogenic

Table 1: An overview of the key experimental characteristics of HCC rodent models

Carcinogenic agent	Co-promoter	Tested species	Tumor development duration	Key characteristics	Metastasis	References
TGF-β transgenic mice	-	Mouse	30 weeks	Tumor formation, inflammation, fibrosis	No	[26]
HBx transgenic (DEN)	-	Mouse	80–100 weeks (30–50 weeks)	Hepatitis B virus (HBV)-associated tumorigenesis	No	[27,28]
DEN	PB	Mouse/rat	12–40 weeks	Highly aggressive tumor growth	Yes	[22,23]
Peroxisome proliferators	-	Mouse	50–100 weeks	Strain-dependent mutations not identified in humans	Yes	[29-34]
Core, A, E transgenic mice (DEN)	-	Mouse	60 weeks (30 weeks)	Hepatitis C virus (HCV)-linked cancer progression	No	[35,36]
CCl4	-	Mouse	100 weeks	Fibrosis and chronic inflammation	Yes	[37-41]
DEN	PH	Rat	4–8 weeks	Low reproducibility in tumor formation	No	[24,25,42,43]
TAA	-	Mouse/rat	50–70 weeks	Inflammatory response without metastasis	No	[44]
Aflatoxin	-	Mouse/rat	50 weeks	Tumor formation	Yes	[45-47]
P-TEN	-	Mouse	40 weeks	Cellular proliferation leading to tumors	No	[48,49]
TAK 1	-	Mouse	48 weeks	Tumor development with fibrosis and inflammation	No	[50]
DEN	-	Mouse/rat	100 weeks	Pure tumor formation, absence of fibrosis	No	[13-21]
NEMO	-	Mouse	48 weeks	Inflammation-driven tumor formation with steatohepatitis	No	[51-53]
Choline-deficient diet (ethionine)	-	Mouse/rat	50 weeks (30–40 weeks)	Induces steatohepatitis but no metastasis	No	[54-59]

HCC: Hepatocellular carcinoma, TAA: Thioacetamide

drug such as DEN and concludes with partial hepatectomy (PH) [23], is another well-known "two-step" hepatocarcinogenesis paradigm. This approach aims to reproduce the multistep process of hepatocarcinogenesis, in which a selection pressure that promotes the growth and multiplication of pre-neoplastic cells follows the first genetic damage caused by DEN. The Solt-Farber approach, a popular tool for studying the molecular and cellular processes that underpin hepatocarcinogenesis, provides insights into how promoting factors such as PB contribute to the development and spread of liver cancer. Using these models, scientists may investigate the complex interactions between environmental and genetic factors that contribute to liver cancer and identify potential preventative and therapy targets.

One of the primary disadvantages of the DEN model is the length of the trials; on average, HCC takes 50 weeks to develop. Chemotoxic models, on the other hand, offer a more simple and repeatable procedure because they only require a single dose of DEN. Despite the fact that tumor growth is <100% in these models, single-dose administration has the advantage of eliminating outside influences and more effectively modeling the pathophysiological course of the disease. With less variability and greater accuracy, this method allows researchers to investigate the natural history of HCC development in a more relevant and controlled manner. Furthermore, by focusing on the primary molecular and cellular processes that drive tumor growth and progression, the single-dose DEN model provides a simpler and more effective method for investigating the fundamental mechanisms of liver carcinogenesis. Overall, the single-dose DEN model is a useful alternative to the traditional long-term DEN model, providing a faster and more insightful approach to studying HCC progression and identifying potential treatment targets.

Although long-term regimens have the advantage of promoting tumor growth in a higher proportion of cases, the model's outcome may be influenced by repeated DEN injections. Furthermore, stimulating drugs such as PB might speed cancer development; however, this may alter tumor characteristics and reduce model repeatability [24,25]. However, the surgically based PH approach is less reproducible due to increasing operator dependence. Because of the PH method's complexities, it might be difficult to obtain consistent and reliable data, resulting in varying outcomes. It can also be difficult to analyze the results and draw conclusions about the underlying mechanisms of liver carcinogenesis because the PH method and the addition of promoting chemicals can introduce new variables that can alter the model's outcome. Simpler models, such as the single-dose DEN model, may give a more direct and reliable means of researching liver cancer, as they have fewer variables to manage and less operator-dependent bias. The specific research topic and study objectives ultimately determine which model is appropriate, and each has advantages and disadvantages that must be carefully considered.

THE MODEL OF "PEROXISOME PROLIFERATORS"

Peroxisome proliferator-activated receptors (PPARs) are nuclear receptors that bind to fatty acid ligands and induce the transcription of target genes, making them critical for managing lipid metabolism [42,43]. When PPARs are activated by their ligands, ROS and peroxisomal oxidase may be increased, thereby promoting the progression of HCC [29,30]. This PPARs ligand-induced experimental model of HCC has distinguishing characteristics, including a trabecular histological appearance, metastasis in 20–40% of patients, and the possibility of gene mutations [31]. However, because hepatocarcinogenesis produced by PPAR ligands may be a species-specific process, caution should be exercised when applying the findings from this model to human disease. Furthermore, the genetic profiles of PPARs-induced HCCs in animal models may differ from those of human HCCs, limiting the paradigm's translational usefulness. As a result, while interpreting the findings and applying them to human disease, it is critical to consider the potential limitations and species-specific differences, even if the PPARs ligand-induced model can provide valuable information regarding the molecular pathways of HCC development.

MODEL "AFLATOXIN"

A tiny number of studies have employed aflatoxin exposure to investigate the development of HCC in rats and mice. AFB, a hepatotoxin produced by some fungi of the *Aspergillus* genus, including *Aspergillus flavus*, has carcinogenic activity and is a significant risk factor for HCC, especially in areas where both AFB and HBV are prevalent [32]. AFB's ability to generate chromosomal aberrations, chromosomal strand breakage, DNA adducts, micronuclei, and uncontrolled DNA synthesis is closely linked to its carcinogenic effects [33]. Studies have shown that injecting 6 mg/kg AFB into 7-day-old mice results in the development of HCC after 52 weeks with a nearly 100% success rate [45], and this model has been employed in both mice and rats [34]. Although AFB-administration experimental models can assist in elucidating the mechanisms of AFB-induced hepatocarcinogenesis, their relevance is limited to specific conditions where more research is needed to understand the mechanisms underlying AFB-induced HCC. Although these models may not be directly relevant to other kinds of HCC, they can provide crucial information on the molecular and cellular pathways that drive AFB-induced liver cancer. They can, however, increase our understanding of the complex links between genetics, environmental contaminants, and the initiation of liver cancer, guiding the development of targeted preventative and treatment methods.

MODEL "CARBON TETRACHLORIDE"

Carbon tetrachloride (CCl_4), a strong chemotoxin, has been widely utilized to cause liver injury in rats and mice [46]. The hepatotoxic effects of CCl_4 are caused by two main processes: first, cytochrome P450 induction, which increases the production of ROS [37,47]; second, Kupffer cell activation, which initiates an inflammatory response by producing cytokines, chemokines, and other proinflammatory factors [38]. Because of its dual mode of action, CCl_4 is an effective tool for studying liver disease and injury, particularly HCC. The fact that CCl_4 can produce liver damage, fibrosis, and, finally, HCC in rodents provides a good model for studying the molecular and cellular pathways involved in liver carcinogenesis. CCl_4 is a reliable and popular model for investigating liver illness since its application has been extensively reported in the literature, as have its effects on the liver. Frequent exposure to CCl_4 causes a cycle of liver injury, inflammation, and healing, which eventually leads to fibrosis and increases the risk of developing HCC. To cause HCC in animal models, CCl_4 has been utilized in a number of investigations in conjunction with other drugs, including alcohol. For example, after 104 weeks of weekly CCl_4 injections and consuming alcohol-containing water, mice develop HCC [38,39,46]. Furthermore, CCl_4 therapy was reported in rat trials to be 30% effective in HCC formation after 30 weeks [40]. According to these findings, CCl_4 could be a useful tool for studying the processes that underpin liver disease and the development of HCC, particularly when paired with other chemicals that may exacerbate liver damage. Using CCl_4 in animal models can provide insights into the complex interactions between environmental contaminants, lifestyle decisions, and genetic vulnerability that contribute to the development of liver cancer. Researchers can identify potential therapeutic targets and develop more effective preventative and therapeutic approaches for liver cancer by understanding how CCl_4 affects the liver and contributes to the development of HCC.

MODELS OF DIET-INDUCED HCC

In animal models, studies have demonstrated that a choline-deficient diet (CDD) can lead to HCC. This diet was initially developed to study cirrhosis, fibrosis, and steatohepatitis in mice and rats [41,54]. More recent research, however, has revealed that rats on a comparable diet had a significantly higher incidence of tumor formation, whereas mice fed a CDD developed HCC after 50–52 weeks [41]. One of the primary factors driving the development of HCC in CDD-treated animals is the stimulation of oval cells, which causes increased oxidative stress, DNA damage, and genetic mutations or changes. The CDD model has been shown to accurately recapitulate the primary hallmarks of clinical HCC, including the development of cirrhosis, fibrosis, and

tumor formation following steatohepatitis. In addition to being a useful tool for investigating the molecular and cellular processes that contribute to the formation of HCC, the use of CDD in animal models may aid in the identification of potential therapeutic targets for liver cancer prevention and treatment. Furthermore, the CDD model can be used to explore how nutritional factors influence liver health and sickness, which could aid in the development of new dietary strategies to prevent HCC.

When chemotoxic chemicals such as DEN or CCl_4 are used, the effects of CDD have been investigated [55]. It has been shown that adding ethionine to CDD promotes oval cell activation, increasing the risk of carcinogenesis [56,57]. Furthermore, combining the DEN and CDD models has been demonstrated to accelerate the development of HCC while preserving the markers of diet-induced liver injury, such as inflammation and steatosis [55]. Similarly, when CDD was delivered with alcohol or CCl_4, the frequency and size of liver tumors increased [55]. The choline-deficient and iron-supplemented L-amino acid-defined diet is a modified version of the CDD that has been proven to mimic the effects of CDD in a shorter period of time (Fig. 1) [54,58]. This modified diet is an important tool for studying the processes of liver disease and cancer since it has been shown to promote liver damage and HCC development faster than the standard CDD [54,58]. Overall, the CDD and its variants have been shown to be useful models for investigating the complex interactions between nutrition, environment, and genetics that contribute to cancer and liver disease.

"THIOACETAMIDE (TAA)" MODEL

TAA, a hepatotoxin that can be delivered intraperitoneally (IP) or through drinking water (0.02–0.05%), is another model used in the study of HCC. According to studies, mice that are regularly exposed to TAA develop fibrosis within 10–15 weeks [59]. The main carcinogenic effect of TAA is assumed to be due to oxidative stress, which increases the quantity of ROS in the liver. ROS gradually damages DNA as levels rise, contributing to the establishment of HCC [59]. The molecular pathways causing HCC have been studied using this model, which has also shed light on the part oxidative stress plays in liver carcinogenesis. The TAA model has been demonstrated to be a valuable instrument for assessing the effectiveness of possible treatment drugs and has also been utilized to investigate the impact of different genetic and environmental variables on the development of HCC. All things considered, the TAA model has greatly advanced our knowledge of HCC and is a tried-and-true method for researching the disease.

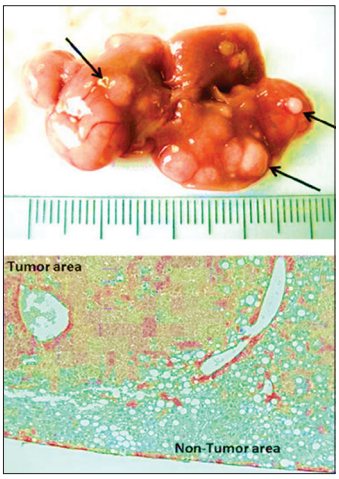

Fig. 1: Representative images show the macroscopic (top) and microscopic H&E staining (bottom) characteristics of hepatocellular carcinoma nodules induced by a choline-deficient and iron-supplemented l-amino acid-defined (CDAA) diet combined with carbon tetrachloride (CCl_4) exposure. After 6 months on the CDAA diet, along with weekly low-dose CCl_4 injections (0.2 mg/kg body weight), large nodules became evident on the liver surface of mice. (Source: De Minicis *et al.*, unpublished observations, 2011).[133]

XENOGRAFT MODELS

Cancer cell proliferation, collagen deposition, and neo-angiogenesis (the formation of new blood vessels) all contribute to xenograft tumors' rapid growth. Two of the key advantages of this model are the rapid creation of tumors and the ease with which they can be tracked, allowing for direct monitoring of tumor nodules over time [60]. To develop xenograft models, immunocompromised mice, such as athymic (nude) or severe combined immunodeficient mice, are injected with human cancer cells, which stimulate tumor formation [44]. In the ectopic model, human cancer cells are injected subcutaneously into mice's hind flanks, whereas in the orthotopic model, tumor cells are injected directly into the mouse liver. These are the two primary categories of xenograft models [61]. The orthotopic model, which provides a more realistic picture of cancer metastatic spread, allows for a better understanding of the intricate interactions between the tumor and its surrounding environment [61]. Xenograft models allow researchers to investigate the fundamental principles of tumor growth and dissemination, as well as evaluate the efficacy of potential treatment medications, in a realistic and controlled environment.

In the ectopic model, various cell lines are frequently utilized to screen for prominent chemotherapeutic drugs. However, there are significant variances in tumor growth inhibition across the literature [62,63]. Orthotopic implantation of HCC cells in fibrotic livers is a more reliable and consistent approach [64]. Using a fibrotic liver model, researchers discovered that tumors grow faster and have a greater risk of metastasis and satellite nodule formation [65]. This orthotopic model enables a more precise portrayal of the tumor microenvironment and a more reliable assessment of the efficacy of chemotherapeutic agents. This model's use of fibrotic livers also allows for an examination of the complicated interactions that exist between the tumor and the surrounding liver tissue, which is critical for understanding how HCC develops. Using this model, scientists can develop more effective HCC treatment strategies and gain valuable insights into the processes that drive tumor growth and metastasis.

To summarize, the xenograft model's key advantage is its ability to rapidly induce tumor growth, with a relatively short time between injection and tumor development. Nonetheless, the underlying pathophysiological mechanisms that drive tumor growth in this model do not accurately reflect the complex modifications that occur during tumor development in humans [66]. As a result, the xenograft model has limitations in reproducing the natural course of real tumors despite its widespread use and utility for studying treatment responses and tumor characteristics. The model cannot accurately recreate the human tumor microenvironment and related disease processes, but it is useful for preliminary studies on pharmaceutical efficacy and tumor biology. As a result, to develop effective cancer therapies and gain a better knowledge of tumor progression, the xenograft model should be used in conjunction with other models and approaches.

Another method for investigating cancer is the "hollow fiber assay (HFA)" [67]. Tumor cell lines are inoculated into tiny, hollow polyvinylidene fluoride fibers with a 1 mm internal diameter. The fibers are then sealed and separated into 2 cm pieces [68]. Multiple fibers can be implanted subcutaneously or IP in a single athymic mouse after a brief *in vitro* culture period of 24–48 h. The HFA method's ability to investigate multiple cell lines in a single mouse at the same time is a significant advantage over previous xenograft models [69]. This capacity enables researchers to analyze the development and behavior of multiple cell lines in a single experiment, making tumor cell line screening more effective and high-throughput. The HFA technique provides a unique opportunity to explore how tumor cells interact with their surroundings and evaluate the efficacy of potential

treatment options in a relevant and controlled setting. Employing this strategy allows researchers to understand a lot about cancer biology and develop more effective cancer treatment regimens.

MODELS THAT HAVE UNDERGONE GENETIC MODIFICATION

Genetically modified mice (GMMs) are an effective way to simulate the pathophysiology and molecular aspects of HCC [70]. This technique provides valuable insights into the fundamental mechanisms of HCC development by allowing researchers to compare the impact of oncogenes with and without carcinogenic agents. Researchers can use complementary DNA constructs with promoters that can target certain cell types to improve GMM selectivity and accuracy [71]. This allows particular genes to express themselves in diverse tissues, which helps to elucidate their role in HCC. Mice with an albumin promoter, for example, are widely used in this study because they allow for the focused expression of genes in hepatocytes, the primary cell type affected by HCC. Using GMMs, researchers can gain a better understanding of the complex interactions between genetic and environmental factors that contribute to HCC and develop more effective treatment approaches for this devastating illness.

Transgenic mice, which allow for the induction of specific genes, offer an alternative to constitutive tissue-specific gene deletion or expression. This model allows researchers to investigate how various oncogenes contribute to the maintenance of malignancies. As shown in Table 2, several transgenic mice models have been developed to study HCC. Notably, transgenic mice models that express hepatitis-related viral genes may replicate the viral hepatitis-induced HCC found in humans, making them extremely relevant. These models can be used to explore how certain oncogenes influence tumor growth and maintenance, and they provide a valuable tool for understanding the molecular pathways behind HCC creation and progression. Scientists can utilize transgenic mice to understand better the complex interactions between genetic changes, viral infections, and environmental factors that lead to HCC, allowing them to develop more effective treatment approaches. The use of transgenic mouse models can help to understand the fundamental mechanisms of tumor growth and progression, as well as aid in the discovery of potential biomarkers and therapeutic targets for HCC.

The HBx gene is expressed in the majority of transgenic animals associated with HBV in viral models, and HCC develops after 52–104 weeks [27,28,72]. In contrast, HCV transgenic animal models that express core, E1, and E2 structural proteins develop HCC after a 60-week period [35]. DEN injections, on the other hand, significantly accelerate the development of HCC, with some cases appearing in as short as 32 weeks [36]. This suggests that exposure to carcinogenic chemicals and HCV infection may work together to raise the risk of developing HCC. In addition to emphasizing the importance of considering the interaction of viral infections, genetic changes, and environmental factors in the pathogenesis of this illness, the use of these viral models provides important insights into the molecular mechanisms underlying the development of HCC. Examining these models allows researchers to gain a better understanding of the complex links between HBV and HCV infections and HCC development. They can also identify potential therapeutic targets for the prevention and treatment of this devastating condition.

Transgenic mice expressing oncogenes such as c-Myc and β-catenin were employed to produce further mouse models of HCC [26]. PDGF, TGFβ1, NEMO, TAK1, alpha-1 antitrypsin, and PTEN, a tumor suppressor gene controlling the PKB/AKT pathway, have been altered or deleted in mice to provide alternative models [50-53,73]. PTEN-deficient mice are one of the models that have greatly aided cancer research [19,48,74,75]. Notably, at 40–44 weeks, liver-specific PTEN-deficient mice develop HCC, fibrosis, inflammation, and hepatic steatosis [49]. This model has helped to explain the molecular mechanisms that underpin HCC and has provided crucial insights into the role of PTEN in the development of liver cancer. The use of PTEN-deficient mice to study the effects of various genetic and environmental variables on the development of HCC has made it easier to identify potential therapeutic targets. Overall, these mouse models have considerably increased our understanding of HCC, paving the way for the development of more effective therapeutic and diagnostic techniques for this devastating disease.

CCA-EXPERIMENTAL MODELS

CCA rat models

The "Syngenic" paradigm

Sirica *et al*. [76] first described a syngeneic model of CCA in which cells from a rat-derived CCA cell line (BDEneu) were implanted intrahepatically into Fisher 344 rats. With a highly consistent tumor mass forming within 20–22 days following inoculation, this approach resulted in a 100% tumor formation rate in the injected animals (Table 3). Between 15–16 days and 25–26 days after cell injection, the tumor size rose considerably, displaying an exponential development tendency. Furthermore, bilirubin levels in the blood increased considerably. A continuous loss of body weight and the appearance of peritoneal metastases coincided with the tumors' intrahepatic growth. This model provides a reliable and consistent framework for studying the start and progression of CCA, allowing for the exploration of underlying molecular pathways and the evaluation of potential treatment methods. The syngeneic model also allows researchers to investigate the relationship between tumor growth and the host's physiological reactions, such as variations in body weight and blood

Table 2: An overview of the primary experimental characteristics of HCC rodent models

Experimental model	Inducing agent	Orthotopic presence	Genetic factor	Toxicity	Surgical procedure	Inflammatory response	References
TAK 1	Present	Yes	No	Yes	No	Yes	[50]
Aflatoxin	Present	No	No	Yes	No	Yes	[45-47]
DEN PH	Present	Yes	No	Yes	No	Yes	[24,25,42,43]
Core, A, E transgenic mice (DEN)	Present	Yes	Partial	No	No	Yes	[35,36]
TGF-β transgenic mice	Present	Yes	No	No	No	Yes	[26]
P-TEN	Present	Yes	No	Yes	No	Yes	[48,49]
HBx transgenic (DEN)	Present	No	Partial	No	No	Yes	[27,28]
Choline-deficient diet (ethionine)	Present	Yes	No	Yes	No	Yes	[58,59]
CCl4	Present	Yes	No	Yes	No	Yes	[37-41]
Peroxisome proliferators	Present	Yes	No	Yes	No	Yes	[29-34]
TAA	Present	Yes	No	Yes	No	Yes	[44]
DEN PB	Present	Yes	No	Yes	No	Yes	[22,23]
DEN	Present	Yes	No	Yes	No	Yes	[13-21]
NEMO	Present	Yes	No	Yes	No	Yes	[51-53]
Choline-deficient diet	Present	Yes	No	Yes	No	Yes	[54-57]

HCC: Hepatocellular carcinoma

Table 3: An overview of the experimental procedures and results for the CCA rodent model

Experimental model	Animal strain	Procedure	Tumor development timeline	Tumor formation rate	Metastasis	References
DEN-LMBDL	Balb/c mouse	Two intraperitoneal (IP) injections of DEN per week Oral gavage (25 mg/kg, once per week)	8th week: Cyst formation 12th week: Biliary hyperplasia 16th week: Cholangiomas and adenomas 28th week: Cholangiocarcinoma (CCA)	100%	Not documented	[129]
TAA-induced model	Fisher rat, Sprague-Dawley, Zucker rat	0.03% TAA administered through drinking water for 24 weeks	9th week: Proliferation and dysplasia of cholangiocytes 12th week: Microfoci of cancer 16th week: visible CCA tumors 24th week: Fully developed CCA tumors	100%	Peritoneal involvement	[76,78,79]
p53 Knockout (CCl4 exposure)	p53−/− C57B16 mouse	Crossbreeding p53+/− mice to generate p53 +/+, +/−, and −/− genotypes Intraperitoneal injections of CCl4 (10 μL/g body weight) 3 times per week for 4 months	17 days: Initial tumor formation 16–24 weeks: Tumor progression 24–28 weeks: Advanced-stage tumors 53 weeks: Tumor development in p53+/− mice	18% (p53+/−) 54% (p53−/−)	Lung involvement	[91,98]
BDEneu Cell line model	Fischer 344 rat	Injection of 4×10^6 BDEneu cells into the left hepatic duct	Tumor onset within 20–22 days	100%	Not recorded	[91]
Xenograft model	Nude mouse	Subcutaneous implantation of human-derived cancer cell lines	Rapid tumor progression detectable size increase from the second week	Not specified	Not documented	[105-113]
Smad4-Pten knockout	Smad4Co/ Co PtenCo/Co Alb-Cre mouse	Genetic modification through crossbreeding Smad4Co and/or PtenCo mice with Alb-Cre mice	2–3 months: Formation of hyperplastic foci in biliary epithelium progressive transition to dysplasia and carcinoma *in situ*	100%	Not specified	[80,81-88]

markers. Overall, this model has the potential to significantly improve our understanding and approach to CCA.

Furthermore, the authors developed a modified paradigm in which mice who had undergone common bile duct ligation (BDL) had BDEneu cells transplanted into their livers. After 21 days, the findings revealed that, in comparison to the animals who did not receive BDL, tumor growth was significantly higher in the former group. The researchers also discovered that mice who received BDEneu cell injections and had sham surgery did not develop extra-hepatic, peritoneal tumor nodules, whereas those who received cell injections and underwent BDL did. This suggests that the BDL approach may have created a microenvironment conducive to tumor growth and spread. The modified model provides a more complete understanding of the complex connections that exist between the hepatic milieu and tumor cells, as well as the impact of BDL on tumor initiation and spread. The findings of this study reveal the model's ability to investigate the molecular processes behind CCA and develop novel treatment methods for the disease's management.

One of the many advantages of the current model is the use of cells with biological features similar to human CCA, such as TRAIL production, COX-2 overexpression, and ERK1/2 hyperphosphorylation [7,76-78]. The model similarly resembles human CCA in that it is associated with increased body weight loss and biliary obstruction, both of which encourage tumor growth. This means that the model is an effective tool for illness study since it accurately depicts the essential components of human CCA. The use of cells with biological characteristics similar to human CCA, as well as the model's ability to replicate the disease's clinical symptoms, such as weight loss and biliary obstruction, provides a strong and relevant system for investigating the underlying mechanisms of CCA and developing effective treatment plans. Overall, the model is a promising platform for translational research and pharmaceutical development because of its ability to simulate the complex biology of human CCA.

This model has two important experimental advantages: (i) tumor nodules form consistently, and (ii) they do so in a relatively short period of time (Table 3). Because of these characteristics, the model is ideal for pre-clinical research testing of novel therapeutic medicines. Because of the model's stability and rapid tumor growth, therapeutic efficacy can be measured efficiently and consistently. For example, the model has been used to demonstrate how well sorafenib works in slowing the course of CCA. Tumor liver invasion was dramatically reduced after sorafenib treatment; in 22% of treated rats, full regression occurred [79]. The model was also used to examine the efficacy of JP1584, a small molecule second mitochondria-derived activator of caspase mimic [78]. In contrast to the vehicle-treated controls, rats treated with JP1584 and implanted with BDEneu cells showed a significant decrease in peritoneal metastasis in this study [78]. These findings highlight the model's potential as a valuable tool for therapeutic agent pre-clinical testing and hastening the development of effective CCA treatments. Because of its ability to grow tumors reliably and its short trial duration, the model is an attractive platform for investigating new therapeutic methods and identifying promising candidates for clinical translation.

One of the current model's limitations is the implantation of malignant cells in the absence of chronic biliary or liver injury, as it does not account for the de novo creation of CCA, which is not symptomatic of the disease in patients. From an experimental standpoint, the model requires invasive methods such as left bile duct cannulation and abdominal manipulation, which may alter the liver's cytokine milieu and limit its use in extended research. Furthermore, the model's application to pathophysiology research in transgenic animals may be limited because it was developed in rats. This is a significant disadvantage because transgenic animals are regularly used to research

the underlying causes of disease and develop novel treatment options. The model's inadequacies demonstrate how pre-clinical models of CCA must be developed and refined to produce more accurate and representative systems for studying this complex and severe condition. Furthermore, enhancing our understanding of CCA and developing effective treatments requires the development of new models that more accurately mimic the actual illness, such as those that include de novo CCA formation and chronic biliary or liver injury.

The model of "Thioacetamide"

TAA injection is a well-established paradigm for inducing liver cirrhosis and fibrosis in animals [59]. More than 20 years ago, scientists discovered that giving rats oral TAA resulted in CCA and biliary dysplasia [80,81]. The TAA rat model has now become the most widely used and thoroughly explored paradigm for CCA studies. According to this theory, TAA is regularly added to drinking water at a normal concentration of 0.03%, causing fibrosis, liver damage, and increased weight loss [81-84]. Signs of cholangiocyte dysplasia and proliferation develop around 9 weeks, and malignant cell microfoci form by 12 weeks [81,83,84]. This model is an important tool for understanding the disease and developing effective treatments because it has been thoroughly characterized and shown to mirror the fundamental aspects of human CCA. The TAA rat model was used to explore the molecular processes underlying the development of CCA, including the roles of inflammation, fibrosis, and chronic liver injury, which provided important insights into the pathogenesis of this devastating condition.

By the 16th week of TAA administration, whitish CCA tumors become evident, and by the 24th week, the incidence of larger, more invasive tumors has steadily increased to 100% of the animals (Fig. 2a) [81,83-86]. Surprisingly, the rat strain employed had no effect on the CCA production route (Table 3) [81,83-86]. Long-term research is possible due to the animals' low death rate [81,83-86], with some experiments lasting up to 40 weeks [87]. Lung metastases appear after 24 weeks (Fig. 2a) [86], and intra-hepatic CCA nodules persist even after TAA treatment is discontinued, at least for an 8-week monitoring period [86]. This makes the model a valuable tool for studying the biology of CCA and evaluating potential treatment methods because it implies that the CCA tumors that grow in this model are exceedingly aggressive and capable of spreading to other parts of the body. The fact that the tumors continue to grow long after TAA administration is withdrawn implies that the model could be utilized to study the mechanisms behind tumor growth and maintenance, as well as to identify potential therapeutic targets. Overall, the TAA rat model of CCA provides a robust and reliable framework for investigating the genesis and progression of this severe illness.

To expedite the development of CCA, researchers attempted to change the standard protocol by increasing the daily dosage of TAA. Increasing the TAA dose to 0.05–0.1% resulted in quicker CCA formation, with tumors developing as early as 11–13 weeks, according to Al-Bader *et al.*'s dose-response study [82]. However, before CCA could form, higher doses of 0.15% TAA caused significant mortality rates. Mansuroglu *et al*. confirmed these findings by demonstrating that rats fed 0.05% TAA developed CCA nodules in 100% of instances within 18 weeks [88]. This suggests that increasing the TAA dosage may speed up the onset of CCA, but it also increases the risk of death. 0.05% TAA is a useful supplement to the traditional method for studying CCA since it appears to reach a compromise between reducing mortality and hastening CCA development. Using this new technique, researchers may be able to gain a better understanding of the underlying causes and progression of CCA while saving time and money.

The TAA model accurately mimics a number of key features of human CCA, including persistent inflammation of the liver parenchyma and bile ducts (Fig. 2b), a strong desmoplastic reaction surrounding the tumor, and a link to chronic liver injury and fibrosis [5,7]. Furthermore, the presence of numerous biomarkers, such as COX-2, EGFR, MUC1, MMP-2, MMP-9, c-Met, c-erb-B2, c-Kit, and estrogen receptors, suggests that the malignant cells in this model have molecular similarities to human

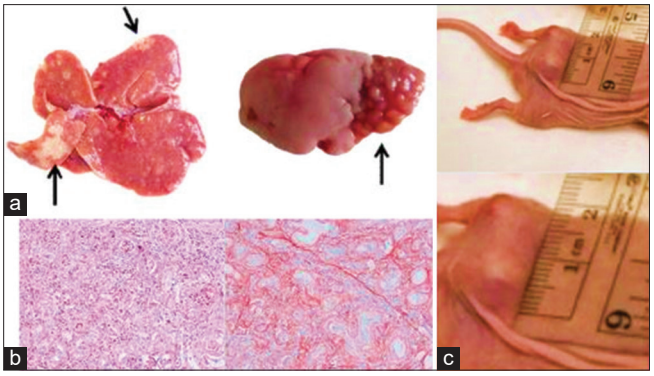

Fig. 2: (a) A representative macroscopic image of thioacetamide (TAA)-induced intrahepatic cholangiocarcinoma (IH-CCA) nodules shows large white-yellowish lesions (arrows) consistently appearing on the liver surface of treated rats after 24 weeks of 0.03% TAA administration (left). In addition, a representative image of lung metastases from TAA-induced IH-CCA reveals visible nodules in the lower segments of the left lung after the same treatment duration (right). (Source: Marzioni and Nilsson, unpublished observations, 2011). (b) Representative hematoxylin and eosin (H&E) stained image of a TAA-induced IH-CCA nodule shows a tumor composed of disorganized, irregular duct-forming tissue with a dense inflammatory infiltrate (original magnification ×20, left). A Sirius-Red stained image of a TAA-induced IH-CCA nodule highlights an intense desmoplastic reaction (stained red), resembling human disease (original magnification ×20, right). (Source: Marzioni and Nilsson, unpublished observations, 2011). (c) Mz-ChA-1 cells, a Cholangiocarcinoma cell line implanted subcutaneously in the flank of a nude mouse, form a clearly visible tumor. Tumor size changes can be easily tracked over time (top). An enlarged view of the same nodule is shown (bottom). (Source: Francis and Alpini, unpublished observations, 2009). [133]

CCA [83,84,87,88]. According to this shared genetic phenotype, the TAA model is a valid and relevant method for investigating the underlying processes of CCA and developing effective treatment plans. Because it can reproduce the complicated interactions between inflammation, tumor growth, and chronic liver injury, the model is an effective tool for understanding the pathophysiology of CCA and identifying potential treatment targets. Overall, the TAA model provides a rigorous and precise framework for studying the biology of CCA and applying the findings to therapeutic settings.

The TAA paradigm offers several experimental advantages, one of which is that it does not require invasive abdominal manipulation or surgery. Furthermore, CCA nodules are consistently produced by a simple injection of TAA-enriched water, making it a reliable and reproducible model. The model has been frequently utilized in pre-clinical trials to assess new CCA diagnostic and treatment procedures, demonstrating its reliability and applicability. To discriminate between tumor nodules and liver cirrhosis, the positron emission tomography tracer [18F] fluoro-2-deoxyglucose has been shown to accumulate in TAA-induced CCA [89,90]. Administering an estrogen receptor-β selective agonist can prevent and reduce the growth of TAA-induced CCA, even after the tumor has been established [86]. These findings demonstrate the model's potential as a translational research platform, as well as its utility in evaluating the efficacy of future CCA treatment medicines and diagnostic tools. Because of its simplicity, reproducibility, and stability, the model is an appealing approach for studying CCA biology and developing effective treatments for this severe condition.

One of the biggest disadvantages of the TAA paradigm is that it is currently only standardized in rats. The animals' rapid gain in size and weight after 16–24 weeks of therapy creates a variety of challenges, including handling and care issues, as well as the need for larger amounts

of test chemicals. This is particularly remarkable when compared to mice, which are widely used in pre-clinical research due to their smaller size and lower maintenance requirements. Furthermore, the restricted availability of rats with genetic knockdowns of specific genes makes it difficult to investigate the precise function of molecules implicated in the etiology of CCA. Because it is difficult to determine the particular roles that different genes or pathways play in the start and progression of CCA, mechanistic research and the development of targeted therapies are limited. The absence of genetically modified rats further limits the model's flexibility and adaptability, making it unsuitable for a variety of studies, including gene function investigations and the evaluation of gene therapy procedures. Overall, standardizing the TAA model in rats is important, but it also highlights the importance of developing replacement models, such as mouse models, that can provide greater adaptability and accessibility for pre-clinical research.

CCA mouse models

The model known as "Smad4-Pten knockout"

The "Smad4-Pten knockout" mouse model of CCA was initially described by Xu *et al*. [91]. It uses the Cre-loxP method to conditionally disrupt the Smad4 and Pten genes. The researchers created this model by crossing albumin-Cre (Alb-Cre) mice with mice carrying conditional alleles for Smad4 (Smad4Co) and/or Pten (PtenCo). At 2–3 months of age, the Smad4Co/CoPtenCo/CoAlb-Cre animals developed hyperplastic foci in the biliary epithelium. Interestingly, by the time they were 4–7 months old, every animal in this group had developed full-blown CCA, as evidenced by a steady rise in intra-hepatic tumor nodules (Table 3). Because this model allows for the targeted deletion of two critical tumor suppressor genes in the liver, Smad4, and Pten, it is a valuable tool for studying the molecular mechanisms underlying CCA. This model's consistent and rapid development of CCA makes it an ideal platform for pre-clinical research and the evaluation of potential therapeutic methods for this devastating condition. The use of this model can help to identify new therapeutic targets and elucidate the complex interactions between genetic changes and environmental factors that influence the start and progression of CCA.

The Smad4-Pten deletion animal model allows researchers to understand better the genetic and molecular processes that contribute to the development of CCA. PTEN (phosphatase and tensin homolog deleted chromosome 10) has been connected to the pathogenesis of several cancers, including CCA [92], and the tumor suppressor gene SMAD4 is frequently altered in CCA [93]. PTEN loss causes constitutive activation of the pro-proliferative and anti-apoptotic PI3K pathway, which is critical in the development of human CCA [77,94,95]. The tumor cells in the Smad4Co/CoPtenCo/CoAlb-Cre mice exhibited ERK1/2 hyperphosphorylation, nuclear cyclin D1 overexpression, AKT hyperphosphorylation, and nuclear translocation. Following these findings, the researchers examined human CCA samples and discovered that 71% of phosphorylated-AKT-positive tumors had PTEN inactivation due to epigenetic changes, whereas 48% had lost SMAD expression. This model's capacity to reliably develop tumors at a relatively young age (4–5 months) without requiring additional manipulation is a significant advantage. This makes it an ideal environment for studying the onset and evolution of CCA and evaluating potential treatment methods. The model's usefulness and importance for pre-clinical research are further confirmed by its ability to reproduce the molecular mechanisms that drive human CCA, including the silencing of tumor suppressor genes and the activation of key signaling networks.

One of the numerous downsides of the Smad4-Pten knockout mice model is the lack of inflammation and sustained liver damage, both of which are common in human CCA. Furthermore, even in older animals, the model does not exhibit metastases, which are an important component of CCA progression. In addition, a tiny fraction of mice in this paradigm develop salivary gland tumors, which could be an independent event. Other options include utilizing Alb-Cre mice to deliver conditional gene knockouts. A recent study has shown that conditional knockouts in Alb-Cre animals are highly selective for hepatocytes, with low recombination in other liver cells, despite the authors' showing that Cre-mediated recombination occurs in both hepatocytes and cholangiocytes [96,97]. Given that the existing model may not effectively depict the disease's cellular genesis, it is worth investigating how hepatocyte-specific mutations contribute to the development of CCA. It is yet unknown how hepatocyte-specific mutations contribute to the development of CCA, and further research is needed to fully understand the relationship between hepatocyte and cholangiocyte biology in the context of CCA. The model's inadequacies highlight the importance of continuous pre-clinical model development and refinement to accurately recreate the complex biology of human CCA.

The "Carbon tetrachloride-P53 knockout" model

Farazi *et al*. [98] created a mouse model of CCA by administering CCl_4 3 times per week for 4 months. The purpose of this model was to reproduce the p53 gene mutation, which is frequent in human intra-hepatic CCA (IH-CCA) [5,99-101]. As expected, the mice grew bile ducts while suffering from increased liver damage and fibrosis. Initially, p53+/+ and +/− animals had cholangiocyte apoptosis; however, p53−/− mice did not. However, during the trial, only the p53−/− rats developed cytological abnormalities and early cancer foci after the CCl_4 injection was withdrawn. This means that CCA develops as a result of p53 function loss and that the molecular mechanisms underlying the disease can be studied using this model. Because it can reproduce the disease's genetic and histological traits, the model is an important tool for understanding its pathogenesis and developing effective treatment regimens. The use of p53 mutant mice in this paradigm allows researchers to investigate how p53 contributes to the formation of CCA, as well as how CCl_4 administration affects the liver and bile ducts. Overall, this model provides a useful framework for studying the complex interactions between environmental variables, genetic alterations, and liver disease in the development of CCA.

A group of mice was watched for an extended period of time after CCl_4 treatment was discontinued. IH-CCA nodules that had fully developed appeared. The tumors were characterized by disorganized, infiltrating ducts and tubules that tested positive for cytokeratin 19 and were surrounded by a thick collagenous stroma. Tumor formation was greatly impacted by the p53 genotype; IH-CCA was only observed in mice with a p53−/− or p53+/− genotype (54% and 18%, respectively). The p53 genotype had a significant impact on tumor latency, with p53−/− mice developing tumors after 29 weeks and p53+/− mice after 52 weeks (Table 3). Loss of p53 activity accelerates the development of IH-CCA. Even partial loss of p53 function (as in p53+/− mice) increases the probability of tumor formation. The findings of this study show that this mouse model has the ability to investigate the molecular pathways producing IH-CCA and provide crucial insights into the role of p53 in the development of this disease.

This model has a significant pathophysiological advantage because it combines a genetic predisposition (p53 knockout) with chronic liver injury caused by CCl_4, which is thought to mimic the conditions that cause CCA to develop in humans [5]. The development of iNOS, COX-2, c-Met, and cErbB2 in malignant cholangiocytes is one of the molecular characteristics of tumor nodules in this model that are similar to those in human CCA [7,102-104]. However, the model has a number of experimental limitations. One notable downside is the lengthy period of time required for tumor establishment, which might last between 29 and 52 weeks. Another disadvantage of the model is the unequal development of IH-CCA, which could complicate experiment planning and execution. Despite these limitations, the model remains a valuable resource for studying the pathophysiology of CCA and the molecular processes that underpin this disease. This model's molecular properties of the tumor nodules make it an appropriate model for studying human CCA, and the combination of genetic and environmental factors provides a unique opportunity to analyze the complicated interactions that contribute to CCA formation.

Table 4: An overview of the primary experimental characteristics of CCA rodent models

Animal model	Experimental type	Liver involvement	Orthotopic tumor formation	Genetic alterations	Toxic exposure	Surgical intervention	Inflammatory response	References
Mouse	DEN-LMBDL	Intrahepatic	Yes	Yes	Yes	Yes	Yes	[129]
Mouse	p53 Knockout (CCl4)	Intrahepatic	Yes	Yes	Yes	Yes	Yes	[98]
Rat	TAA-Induced Model	Intrahepatic	Yes	No	Yes	No	Yes	[80-88]
Mouse	Smad4-Pten Knockout	Intrahepatic	Yes	Yes	No	No	Yes	[91]
Mouse	Xenograft	Intrahepatic	No	No	No	No	No	[105-113]
Rat	Syngeneic Model	Intrahepatic	Yes	Yes	No	No	No	[76,78,79]

CCA: Cholangiocarcinoma

The "Xenograft" paradigm

In 1985, researchers used a human CCA cell line in a nude mouse model, injecting cells from a CCA metastasis into the flanks of immunocompromised mice [105]. Since then, this model has been utilized to study the pathophysiology and other aspects of CCA [106-113]. One of the model's key strengths is its capacity to analyze the efficacy of novel therapy choices for CCA. With obvious changes in tumor size occurring within 2 weeks following cell implantation, the model allows for rapid monitoring of tumor growth (Fig. 2c). Because trials can last up to 11 weeks, this strategy provides a relatively rapid and effective way to assess new medications (Table 3). This model is an ideal tool for pre-clinical CCA research because it allows for the control and replication of tumor growth and drug response. Using this model, researchers may quickly and efficiently examine the feasibility of novel therapy techniques, accelerating the development of more robust remedies for this severe condition.

Tannic acid, resveratrol, caffeic acid, anandamide, tamoxifen, felodipine, melatonin, and clobenpropit have all been shown to inhibit the growth of CCA xenograft tumors in naked mice [114-126]. Furthermore, it has been revealed that medicines such as oncolytic gene therapy and photodynamic therapy using hematoporphyrin derivatives are effective in reducing tumor growth. Furthermore, it has been proven that Slug si-RNA makes CCA cells more susceptible to cisplatin [127]. It is critical to recall that the subcutaneous xenograft model has significant limitations, including species-specific changes in pharmacodynamics and microenvironment when compared to liver-derived cancers [44]. Yokomuro *et al*. proposed an alternative approach to dealing with this issue, which entails injecting CCA cells directly into the livers of nude mice [128]. Despite the fact that this strategy requires an abdominal incision, it may provide a more accurate representation of the tumor microenvironment and allow for more relevant pre-clinical research. This orthotopic model may provide a more reliable method for assessing the efficacy of potential treatment options and understanding the complex interactions that occur between CCA cells and their surroundings.

The "den-left median bile duct ligation (LMBDL)" model

Yang *et al*. [129] presented a novel mouse model of CCA that combines chemical carcinogenesis and surgical modification. To develop the model, young adult Balb/c mice were given two weekly intraperitoneal (IP) injections of DEN. The mice are given DEN through oral gavage 1 week after the LMBDL, which occurs 2 weeks later. The experiment lasts 28 weeks (Table 3), and by the end of it, the overall animal survival rate is approximately 70%. Histopathological study of the livers at week 8 reveals multifocal cyst formation as well as multifocal cystic hyperplasia of the intrahepatic bile ducts. The LMBDL causes chronic biliary obstruction, an established risk factor for CCA, and the DEN injections initiate carcinogenesis; hence, this model is designed to mirror the human state. The oral gavage with DEN causes the liver to acquire CCA-like lesions, which promotes tumor growth. This new model provides a more complete view of the disease and its progression, making it an essential tool for studying the pathophysiology of CCA and evaluating potential treatment strategies. The biliary epithelium in the hyperplastic foci, as well as the epithelium lining the cysts, changed significantly throughout the experiment. By week 12, these cells' nuclei had grown to a size that indicated abnormal cell proliferation. Cholangiomas and biliary adenomas, which are benign tumors with the potential to become cancerous, first appeared in week 16. By week 28, these regions had developed into full-blown CCA. Interestingly, CCA did not develop in control rats that received any one or two of these therapies; rather, it only happened in animals who received a combination of DEN injection, LMBDL, and DEN feeding.

In control rats, the biliary epithelium was abnormal, but it did not progress to cancer. Throughout the experiment, the expression of c-Myc, a protein involved in cell proliferation, was monitored. In rats with CCA, the number of c-Myc-positive cells in the liver increased and remained continuously high, showing that c-Myc contributes to the development and spread of this cancer. However, in control rats, c-Myc expression increased before decreasing, indicating that the combo treatment was required to stimulate the formation of CCA.

Among its many advantages, this model is the only one capable of causing tumors to grow in non-GMMs, as it can generate CCA in wild-type mice. Furthermore, the model is associated with biliary obstruction and the production of oncogenes such as c-Myc, both of which are thought to be critical for the formation of primary liver cancers in people [130]. Understanding the role of c-Myc in the malignant transformation of cholangiocytes is hampered by the model's pathologic showing that the molecule is overexpressed not only in cholangiocytes but also in hepatocytes and inflammation cells. One of the model's experimental advantages is that tumor formation takes approximately 28 weeks. The model, however, is complex, requiring long-term weekly gavage of the mice as well as sensitive abdominal manipulation, both of which can be difficult and time-consuming. Although the model's complexities and technological limits must be carefully evaluated, it provides a useful tool for studying the development of CCA in a more physiologically realistic environment. Despite its limitations, researchers like this model because it allows them to study CCA formation in wild-type mice and shows the function of c-Myc and biliary obstruction.

CONCLUSION

Animal models, particularly those involving rats, are critical in cancer research because they allow researchers to replicate the genetic, pathophysiological, and environmental elements that contribute to cancer formation. Before moving forward with human clinical trials, these models provide a platform for pre-clinical testing of novel therapeutic methods, allowing for the evaluation of their safety and effectiveness. Rodents, such as mice, are appropriate for these studies due to their small size, ease of reproduction, and relatively low care costs. Since GMMs may be bred to imitate specific genetic mutations found in human cancers, their availability has increased their attractiveness in cancer research. This has allowed scientists to investigate the underlying causes of the disease and create precise models of CCA and other cancer types. Animal models, particularly mice, have altered cancer research, allowing us to develop new treatments and therapies while also improving our understanding of the disease.

Numerous rat models of CCA and HCC have recently been developed, each with unique characteristics. Several models have been shown

to be valuable resources for studying the pathophysiology of various cancers. However, determining how well these mouse models mirror the characteristics of important human diseases is difficult. To address this issue, Prof. Thorgeirsson's team conducted a thorough analysis, comparing the global gene expression patterns of 91 human HCCs from specified subclasses to those of 68 HCCs from seven different mouse models. The study found that HCCs from specific mouse models, such as Myc, E2f1, and Myc E2f1 transgenic mice, displayed gene expression patterns that were strikingly similar to those of human HCCs with greater survival rates. Animal models of HCCs, including DEN-induced mouse HCCs and Myc Tgfα transgenic mice, showed expression patterns similar to human HCCs with worse survival rates. This study demonstrates the need to carefully assess the relevance of mice models to human disease, as well as how gene expression patterns may be exploited to identify the most precise and instructional models for studying HCC and CCA.

Prof. Thorgeirsson's group found that gene expression patterns in ciprofibrate-induced HCCs and Acox1−/−mice were most dissimilar to those in human HCCs. Unfortunately, no equivalent study has been conducted for CCA, leaving a vacuum in our understanding of the similarities and differences between experimental models and actual CCA. A detailed review of the available models reveals that important characteristics of human CCA, such as genetic background, chronic liver damage, and cholestasis, are not reliably reproduced (Table 4). Furthermore, models of extra-hepatic CCA are uncommon. To use mouse models in future research, it is critical to understand their experimental properties and how they relate to the underlying human disease. The models' differences from human disease highlight the importance of more research and the development of new models that better depict the complexities of primary liver cancers. The scientific community is obligated to seek out the "ideal" model, and resolving these discrepancies is critical to increasing our understanding and treatment of these serious diseases. Researchers can improve the transfer of pre-clinical study results to clinical practice by acknowledging the limitations of existing models and attempting to develop more precise and relevant models.

AUTHORS' CONTRIBUTIONS

Lokeshvar Ravikumar: Conceptualization, Literature Review, Drafting of the Manuscript, and Data Compilation. Ramaiyan Velmurugan: Supervision, Critical Review and Editing, and Final Approval of the Manuscript. Yokesh S and Maha Swetha K: Data Collection, Formatting, Reference Management, and Technical Support.

CONFLICTS OF INTEREST

Nil.

FUNDING

Nil.

REFERENCES

1. El-Serag HB. Hepatocellular carcinoma. N Engl J Med. 2011 Sep 22;365(12):1118-27. doi: 10.1056/NEJMra1001683. PMID: 21992124
2. Lavanya M, Srinivasan P, Padmini R. Unveiling the anticancer effect of syringic acid and its derivatives in hepatocellular carcinoma. Int J Appl Pharm. 2023;15(4):114-24. doi: 10.22159/ijap.2023v15i4.47773
3. Acharya NS, Shah UR, Shah RG, Acharya S, Hingorani L. Evaluation of *in vitro* anticancer activity of *Symplocos racemosa* bark against hepatocellular carcinoma. Int J Pharm Pharm Sci. 2015 Nov;7(11):384-5.
4. Jayaraman MR, V L, Grace Priyadarshini S. An Unusual Case of Hepatocellular Carcinoma in a Healthy Adolescent Male. Cureus. 2024 Apr 15;16(4):e58357. doi: 10.7759/cureus.58357. PMID: 38756304.
5. Khan SA, Thomas HC, Davidson BR, Taylor-Robinson SD. Cholangiocarcinoma. Lancet. 2005 Oct 8;366(9493):1303-14. Erratum in: Lancet. 2006 May 20;367(9523):1656. doi: 10.1016/S0140-6736(05)67530-7. PMID: 16214602
6. Wolpin BM, Mayer RJ. A step forward in the treatment of advanced biliary tract cancer. N Engl J Med. 2010 Apr 8;362(14):1335-7. doi: 10.1056/NEJMe1001183. PMID: 20375411
7. Blechacz B, Gores GJ. Cholangiocarcinoma: Advances in pathogenesis, diagnosis, and treatment. Hepatology. 2008 Jul;48(1):308-21. doi: 10.1002/hep.22310. PMID: 18536057; PMC2547491
8. Pitot HC, Dragan YP. Facts and theories concerning the mechanisms of carcinogenesis. FASEB J. 1991 Jun;5(9):2280-6. PMID: 1860619
9. Pandey P, Elsori D, Kumar R, Lakhanpal S, Rautela I, Alqahtani TM, *et al*. Ferroptosis targeting natural compounds as a promising approach for developing potent liver cancer agents. Front Pharmacol. 2024 Apr 26;15:1399677. doi: 10.3389/fphar.2024.1399677. PMID: 38738178
10. Gray R, Peto R, Brantom P, Grasso P. Chronic nitrosamine ingestion in 1040 rodents: The effect of the choice of nitrosamine, the species studied, and the age of starting exposure. Cancer Res. 1991 Dec 1;51(23 Pt 2):6470-91. PMID: 1933908
11. Kawanishi S, Hiraku Y, Murata M, Oikawa S. The role of metals in site-specific DNA damage with reference to carcinogenesis. Free Radic Biol Med. 2002 May 1;32(9):822-32. doi: 10.1016/s0891-5849(02)00779-7. PMID: 11978484
12. Valko M, Rhodes CJ, Moncol J, Izakovic M, Mazur M. Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chem Biol Interact. 2006 Mar 10;160(1):1-40. doi: 10.1016/j.cbi.2005.12.009. PMID: 16430879
13. Lampimukhi M, Qassim T, Venu R, Pakhala N, Mylavarapu S, Perera T, *et al*. A Review of incidence and related risk factors in the development of hepatocellular carcinoma. Cureus. 2023 Nov 26;15(11):e49429. doi: 10.7759/cureus.49429. PMID: 38149129
14. Park TJ, Kim JY, Oh SP, Kang SY, Kim BW, Wang HJ, *et al*. TIS21 negatively regulates hepatocarcinogenesis by disruption of cyclin B1-Forkhead box M1 regulation loop. Hepatology. 2008 May;47(5):1533-43. doi: 10.1002/hep.22212. PMID: 18393292
15. Teoh NC, Dan YY, Swisshelm K, Lehman S, Wright JH, Haque J, *et al*. Defective DNA strand break repair causes chromosomal instability and accelerates liver carcinogenesis in mice. Hepatology. 2008 Jun;47(6):2078-88. doi: 10.1002/hep.22194. PMID: 18506893
16. Williams GM, Iatropoulos MJ, Jeffrey AM. Mechanistic basis for nonlinearities and thresholds in rat liver carcinogenesis by the DNA-reactive carcinogens 2-acetylaminofluorene and diethylnitrosamine. Toxicol Pathol. 2000 May-Jun;28(3):388-95. doi: 10.1177/019262330002800306. PMID: 10862555
17. Zimmers TA, Jin X, Gutierrez JC, Acosta C, McKillop IH, Pierce RH, *et al*. Effect of *in vivo* loss of GDF-15 on hepatocellular carcinogenesis. J Cancer Res Clin Oncol. 2008 Jul;134(7):753-9. doi: 10.1007/s00432-007-0336-4, PMID: 18210153
18. Finnberg N, Stenius U, Högberg J. Heterozygous p53-deficient (+/-) mice develop fewer p53-negative preneoplastic focal liver lesions in response to treatment with diethylnitrosamine than do wild-type (+/+) mice. Cancer Lett. 2004 Apr 30;207(2):149-55. doi: 10.1016/j.canlet.2003.11.013. PMID: 15072823
19. Shiota G, Harada K, Ishida M, Tomie Y, Okubo M, Katayama S, *et al*. Inhibition of hepatocellular carcinoma by glycyrrhizin in diethylnitrosamine-treated mice. Carcinogenesis. 1999 Jan;20(1):59-63. doi: 10.1093/carcin/20.1.59. PMID: 9934850
20. Hsu HC, Jeng YM, Mao TL, Chu JS, Lai PL, Peng SY. Beta-catenin mutations are associated with a subset of low-stage hepatocellular carcinoma negative for hepatitis B virus and with favorable prognosis. Am J Pathol. 2000 Sep;157(3):763-70. doi: 10.1016/s0002-9440(10)64590-7. PMID: 10980116; PMCID: PMC1885685
21. Mao TL, Chu JS, Jeng YM, Lai PL, Hsu HC. Expression of mutant nuclear beta-catenin correlates with non-invasive hepatocellular carcinoma, absence of portal vein spread, and good prognosis. J Pathol. 2001 Jan;193(1):95-101. doi: 10.1002/1096-9896(2000)9999:9999<:AID-PATH720>3.0.CO;2-3. PMID: 11169521
22. Farber E, Solt D, Cameron R, Laishes B, Ogawa K, Medline A. Newer insights into the pathogenesis of liver cancer. Am J Pathol. 1977 Nov;89(2):477-82. PMID: 920780; PMC2032235
23. Klinman NR, Erslev AJ. Cellular response to partial hepatectomy. Proc Soc Exp Biol Med. 1963 Feb;112:338-40. doi: 10.3181/00379727-112-28037. PMID: 14033567
24. Rignall B, Braeuning A, Buchmann A, Schwarz M. Tumor formation in liver of conditional β-catenin-deficient mice exposed to a diethylnitrosamine/phenobarbital tumor promotion regimen. Carcinogenesis. 2011 Jan;32(1):52-7. doi: 10.1093/carcin/bgq226, PMID: 21047994
25. Puatanachokchai R, Kakuni M, Wanibuchi H, Kinoshita A, Kang JS,

Salim EI, *et al*. Lack of promoting effects of phenobarbital at low dose on diethylnitrosamine-induced hepatocarcinogenesis in TGF-alpha transgenic mice. Asian Pac J Cancer Prev. 2006 Apr-Jun;7(2):274-8. PMID: 16839222

26. Baek HJ, Lim SC, Kitisin K, Jogunoori W, Tang Y, Marshall MB, *et al*. Hepatocellular cancer arises from loss of transforming growth factor beta signaling adaptor protein embryonic liver fodrin through abnormal angiogenesis. Hepatology. 2008 Oct;48(4):1128-37. doi: 10.1002/hep.22460. PMID: 18704924; PMC2747753
27. Lakhtakia R, Kumar V, Reddi H, Mathur M, Dattagupta S, Panda SK. Hepatocellular carcinoma in a hepatitis B "x" transgenic mouse model: A sequential pathological evaluation. J Gastroenterol Hepatol. 2003 Jan;18(1):80-91. doi: 10.1046/j.1440-1746.2003.02902.x. PMID: 12519229
28. Xiong J, Yao YC, Zi XY, Li JX, Wang XM, Ye XT, *et al*. Expression of hepatitis B virus X protein in transgenic mice. World J Gastroenterol. 2003 Jan;9(1):112-6. doi: 10.3748/wjg.v9.i1.112. PMID: 12508363; PMC4728222
29. Hays T, Rusyn I, Burns AM, Kennett MJ, Ward JM, Gonzalez FJ, *et al*. Role of peroxisome proliferator-activated receptor-alpha (PPARalpha) in bezafibrate-induced hepatocarcinogenesis and cholestasis. Carcinogenesis. 2005 Jan;26(1):219-27. doi: 10.1093/carcin/bgh285. PMID: 15447978
30. Zhao W, Iskandar S, Kooshki M, Sharpe JG, Payne V, Robbins ME. Knocking out peroxisome proliferator-activated receptor (PPAR) alpha inhibits radiation-induced apoptosis in the mouse kidney through activation of NF-kappaB and increased expression of IAPs. Radiat Res. 2007 May;167(5):581-91. doi: 10.1667/RR0814.1. PMID: 17474796
31. Yin PH, Lee HC, Chau GY, Wu YT, Li SH, Lui WY, *et al*. Alteration of the copy number and deletion of mitochondrial DNA in human hepatocellular carcinoma. Br J Cancer. 2004 Jun 14;90(12):2390-6. doi: 10.1038/sj.bjc.6601838. PMID: 15150555; PMC2409531
32. Liu SP, Li YS, Chen YJ, Chiang EP, Li AF, Lee YH, *et al*. Glycine N-methyltransferase-/-mice develop chronic hepatitis and glycogen storage disease in the liver. Hepatology. 2007 Nov;46(5):1413-25. Erratum in: Hepatology. 2008 Feb;47(2):769. Hwang, Shiu-Feng [corrected to Huang, Shiu-Feng]. doi: 10.1002/hep.21863. PMID: 17937387
33. Woo LL, Egner PA, Belanger CL, Wattanawaraporn R, Trudel LJ, Croy RG, *et al*. Aflatoxin B1-DNA adduct formation and mutagenicity in livers of neonatal male and female B6C3F1 mice. Toxicol Sci. 2011 Jul;122(1):38-44. Erratum in: Toxicol Sci. 2012 Jan;125(1):326. Bouhenguel, Jason T [added]. doi: 10.1093/toxsci/kfr087. PMID: 21507988; PMC3143467
34. Hulla JE, Chen ZY, Eaton DL. Aflatoxin B1-induced rat hepatic hyperplastic nodules do not exhibit a site-specific mutation within the p53 gene. Cancer Res. 1993 Jan 1;53(1):9-11. PMID: 8380129
35. Naas T, Ghorbani M, Alvarez-Maya I, Lapner M, Kothary R, De Repentigny Y, *et al*. Characterization of liver histopathology in a transgenic mouse model expressing genotype 1a hepatitis C virus core and envelope proteins 1 and 2. J Gen Virol. 2005 Aug;86(Pt 8):2185-2196. doi: 10.1099/vir.0.80969-0. PMID: 16033966
36. Kamegaya Y, Hiasa Y, Zukerberg L, Fowler N, Blackard JT, Lin W, *et al*. Hepatitis C virus acts as a tumor accelerator by blocking apoptosis in a mouse model of hepatocarcinogenesis. Hepatology. 2005 Mar;41(3):660-7. doi: 10.1002/hep.20621. PMID: 15723444
37. Domenicali M, Caraceni P, Principe A, Pertosa AM, Ros J, Chieco P, *et al*. A novel sodium overload test predicting ascites decompensation in rats with CCl4-induced cirrhosis. J Hepatol. 2005 Jul;43(1):92-7. doi: 10.1016/j.jhep.2005.01.034. PMID: 15893844
38. Sheweita SA, Abd El-Gabar M, Bastawy M. Carbon tetrachloride-induced changes in the activity of phase II drug-metabolizing enzyme in the liver of male rats: Role of antioxidants. Toxicology. 2001 Aug 28;165(2-3):217-24. doi: 10.1016/s0300-483x(01)00429-2. PMID: 11522380
39. Farazi PA, Glickman J, Horner J, Depinho RA. Cooperative interactions of p53 mutation, telomere dysfunction, and chronic liver damage in hepatocellular carcinoma progression. Cancer Res. 2006 May 1;66(9):4766-73. doi: 10.1158/0008-5472.CAN-05-4608. PMID: 16651430
40. Frezza EE, Gerunda GE, Farinati F, DeMaria N, Galligioni A, Plebani F, *et al*. CCL4-induced liver cirrhosis and hepatocellular carcinoma in rats: Relationship to plasma zinc, copper and estradiol levels. Hepatogastroenterology. 1994 Aug;41(4):367-9. PMID: 7959573
41. Knight B, Yeoh GC, Husk KL, Ly T, Abraham LJ, Yu C, *et al*. Impaired preneoplastic changes and liver tumor formation in tumor necrosis factor receptor type 1 knockout mice. J Exp Med. 2000 Dec 18;192(12):1809-18. doi: 10.1084/jem.192.12.1809. PMID: 11120777; PMC2213505
42. Hasmall SC, James NH, Macdonald N, Gonzalez FJ, Peters JM, Roberts RA. Suppression of mouse hepatocyte apoptosis by peroxisome proliferators: Role of PPARalpha and TNFalpha. Mutat Res. 2000 Mar 17;448(2):193-200. doi: 10.1016/s0027-5107(99)00236-5. PMID: 10725472
43. Reddy JK, Rao S, Moody DE. Hepatocellular carcinomas in acatalasemic mice treated with nafenopin, a hypolipidemic peroxisome proliferator. Cancer Res. 1976 Apr;36(4):1211-7. PMID: 177202
44. Newell P, Villanueva A, Friedman SL, Koike K, Llovet JM. Experimental models of hepatocellular carcinoma. J Hepatol. 2008 May;48(5):858-79. doi: 10.1016/j.jhep.2008.01.008. PMID: 18314222; PMC2990959
45. McGlynn KA, Hunter K, LeVoyer T, Roush J, Wise P, Michielli RA, *et al*. Susceptibility to aflatoxin B1-related primary hepatocellular carcinoma in mice and humans. Cancer Res. 2003 Aug 1;63(15):4594-601. PMID: 12907637
46. Weisburger EK. Carcinogenicity studies on halogenated hydrocarbons. Environ Health Perspect. 1977 Dec;21:7-16. doi: 10.1289/ehp.77217. PMID: 206428; PMC1475344
47. Campo GM, Avenoso A, Campo S, Nastasi G, Traina P, D'Ascola A, *et al*. The antioxidant activity of chondroitin-4-sulphate, in carbon tetrachloride-induced acute hepatitis in mice, involves NF-kappaB and caspase activation. Br J Pharmacol. 2008 Nov;155(6):945-56. doi: 10.1038/bjp.2008.338. PMID: 18724385; PMC2597242
48. Nicholes K, Guillet S, Tomlinson E, Hillan K, Wright B, Frantz GD, *et al*. A mouse model of hepatocellular carcinoma: Ectopic expression of fibroblast growth factor 19 in skeletal muscle of transgenic mice. Am J Pathol. 2002 Jun;160(6):2295-307. doi: 10.1016/S0002-9440(10)61177-7. PMID: 12057932; PMC1850847
49. Watanabe S, Horie Y, Kataoka E, Sato W, Dohmen T, Ohshima S, *et al*. Non-alcoholic steatohepatitis and hepatocellular carcinoma: Lessons from hepatocyte-specific phosphatase and tensin homolog (PTEN)-deficient mice. J Gastroenterol Hepatol. 2007 Jun;22 Suppl 1:S96-100. doi: 10.1111/j.1440-1746.2006.04665.x. Erratum in: J Gastroenterol Hepatol. 2008 Mar;23(3):501-2. PMID: 17567478
50. Seki E, Brenner DA. The role of NF-kappaB in hepatocarcinogenesis: Promoter or suppressor? J Hepatol. 2007 Aug;47(2):307-9. doi: 10.1016/j.jhep.2007.05.006. PMID: 17566588; PMC2739234
51. Horie Y, Suzuki A, Kataoka E, Sasaki T, Hamada K, Sasaki J, *et al*. Hepatocyte-specific Pten deficiency results in steatohepatitis and hepatocellular carcinomas. J Clin Invest. 2004 Jun;113(12):1774-83. doi: 10.1172/JCI20513. PMID: 15199412; PMC420505
52. Inokuchi S, Aoyama T, Miura K, Osterreicher CH, Kodama Y, Miyai K, *et al*. Disruption of TAK1 in hepatocytes causes hepatic injury, inflammation, fibrosis, and carcinogenesis. Proc Natl Acad Sci U S A. 2010 Jan 12;107(2):844-9. doi: 10.1073/pnas.0909781107. PMID: 20080763; PMC2818947
53. Luedde T, Beraza N, Kotsikoris V, van Loo G, Nenci A, De Vos R, *et al*. Deletion of NEMO/IKKgamma in liver parenchymal cells causes steatohepatitis and hepatocellular carcinoma. Cancer Cell. 2007 Feb;11(2):119-32. doi: 10.1016/j.ccr.2006.12.016. PMID: 17292824
54. Nakano T, Cheng YF, Lai CY, Hsu LW, Chang YC, Deng JY, *et al*. Impact of artificial sunlight therapy on the progress of non-alcoholic fatty liver disease in rats. J Hepatol. 2011 Aug;55(2):415-25. doi: 10.1016/j.jhep.2010.11.028. PMID: 21184788
55. de Lima VM, Oliveira CP, Alves VA, Chammas MC, Oliveira EP, Stefano JT, *et al*. A rodent model of NASH with cirrhosis, oval cell proliferation and hepatocellular carcinoma. J Hepatol. 2008 Dec;49(6):1055-61. doi: 10.1016/j.jhep.2008.07.024. PMID: 18929425
56. Zhong B, Zhou Q, Toivola DM, Tao GZ, Resurreccion EZ, Omary MB. Organ-specific stress induces mouse pancreatic keratin overexpression in association with NF-kappaB activation. J Cell Sci. 2004 Apr 1;117(Pt 9):1709-19. doi: 10.1242/jcs.01016. PMID: 15075232
57. Guest I, Ilic Z, Sell S. Age dependence of oval cell responses and bile duct carcinomas in male Fischer 344 rats fed a cyclic choline-deficient, ethionine-supplemented diet. Hepatology. 2010 Nov;52(5):1750-7. doi: 10.1002/hep.23880. PMID: 20842700; PMC2967670
58. Kodama Y, Kisseleva T, Iwaisako K, Miura K, Taura K, De Minicis S, *et al*. c-Jun N-terminal kinase-1 from hematopoietic cells mediates progression from hepatic steatosis to steatohepatitis and fibrosis in mice. Gastroenterology. 2009 Oct;137(4):1467-77.e5. doi: 10.1053/j.gastro.2009.06.045. PMID: 19549522; PMC2757473
59. Yang MC, Chang CP, Lei HY. Induction of liver fibrosis in a murine hepatoma model by thioacetamide is associated with enhanced tumor growth and suppressed antitumor immunity. Lab Invest.

2010 Dec;90(12):1782-93. doi: 10.1038/labinvest.2010.139. PMID: 20680008

60. Rygaard J, Povlsen CO. Heterotransplantation of a human malignant tumour to "Nude" mice. Acta Pathol Microbiol Scand. 1969;77(4):758-60. doi: 10.1111/j.1699-0463.1969.tb04520.x. PMID: 5383844
61. Sun FX, Tang ZY, Lui KD, Ye SL, Xue Q, Gao DM, *et al*. Establishment of a metastatic model of human hepatocellular carcinoma in nude mice via orthotopic implantation of histologically intact tissues. Int J Cancer. 1996 Apr 10;66(2):239-43. doi: 10.1002/(SICI)1097-0215(19960410)66:2<239:AID-IJC17>3.0.CO;2-7. PMID: 8603818
62. Huynh H, Soo KC, Chow PK, Panasci L, Tran E. Xenografts of human hepatocellular carcinoma: A useful model for testing drugs. Clin Cancer Res. 2006 Jul 15;12(14 Pt 1):4306-14. doi: 10.1158/1078-0432.CCR-05-2568. PMID: 16857806
63. Matsuo M, Sakurai H, Saiki I. ZD1839, a selective epidermal growth factor receptor tyrosine kinase inhibitor, shows antimetastatic activity using a hepatocellular carcinoma model. Mol Cancer Ther. 2003 Jun;2(6):557-61. PMID: 12813135
64. Kornek M, Raskopf E, Tolba R, Becker U, Klöckner M, Sauerbruch T, *et al*. Accelerated orthotopic hepatocellular carcinomas growth is linked to increased expression of pro-angiogenic and prometastatic factors in murine liver fibrosis. Liver Int. 2008 Apr;28(4):509-18. doi: 10.1111/j.1478-3231.2008.01670.x. PMID: 18339078
65. Tang TC, Man S, Xu P, Francia G, Hashimoto K, Emmenegger U, *et al*. Development of a resistance-like phenotype to sorafenib by human hepatocellular carcinoma cells is reversible and can be delayed by metronomic UFT chemotherapy. Neoplasia. 2010 Nov;12(11):928-40. doi: 10.1593/neo.10804. PMID: 21076618; PMC2978915
66. Shao DM, Wang QH, Chen C, Shen ZH, Yao M, Zhou XD, *et al*. N-acetylglucosaminyltransferase V activity in metastatic models of human hepatocellular carcinoma in nude mice. J Exp Clin Cancer Res. 1999 Sep;18(3):331-5. PMID: 10606178
67. Hollingshead MG, Alley MC, Camalier RF, Abbott BJ, Mayo JG, Malspeis L, *et al*. *In vivo* cultivation of tumor cells in hollow fibers. Life Sci. 1995;57(2):131-41. doi: 10.1016/0024-3205(95)00254-4. PMID: 7603295
68. Suggitt M, Bibby MC. 50 years of preclinical anticancer drug screening: Empirical to target-driven approaches. Clin Cancer Res. 2005 Feb 1;11(3):971-81. PMID: 15709162
69. Shnyder SD, Cooper PA, Scally AJ, Bibby MC. Reducing the cost of screening novel agents using the hollow fibre assay. Anticancer Res. 2006 May-Jun;26(3A):2049-52. PMID: 16827143
70. Frese KK, Tuveson DA. Maximizing mouse cancer models. Nat Rev Cancer. 2007 Sep;7(9):645-58. doi: 10.1038/nrc2192. PMID: 17687385
71. Tuveson DA, Jacks T. Technologically advanced cancer modeling in mice. Curr Opin Genet Dev. 2002 Feb;12(1):105-10. doi: 10.1016/s0959-437x(01)00272-6. PMID: 11790563
72. Koo JS, Seong JK, Park C, Yu DY, Oh BK, Oh SH, *et al*. Large liver cell dysplasia in hepatitis B virus x transgenic mouse liver and human chronic hepatitis B virus-infected liver. Intervirology. 2005;48(1):16-22. doi: 10.1159/000082090. PMID: 15785085
73. Beraza N, Malato Y, Sander LE, Al-Masaoudi M, Freimuth J, Riethmacher D, *et al*. Hepatocyte-specific NEMO deletion promotes NK/NKT cell- and TRAIL-dependent liver damage. J Exp Med. 2009 Aug 3;206(8):1727-37. doi: 10.1084/jem.20082152. PMID: 19635861; PMC2722179
74. Harada N, Oshima H, Katoh M, Tamai Y, Oshima M, Taketo MM. Hepatocarcinogenesis in mice with beta-catenin and Ha-ras gene mutations. Cancer Res. 2004 Jan 1;64(1):48-54. doi: 10.1158/0008-5472.can-03-2123. PMID: 14729607
75. Merle P, Kim M, Herrmann M, Gupte A, Lefrançois L, Califano S, *et al*. Oncogenic role of the frizzled-7/beta-catenin pathway in hepatocellular carcinoma. J Hepatol. 2005 Nov;43(5):854-62. doi: 10.1016/j.jhep.2005.05.018. PMID: 16098625
76. Sirica AE, Zhang Z, Lai GH, Asano T, Shen XN, Ward DJ, *et al*. A novel "patient-like" model of cholangiocarcinoma progression based on bile duct inoculation of tumorigenic rat cholangiocyte cell lines. Hepatology. 2008 Apr;47(4):1178-90. doi: 10.1002/hep.22088. PMID: 18081149
77. Fava G, Marzioni M, Benedetti A, Glaser S, DeMorrow S, Francis H, *et al*. Molecular pathology of biliary tract cancers. Cancer Lett. 2007 Jun 8;250(2):155-67. doi: 10.1016/j.canlet.2006.09.011. PMID: 17069969
78. Fingas CD, Blechacz BR, Smoot RL, Guicciardi ME, Mott J, Bronk SF, *et al*. A smac mimetic reduces TNF related apoptosis inducing ligand (TRAIL)-induced invasion and metastasis of cholangiocarcinoma cells. Hepatology. 2010 Aug;52(2):550-61. doi: 10.1002/hep.23729. PMID: 20683954; PMC2957364
79. Blechacz BR, Smoot RL, Bronk SF, Werneburg NW, Sirica AE, Gores GJ. Sorafenib inhibits signal transducer and activator of transcription-3 signaling in cholangiocarcinoma cells by activating the phosphatase shatterproof 2. Hepatology. 2009 Dec;50(6):1861-70. doi: 10.1002/hep.23214. PMID: 19821497; PMC2891152
80. Praet MM, Roels HJ. Histogenesis of cholangiomas and cholangiocarcinomas in thioacetamide fed rats. Exp Pathol. 1984;26(1):3-14. doi: 10.1016/s0232-1513(84)80063-8. PMID: 6207043
81. Dashti H, Jeppsson B, Hägerstrand I, Hultberg B, Srinivas U, Abdulla M, *et al*. Thioacetamide-and carbon tetrachloride-induced liver cirrhosis. Eur Surg Res. 1989;21(2):83-91. doi: 10.1159/000129007. PMID: 2767088
82. Al-Bader A, Mathew TC, Abul H, Al-Sayer H, Singal PK, Dashti HM. Cholangiocarcinoma and liver cirrhosis in relation to changes due to thioacetamide. Mol Cell Biochem. 2000 May;208(1-2):1-10. doi: 10.1023/a:1007082515548. PMID: 10939622
83. Jan YY, Yeh TS, Yeh JN, Yang HR, Chen MF. Expression of epidermal growth factor receptor, apomucins, matrix metalloproteinases, and p53 in rat and human cholangiocarcinoma: Appraisal of an animal model of cholangiocarcinoma. Ann Surg. 2004 Jul;240(1):89-94. doi: 10.1097/01.sla.0000129492.95311.f2. PMID: 15213623; PMC1356379
84. Yeh CN, Maitra A, Lee KF, Jan YY, Chen MF. Thioacetamide-induced intestinal-type cholangiocarcinoma in rat: An animal model recapitulating the multi-stage progression of human cholangiocarcinoma. Carcinogenesis. 2004 Apr;25(4):631-6. doi: 10.1093/carcin/bgh037. PMID: 14656942
85. Fava G, Alpini G, Rychlicki C, Saccomanno S, DeMorrow S, Trozzi L, *et al*. Leptin enhances cholangiocarcinoma cell growth. Cancer Res. 2008 Aug 15;68(16):6752-61. doi: 10.1158/0008-5472.CAN-07-6682. PMID: 18701500; PMC2556377
86. Marzioni M, Torrice A, Saccomanno S, Rychlicki C, Agostinelli L, Pierantonelli I, *et al*. An oestrogen receptor β-selective agonist exerts anti-neoplastic effects in experimental intrahepatic cholangiocarcinoma. Dig Liver Dis. 2012 Feb;44(2):134-42. doi: 10.1016/j.dld.2011.06.014. PMID: 21782536
87. Liu KH, Liao LM, Ro LS, Wu YL, Yeh TS. Thalidomide attenuates tumor growth and preserves fast-twitch skeletal muscle fibers in cholangiocarcinoma rats. Surgery. 2008 Mar;143(3):375-83. doi: 10.1016/j.surg.2007.09.035. PMID: 18291259
88. Mansuroglu T, Ramadori P, Dudás J, Malik I, Hammerich K, Füzesi L, *et al*. Expression of stem cell factor and its receptor c-Kit during the development of intrahepatic cholangiocarcinoma. Lab Invest. 2009 May;89(5):562-74. doi: 10.1038/labinvest.2009.15. PMID: 19255573
89. Laverman P, Blokx WA, Te Morsche RH, Frielink C, Boerman OC, Oyen WJ, *et al*. [(18)F]FDG accumulation in an experimental model of multistage progression of cholangiocarcinoma. Hepatol Res. 2007 Feb;37(2):127-32. doi: 10.1111/j.1872-034X.2007.00016.x. PMID: 17300708
90. Yeh CN, Lin KJ, Hsiao IT, Yen TC, Chen TW, Jan YY, *et al*. Animal PET for thioacetamide-induced rat cholangiocarcinoma: A novel and reliable platform. Mol Imaging Biol. 2008 Jul-Aug;10(4):209-16. doi: 10.1007/s11307-008-0141-8. PMID: 18491193
91. Xu X, Kobayashi S, Qiao W, Li C, Xiao C, Radaeva S, *et al*. Induction of intrahepatic cholangiocellular carcinoma by liver-specific disruption of Smad4 and Pten in mice. J Clin Invest. 2006 Jul;116(7):1843-52. doi: 10.1172/JCI27282. PMID: 16767220; PMCID: PMC1474816
92. Sansal I, Sellers WR. The biology and clinical relevance of the PTEN tumor suppressor pathway. J Clin Oncol. 2004 Jul 15;22(14):2954-63. doi: 10.1200/JCO.2004.02.141. PMID: 15254063
93. Kang YK, Kim WH, Jang JJ. Expression of G1-S modulators (p53, p16, p27, cyclin D1, Rb) and Smad4/Dpc4 in intrahepatic cholangiocarcinoma. Hum Pathol. 2002 Sep;33(9):877-83. doi: 10.1053/hupa.2002.127444. PMID: 12378511
94. Kobayashi S, Werneburg NW, Bronk SF, Kaufmann SH, Gores GJ. Interleukin-6 contributes to Mcl-1 up-regulation and TRAIL resistance via an Akt-signaling pathway in cholangiocarcinoma cells. Gastroenterology. 2005 Jun;128(7):2054-65. doi: 10.1053/j.gastro.2005.03.010. PMID: 15940637
95. Tanno S, Yanagawa N, Habiro A, Koizumi K, Nakano Y, Osanai M, *et al*. Serine/threonine kinase AKT is frequently activated in human bile duct cancer and is associated with increased radioresistance. Cancer Res. 2004 May 15;64(10):3486-90. doi: 10.1158/0008-5472.CAN-03-1788. PMID: 15150102

96. Taura K, Miura K, Iwaisako K, Osterreicher CH, Kodama Y, Penz-Osterreicher M, *et al*. Hepatocytes do not undergo epithelial-mesenchymal transition in liver fibrosis in mice. Hepatology. 2010 Mar;51(3):1027-36. doi: 10.1002/hep.23368. PMID: 20052656; PMC2906231
97. Zeisberg M, Yang C, Martino M, Duncan MB, Rieder F, Tanjore H, *et al*. Fibroblasts derive from hepatocytes in liver fibrosis via epithelial to mesenchymal transition. J Biol Chem. 2007 Aug 10;282(32):23337-47. doi: 10.1074/jbc.M700194200. PMID: 17562716
98. Farazi PA, Zeisberg M, Glickman J, Zhang Y, Kalluri R, DePinho RA. Chronic bile duct injury associated with fibrotic matrix microenvironment provokes cholangiocarcinoma in p53-deficient mice. Cancer Res. 2006 Jul 1;66(13):6622-7. doi: 10.1158/0008-5472.CAN-05-4609. PMID: 16818635
99. Momoi H, Itoh T, Nozaki Y, Arima Y, Okabe H, Satoh S, *et al*. Microsatellite instability and alternative genetic pathway in intrahepatic cholangiocarcinoma. J Hepatol. 2001 Aug;35(2):235-44. doi: 10.1016/s0168-8278(01)00106-4. PMID: 11580146
100. Furubo S, Harada K, Shimonishi T, Katayanagi K, Tsui W, Nakanuma Y. Protein expression and genetic alterations of p53 and ras in intrahepatic cholangiocarcinoma. Histopathology. 1999 Sep;35(3):230-40. doi: 10.1046/j.1365-2559.1999.00705.x. PMID: 10469215
101. Tullo A, D'Erchia AM, Honda K, Kelly MD, Habib NA, Saccone C, *et al*. New p53 mutations in hilar cholangiocarcinoma. Eur J Clin Invest. 2000 Sep;30(9):798-803. doi: 10.1046/j.1365-2362.2000.00717.x. PMID: 10998080
102. Okuda K, Nakanuma Y, Miyazaki M. Cholangiocarcinoma: Recent progress. Part 2: Molecular pathology and treatment. J Gastroenterol Hepatol. 2002 Oct;17(10):1056-63. doi: 10.1046/j.1440-1746.2002.02780.x. PMID: 12201864
103. Okuda K, Nakanuma Y, Miyazaki M. Cholangiocarcinoma: Recent progress. Part 1: Epidemiology and etiology. J Gastroenterol Hepatol. 2002 Oct;17(10):1049-55. doi: 10.1046/j.1440-1746.2002.02781.x. PMID: 12201863
104. Endo K, Yoon BI, Pairojkul C, Demetris AJ, Sirica AE. ERBB-2 overexpression and cyclooxygenase-2 up-regulation in human cholangiocarcinoma and risk conditions. Hepatology. 2002 Aug;36(2):439-50. doi: 10.1053/jhep.2002.34435. PMID: 12143054
105. Hudd C, Euhus DM, LaRegina MC, Herbold DR, Palmer DC, Johnson FE. Effect of cholecystokinin on human cholangiocarcinoma xenografted into nude mice. Cancer Res. 1985 Mar;45(3):1372-7. PMID: 2982488
106. Wang M, Xiao J, Shen M, Yahong Y, Tian R, Zhu F, *et al*. Isolation and characterization of tumorigenic extrahepatic cholangiocarcinoma cells with stem cell-like properties. Int J Cancer. 2011 Jan 1;128(1):72-81. doi: 10.1002/ijc.25317. PMID: 20232394
107. Lu D, Han C, Wu T. Microsomal prostaglandin E synthase-1 inhibits PTEN and promotes experimental cholangiocarcinogenesis and tumor progression. Gastroenterology. 2011 Jun;140(7):2084-94. doi: 10.1053/j.gastro.2011.02.056. PMID: 21354147; PMC3109169
108. Rozich RA, Mills DR, Brilliant KE, Callanan HM, Yang D, Tantravahi U, *et al*. Accumulation of neoplastic traits prior to spontaneous *in vitro* transformation of rat cholangiocytes determines susceptibility to activated ErbB-2/Neu. Exp Mol Pathol. 2010 Dec;89(3):248-59. doi: 10.1016/j.yexmp.2010.07.005. PMID: 20655306; PMC4012332
109. Shiraso S, Katayose Y, Yamamoto K, Mizuma M, Yabuuchi S, Oda A, *et al*. Overexpression of adenovirus-mediated p27kip1 lacking the Jab1-binding region enhances cytotoxicity and inhibits xenografted human cholangiocarcinoma growth. Anticancer Res. 2009 Jun;29(6):2015-24. PMID: 19528460
110. Fava G, Demorrow S, Gaudio E, Franchitto A, Onori P, Carpino G, *et al*. Endothelin inhibits cholangiocarcinoma growth by a decrease in the vascular endothelial growth factor expression. Liver Int. 2009 Aug;29(7):1031-42. doi: 10.1111/j.1478-3231.2009.01997.x. PMID: 19291182; PMC2706939
111. Fava G, Marucci L, Glaser S, Francis H, De Morrow S, Benedetti A, *et al*. gamma-Aminobutyric acid inhibits cholangiocarcinoma growth by cyclic AMP-dependent regulation of the protein kinase A/extracellular signal-regulated kinase 1/2 pathway. Cancer Res. 2005 Dec 15;65(24):11437-46. doi: 10.1158/0008-5472.CAN-05-1470. PMID: 16357152
112. Francis H, Onori P, Gaudio E, Franchitto A, DeMorrow S, Venter J, *et al*. H3 histamine receptor-mediated activation of protein kinase Calpha inhibits the growth of cholangiocarcinoma *in vitro* and *in vivo*. Mol Cancer Res. 2009 Oct;7(10):1704-13. doi: 10.1158/1541-7786.MCR-09-0261. PMID: 19825989; PMC2788765
113. Braconi C, Huang N, Patel T. MicroRNA-dependent regulation of DNA methyltransferase-1 and tumor suppressor gene expression by interleukin-6 in human malignant cholangiocytes. Hepatology. 2010 Mar;51(3):881-90. doi: 10.1002/hep.23381. PMID: 20146264; PMC3902044
114. Jing G, Yuan K, Turk AN, Jhala NC, Arnoletti JP, Zhang K, *et al*. Tamoxifen enhances therapeutic effects of gemcitabine on cholangiocarcinoma tumorigenesis. Lab Invest. 2011 Jun;91(6):896-904. doi: 10.1038/labinvest.2011.60. PMID: 21464824
115. Huang L, Ramirez JC, Frampton GA, Golden LE, Quinn MA, Pae HY, *et al*. Anandamide exerts its antiproliferative actions on cholangiocarcinoma by activation of the GPR55 receptor. Lab Invest. 2011 Jul;91(7):1007-17. doi: 10.1038/labinvest.2011.62. PMID: 21464819; PMC3126905
116. DeMorrow S, Onori P, Venter J, Invernizzi P, Frampton G, White M, *et al*. Neuropeptide Y inhibits cholangiocarcinoma cell growth and invasion. Am J Physiol Cell Physiol. 2011 May;300(5):C1078-89. doi: 10.1152/ajpcell.00358.2010. PMID: 21270292; PMC3093951
117. DeMorrow S, Francis H, Gaudio E, Venter J, Franchitto A, Kopriva S, *et al*. The endocannabinoid anandamide inhibits cholangiocarcinoma growth via activation of the noncanonical Wnt signaling pathway. Am J Physiol Gastrointest Liver Physiol. 2008 Dec;295(6):G1150-8. doi: 10.1152/ajpgi.90455.2008. PMID: 18832445; PMC2604798
118. Lang M, Henson R, Braconi C, Patel T. Epigallocatechin-gallate modulates chemotherapy-induced apoptosis in human cholangiocarcinoma cells. Liver Int. 2009 May;29(5):670-7. doi: 10.1111/j.1478-3231.2009.01984.x. PMID: 19226332; PMC3903791
119. Onori P, DeMorrow S, Gaudio E, Franchitto A, Mancinelli R, Venter J, *et al*. Caffeic acid phenethyl ester decreases cholangiocarcinoma growth by inhibition of NF-kappaB and induction of apoptosis. Int J Cancer. 2009 Aug 1;125(3):565-76. doi: 10.1002/ijc.24271. PMID: 19358267; PMC3051346
120. Kojima Y, Honda K, Hamada H, Kobayashi N. Oncolytic gene therapy combined with double suicide genes for human bile duct cancer in nude mouse models. J Surg Res. 2009 Nov;157(1):e63-70. doi: 10.1016/j.jss.2008.12.016. PMID: 19345377
121. Cao LQ, Xue P, Lu HW, Zheng Q, Wen ZL, Shao ZJ. Hematoporphyrin derivative-mediated photodynamic therapy inhibits tumor growth in human cholangiocarcinoma *in vitro* and *in vivo*. Hepatol Res. 2009 Dec;39(12):1190-7. doi: 10.1111/j.1872-034X.2009.00569.x. PMID: 19788692
122. Frampton GA, Lazcano EA, Li H, Mohamad A, DeMorrow S. Resveratrol enhances the sensitivity of cholangiocarcinoma to chemotherapeutic agents. Lab Invest. 2010 Sep;90(9):1325-38. doi: 10.1038/labinvest.2010.99. PMID: 20458282; PMC2921554
123. Marienfeld C, Tadlock L, Yamagiwa Y, Patel T. Inhibition of cholangiocarcinoma growth by tannic acid. Hepatology. 2003 May;37(5):1097-104. doi: 10.1053/jhep.2003.50192. PMID: 12717390
124. Braconi C, Swenson E, Kogure T, Huang N, Patel T. Targeting the IL-6 dependent phenotype can identify novel therapies for cholangiocarcinoma. PLoS One. 2010 Dec 16;5(12):e15195. doi: 10.1371/journal.pone.0015195. PMID: 21179572; PMC3002961
125. Han Y, Demorrow S, Invernizzi P, Jing Q, Glaser S, Renzi A, *et al*. Melatonin exerts by an autocrine loop antiproliferative effects in cholangiocarcinoma: Its synthesis is reduced favoring cholangiocarcinoma growth. Am J Physiol Gastrointest Liver Physiol. 2011 Oct;301(4):G623-33. doi: 10.1152/ajpgi.00118.2011. PMID: 21778461; PMCID: PMC3191557
126. Meng F, Han Y, Staloch D, Francis T, Stokes A, Francis H. The H4 histamine receptor agonist, clobenpropit, suppresses human cholangiocarcinoma progression by disruption of epithelial mesenchymal transition and tumor metastasis. Hepatology. 2011 Nov;54(5):1718-28. doi: 10.1002/hep.24573. PMID: 21793031
127. Zhang K, Chen D, Wang X, Zhang S, Wang J, Gao Y, *et al*. RNA interference targeting slug increases cholangiocarcinoma cell sensitivity to cisplatin via upregulating PUMA. Int J Mol Sci. 2011 Jan 14;12(1):385-400. doi: 10.3390/ijms12010385. Retraction in: Int J Mol Sci. 2017 Jul 10;18(7):E1483. doi: 10.3390/ijms18071483. PMID: 21339993; PMC3039959
128. Yokomuro S, Tsuji H, Lunz JG 3rd, Sakamoto T, Ezure T, Murase N, *et al*. Growth control of human biliary epithelial cells by interleukin 6, hepatocyte growth factor, transforming growth factor beta1, and activin A: comparison of a cholangiocarcinoma cell line with primary cultures of non-neoplastic biliary epithelial cells. Hepatology. 2000 Jul;32(1):26-35. doi: 10.1053/jhep.2000.8535. PMID: 10869285
129. Yang H, Li TW, Peng J, Tang X, Ko KS, Xia M, *et al*. A mouse model

of cholestasis-associated cholangiocarcinoma and transcription factors involved in progression. Gastroenterology. 2011 Jul;141(1):378-88, 388.e1-4. doi: 10.1053/j.gastro.2011.03.044. PMID: 21440549; PMC3129489

130. Marquardt JU, Raggi C, Andersen JB, Seo D, Avital I, Geller D, *et al*. Human hepatic cancer stem cells are characterized by common stemness traits and diverse oncogenic pathways. Hepatology. 2011 Sep 2;54(3):1031-42. doi: 10.1002/hep.24454. PMID: 21618577; PMC3179780
131. de Jong M, Maina T. Of mice and humans: are they the same?--Implications in cancer translational research. J Nucl Med. 2010 Apr;51(4):501-4. doi: 10.2967/jnumed.109.065706. PMID: 20237033
132. Lee JS, Chu IS, Mikaelyan A, Calvisi DF, Heo J, Reddy JK, *et al*. Application of comparative functional genomics to identify best-fit mouse models to study human cancer. Nat Genet. 2004 Dec;36(12):1306-11. doi: 10.1038/ng1481. PMID: 15565109
133. De Minicis S, Kisseleva T, Francis H, Baroni GS, Benedetti A, Brenner D, Alvaro D, *et al*. Liver carcinogenesis: rodent models of hepatocarcinoma and cholangiocarcinoma. Dig Liver Dis. 2013 Jun;45(6):450-9. doi: 10.1016/j.dld.2012.10.008. PMID: 23177172; PMC3716909