REVIEW

doi.org/10.1590/S0004-2803.230302023-58

Preclinical models of liver cancer

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HIGLIGHTS

- In this review, we described different murine models of carcinogenesis: classic models, new transgenic and combined models, that reproduce the key points for HCC and CCA genesis allowing a better understanding of its genetic physiopathological, and environmental abnormalities.
- Each model has its advantages, disadvantages, similarities, and differences with the corresponding human disease and should be chosen according to the specificity of the study. Ultimately, those models can also be used for testing new anticancer therapeutic approaches.
- Cholangiocarcinoma has been highlighted, with an increase in prevalence. This review has an important role in understanding the pathophysiology and the development of new drugs.

Received: 6 April 2023
Accepted: 25 May 2023

Declared conflict of interest of all authors: none
Disclosure of funding: no funding received
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ABSTRACT – Background – This manuscript provides an overview of liver carcinogenesis in murine models of hepatocellular carcinoma (HCC) and cholangiocarcinoma (CCA). **Objective –** A review through MEDLINE and EMBASE was performed to assess articles until August 2022. **Methods –** Search was conducted of the entire electronic databases and the keywords used was HCC, CCA, carcinogenesis, animal models and liver. Articles exclusion was based on the lack of close relation to the subject. Carcinogenesis models of HCC include HCC induced by senescence in transgenic animals, HCC diet-induced, HCC induced by chemotoxicagents, xenograft, oncogenes, and HCC in transgenic animals inoculated with B and C virus. The models of CCA include the use of dimethylnitrosamine (DMN), diethylnitrosamine (DEN), thioacetamide (TAA), and carbon tetrachloride (CCl4). CCA murine models may also be induced by: CCA cells, genetic manipulation, Smad4, PTEN and p53 knockout, xenograft, and DEN-left median bile duct ligation. **Results –** In this review, we described different murine models of carcinogenesis that reproduce the key points for HCC and CCA genesis allowing a better understanding of its genetic, physiopathological, and environmental abnormalities. **Conclusion –** Each model has its advantages, disadvantages, similarities, and differences with the corresponding human disease and should be chosen according to the specificity of the study. Ultimately, those models can also be used for testing new anticancer therapeutic approaches.

Keywords – Animal models; carcinogenesis; cholangiocarcinoma; hepatocellular carcinoma; liver.

INTRODUCTION

Primary liver cancer (PLC) is a common type of cancer worldwide accounting for 1.2 to 5.7% of global malignancies, with more than 80% of the cases occurring in developing countries[1]. There are about 370,000 new cases of PLC per year in men and 150,000 in women around the world[2]. Hepatocellular carcinoma (HCC) is the most common type, accounting for 70–80% of cases, and is responsible for about 80% of the 700,000 annual deaths related to liver cancer[3]. Other types of PLC are cholangiocarcinoma (CCA), hepatoblastoma, and hemangiosarcoma.

About 90% of all HCC is related to environmental risk factors, such as chronic inflammation, fibrosis, and cirrhosis caused by hepatitis B and C virus (HBV and HCV), exposure to aflatoxin B, chronic alcohol consumption and metabolic disorders, such as nonalcoholic steatohepatitis (NASH)[4]. B and C viral hepatitis accounts for over 80% of HCC cases worldwide[1]. HBV DNA serum levels bigger than 10,000 copies/mL and any level of HCV infection are strong independent risk factor for HCC[2]. Diet-induced liver cancer due to obesity and metabolic-related liver diseases is also an emerging public health problem worldwide[5]. Recent analyzes of liver tumors identified numerous oncogenic pathways and a wide variety of alleged driver gene mutations underlying hepatocarcinogenesis; thus, the mechanisms involved in HCC genesis following this broad range of molecular mediators and pathways is still under study[6].

CCA arise at the biliary ducts epithelium (intra or extrahepatic) is currently the second most frequent PLC. CCA etiopathogenesis remains largely unknown and its incidence and mortality are raising globally[1]. Potential etiologies of this cancer, including primary sclerosing cholangitis, congenital fibro polycystic adenomas of the bile duct, liver infestation by fascioliasis worm, intrahepatic lithiasis, biliary papillomatosis, chronic viral hepatitis, contact with cancer-causing chemicals, NASH, cirrhosis and obesity. Furthermore, a wide range of congenital abnormalities increase the risk of developing CCA. Cholestasis caused by genes encoding bile salt transport proteins defects (*BSEP/ABCB11*, *FIC1/ATP8B1* and *MDR3/ABCB4*) produce inflammatory cytokines, chronic inflammation and subsequent cholangiocarcinogenesis[7].

Experimental cancer models may improve the prevention and the treatment of PLC. Experimental models of PLC generate additional information about the causal signaling pathways, preventive factors and effective therapeutic measures. These models mimic the complex multistep process of hepatocarcinogenesis providing unique opportunity to perform several therapeutic experiments evaluating the immunological tumor-host interactions and drug screening. Therefore, animal models are crucial for translational studies of hepatocarcinogenesis; nevertheless, it is important to choose the experiment based on the hypothesis search. Rodents liver carcinogenesis models are the most used due to the facilities of these animals, which include: easy handling, low cost, physiological and molecular similarities to humans, genetic manipulation and implementation of specific methodology allowing more precise studies. Significant advances have been made in genetic modeling of rat cancer, over a spectrum ranging from simple xenograft to more complex models involving genetically modulated animals[8,9].

No single rodent model can capture all aspects of human HCC and CCA, although each can recapitulate at least some of the genetic and/or cellular characteristics of human disease. Likewise, there are few studies that compare mutational landscapes across the genome of rodent cancer models to those seen in human cancer[6]. In this article we describe and discuss the main rodent models of HCC and CCA (FIGURE 1).

METHODS

A review through MEDLINE and EMBASE was performed to assess articles. Search was conducted of the entire electronic databases and the keywords used was HCC, CCA, carcinogenesis, animal models and liver. Articles exclusion was based on the lack of close relation to the subject. Carcinogenesis models of HCC include HCC induced by senescence in transgenic animals, HCC diet-induced, HCC induced by chemotoxicagents, xenograft, oncogenes, and HCC in transgenic animals inoculated with B and C virus. The models of CCA include the use of dimethylnitrosamine

Model of carcinogenesis	Pathophysiology	Outcome
HCC induced by senescence in transgenic animals and immune modulation	Promotes the carcinogenesis through telomere shortening and immune modulation by organ dysfunction and decreases the reproductive activity.	It shows large genomic instability and DNA damage accompanied by the appearance of cancer. Rats with knockout for the telomerase gene (mTerc) with activation of p53 had a tumor suppression.
HCC diet-induced	Realized through a diet deficient in choline (DDC). The mechanisms related to stimulation of oval cells leading to an oxidative stress that increase DNA damage and mutation or genetic modification.	Rats developed HCC after 50–52 weeks. In association with C14 or alcohol, increased the number and the size of liver tumors and rats exposed to NDE the outcomes related to fatty change and cirrhosis.
HCC induced by chemotoxic agents	The carcinogenic compounds were divided into: genotoxic agents that induce tumor formation or tumor forming and promoter agents. The treatment with tumor promoting agent facilitates the clonal expansion of pre-neoplastic cells, which increases the development of tumor and its aggressiveness.	The agents can damage permanently the liver, causing cirrhosis and induce or progress tumor development.
HCC in transgenic animals inoculated with B virus, C virus and oncogenes	Laboratory-bred mice are suitable for studying the role of many oncogenes in tumor genesis and maintenance. It is important to consider the ones with express viral genes for hepatitis.	Animals related to hepatitis B virus with the expression of HBx genes course with development of HCC after 52–104 weeks. Rats related to hepatitis C virus developed HCC after 60 weeks. The major contribution of gene suppression or expression is related to PTEN gene suppression showing the development of HCC in 40–44 weeks, in addition to hepatic steatosis, inflammation and fibrosis.
HCC Xenografts models	Tumors original from other animals (xenografts) have the ability of growing fast, due to cancer cells replication, collagen deposition, and neo-angiogenesis.	Using a fibrosis liver as a model, the authors were able to demonstrated a rapid tumor development, with higher capability of generating metastases and satellites nodules.
CCA induced by chemotoxic agents	Induce a genotoxic effect with structural changes in deoxyribonucleotide acid (DNA) or to increase tumor formation through the expansion of pre-neoplastic cells. Promotes the alkylation of the DNA structure and generation of reactive oxygen species known to induce proteins, lipids and DNA damage. Promote tumorigenesis through DNA methylation.	Induces not only CCA, but also other gastrointestinal tumors, as well as skin, lung and hematopoietic tumors. Results in the formation of multifocal bile cystic lesions in mice and induces CCA in mice.
CCA induced by CCA cells implant	They are types of cells that exhibit chromosomal instability. Essential characteristics of malignancy, including tumor formation after implantation in a syngeneic environment. In this way, the reduced associated mitotic fidelity, promotes the genetic heterogeneity of the tumor and allows the acquisition of metastatic potential.	Promotes formation of tumor in 100% of animals, with high level of consistency of tumor mass after 20–22 day of bile duct inoculation. The development of the tumor has an exponential growth tendency, and important increase of bilirubin levels. The growth of intra-hepatic tumor was accompanied by hepatic ducts obstruction, peritoneal metastasis and progressive loss of weight. Presents the similar biological features as the ones observed in the human disease.
CCA induced by genetic manipulation	Performed through specific mutations in important specific tumor suppressor genes, such as: Smad4 -Pten and p53. Promoting targeted disruption of these tumor suppressors SMAD4, PTEN and p53.Thus, the tumor formed has a very aggressive growth and with a worse prognosis.	Invasive CCA occurred in about 55% of the mice. Metastases represented a very frequent event. At the molecular level, the CCA of the p53 knockout CCl 4 model resembles the human CCA. Smad4 and Pten are associated with significant biliary duct hyperplasia as early as 8 weeks of age. These degenerate into dysplasia and finally into invasive CCA, occurring with high penetrance at 4-7 months of age. The Smad4 / Pten model resembles the human CCA at both the histological and molecular levels of tumors.
CCA Xenografts models	It involves a xenotransplant implanted in the analogue (orthotopic) or different (ectopic) organ from the original (via subcutaneous injection in the flank) of human cells or human tumor tissue in immunodeficient or nude mice cell lines derived from human metastatic CCA tissue.	The tumor can be noticed after 2 weeks of cell implantation, and its growth is progressive. However, there may be a species incompatibility between the tumor and the host microenvironment.
The "DEN-left median bile duct ligation" model	Through the induction of chronic cholestasis, it accelerates the progression of CCA. Use of chemical agent DEN in combination with models of cholestatic liver injury; such as ligation of the left and median bile duct (LMBDL).	It allows the study of the biological behavior of CCAs in the physiological environment of the liver. By day 16, Cholangiomas and biliary adenomas had developed, with full development of CCA in these areas by day 28. The number of positive c-Myc liver cells increased and remained persistently high in animals that developed CCA. Although this combination model is complex due to the need for surgical intervention and long-term nutrition, carcinogenesis is relatively fast (28 weeks).

FIGURE 1. The summary of hepatocellular carcinoma and cholangiocarcinoma models.

(DMN), diethylnitrosamine (DEN), thioacetamide (TAA), and carbon tetrachloride (CCl4). CCA murine models may also be induced by: CCA cells, genetic manipulation, Smad4, PTEN and p53 knockout, xenograft, and DEN-left median bile duct ligation.

Carcinogenesis models of HCC

Studies to improve survival of HCC patients include murine liver cancer models to defined categories of human HCC etiology, comparing genomic changes and histomorphology. Recent studies demonstrate that the stratification of preclinical mouse models across genotypes oriented to an etiology and human-like phenotypes is feasible[8,10].

The main current models of HCC carcinogenesis include: senescence induction and immune modulation, carcinogenesis using specific diet, models using chemotoxic agents, models with transgenic animals inoculated by genetic engineering B and C virus and other oncogenic genes, and the implantation of cancer cells from another species (xenograft) (FIGURE 2).

HCC induced by senescence in transgenic animals and immune modulation

Transgenic mice express oncogenes or dominant-negative tumor suppressor genes due to the ectopic promoter and enhancer elements[11]. This model promotes carcinogenesis by shortening telomere and immune modulation. Telomere is a DNA sequence of the ends of chromosomes that promotes stable increase in the length of telomeres and ensures that each replication cycle is completed warranting cellular replication. Aging and cancer cells express large genomic instability and DNA damage, causing direct relationship between aging and the appearance of cancer[8,12,13].

The incidence of HCC is significantly suppressed in rats with knockout for the telomerase gene (*mTERC*)[14] that was associated with *p53* activation (tumor suppressor gene that prevents the spread of genetically defective cells). Somatic mutations in the *p53* gene occur is the most commonly mutated gene in human carcinogenesis[8,12,13]. In an experimental study, *mTERC* -/- rats were crossed with negative

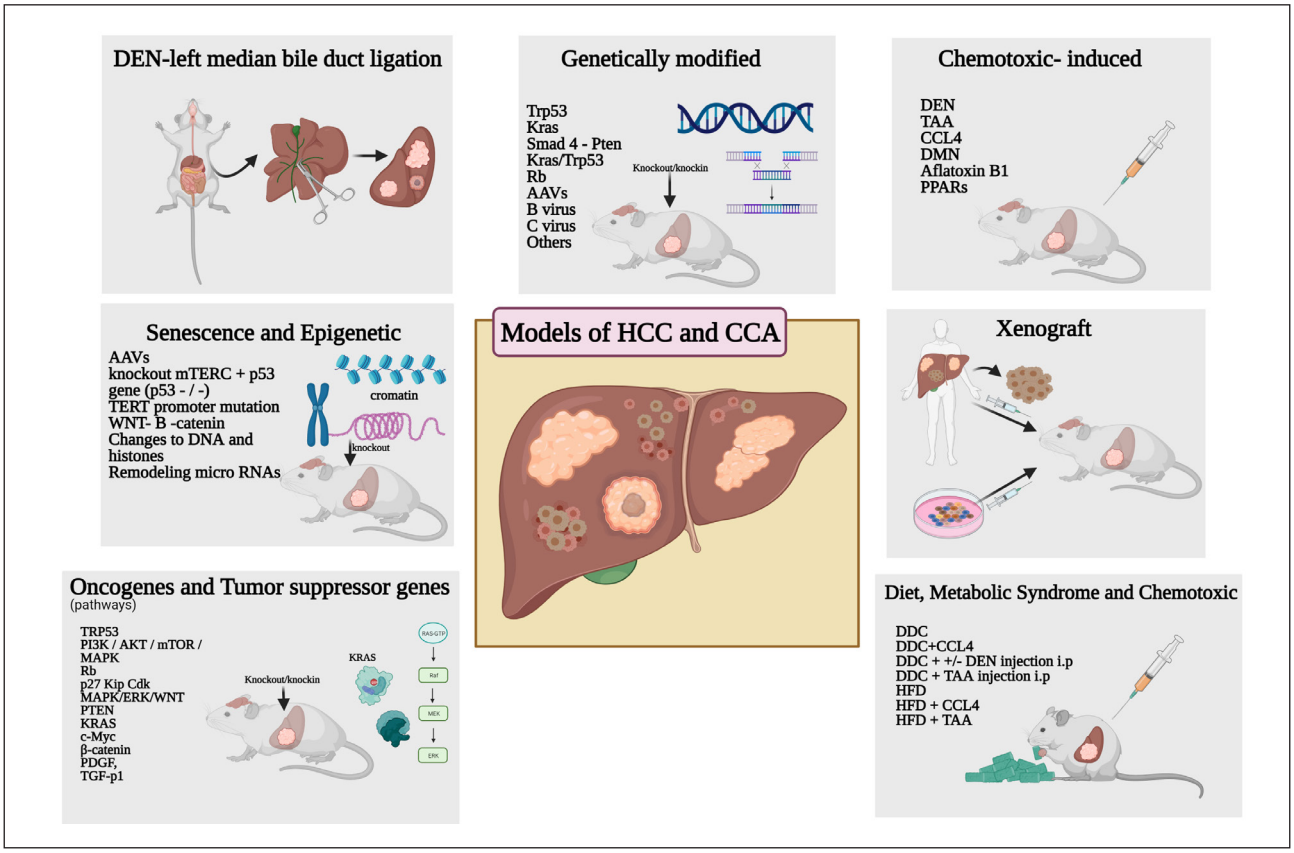

FIGURE 2. Main animal models of Hepatocellular Carcinoma (HCC) and Cholangiocarcinoma (CCA).

knockout rats for *p53* gene (*p53* -/-). Farazi et al. described a CLD model to induce HCC by hepatitis B surface antigen (HBS-Ag) inoculation in transgenic rats with telomere shortening by the deletion of *p53*.

HCC induced by chemotoxic agents and HCC diet-induced

Cirrhosis is a disease with clear environmental components and no hereditary in its etiology[15]. Toxic chemicals can cause cirrhosis and induce the development and progression of tumors. Its main advantage is to induce models similar to the cycle of injury inflammation-fibrosis-malignancy seen in humans[16].

N-nitrosodiethylamine (NDE)

HCC model developed by NDE administration in rats is exercised in two different modes: 1-alkylation of DNA damaging its structure and subsequent cell degeneration; and 2- induction of ROS, formed from the cytochrome P450 activation in hepatocytes[17]. The DEN model has been widely known as a chemical model of liver carcinogenesis for several years. Rats or mice develop histological changes similar to those seen in human HCC[18].

Frequent β-catenin mutations found in choline-deficient l-amino acid-defined diet combined with DEN generates p53 mutation or rearrangement in rats. The liver disease in mice treated with DEN is highly similar to chronic human liver disease for the progression of HCC. DEN administration in a single dose to rats at 15 days of age leads to tumor development in 80% of cases and long-term administration has a 100% success rate in tumor development[19,20].

The disadvantage of the isolated model with DEN, without the combination of other factors, is the long duration of the experiment, with an average of 50 weeks for the development of hepatocellular carcinoma. In addition, there is also heterogeneity in the frequency of appearance of tumors, requiring a longer period for all animals to develop, influencing the need for multiple injections[16,19,20]. DEN with Phenobarbital (PB) promote HCC in rats with considerably variation, depending on its strain, sex and age. The administration of DEN in male rats between 6 and 10 weeks of age, followed by administration of PB in potable water, promoted the formation of HCC after 36 weeks. Agents such as PB can induce a higher rate of carcinogenesis, but tumor characteristics are slightly modified with significant reduction in the model reproducibility[19,20].

Carbon tetrachloride (CCl4) and thioacetamide (TAA)

Carbon tetrachloride (CCl4) produces liver injury by activation of Kupffer cells followed by induction of cytokines and chemokines, by induction of cytochrome 450 and increased oxidative stress, activation of liver fibrogenesis and infiltration of immune cells, which contributes to tissue damage[21-24]. Studies have been using CCl4 associated with other chemical agents, such as alcohol. Weekly injections of CCl4 and oral administration of alcohol cause HCC after 14 weeks in mice[25].

Thioacetamide (TAA) was originally used to induce fibrosis and cirrhosis in mice and rats (it can be administered in drinking water (0.02–0.05%) or by intraperitoneal (IP) injections. Liver injury is possibly caused by increased oxidative stress, progressive DNA damage and, subsequently, the development of HCC[26].

Aflatoxins (AFT)

Mice and rats exposed to AFT is also a model used to study the carcinogenic process of HCC[27]. AFT is an hepatotoxin produced by *Aspergillus flavum* fungi with a carcinogenic activity. AFT promotes the induction of chromosomal aberrations followed by generation of DNA adducts, micronuclei and uncontrolled DNA synthesis. The development of HCC in mice can occur through injections of 6 mg/kg of AFT for 52 weeks in newborn mice, with a success rate of nearly 100%[28].

Diet and combination with chemical agents

The diet deficient in choline (DDC) diet was originally developed to induce steatohepatitis, fibrosis and cirrhosis in rats and mice[29-31]. Recently, mice subjected to DDC were observed to develop HCC after 50–52 weeks[31]. The main mechanisms associated with the development of HCC in DDC are related to the stimulation of oval cells (liver progenitor cells) by oxidative stress followed by DNA damage and genetic modifications. Combining DDC models with carbon tetrachloride (CCl4) or alcohol

resulted in an increase in the number and size of liver tumors[29]. Likewise, the combination of DDC and N-nitrosodiethylamine (NDE) induces HCC faster than DDC alone and maintains the specific features of diet-induced liver injury, which are steatosis and inflammation[29-31]. Our group published a model that combined diet and DEN that replicates the sequence of NASH, fibrosis, cirrhosis and HCC, with anti-CK 19 expression that suggests proliferation of oval cells after 16 weeks[29] and, more recently tested the effectiveness of sorafenib using PET-Scan in this model[32].

Another known model of NASH is the use of a high-fat diet (HFD), which induces NASH and progresses to HCC[33,34]. Also, the combination of stimuli to reduce the time of appearance of HCC, such as the combination of CCl4 and HFD for 24 weeks, recapitulates the progressive stages of NASH and HCC[35].

HCC in transgenic animals inoculated with B virus, C virus and oncogenes

Genetically modified mouse models (GMMs) mimic the physiopathology and the molecular characteristics of HCC. Although many experiments have used rats, due to their tendency of developing fibrosis, mice created in laboratory (*Mus musculus*) are considered one of the best models for cancer. This is because of available methods of genes manipulation, animal size, reproduction capacity, 3-years life, besides the physiological and molecular similarity with humans[36]. GMMs can be improved by cDNA constructions that allows us to generate expression of special genes in determinate tissues[37]. Thus, we can study the roll of many oncogenes in the genesis and maintenance of the tumor[36].

Many models of transgenic mice can be found in literature for HCC. Among them, it is important to consider the ones which express viral genes for hepatitis because they reproduce HCC with viral cause, which is the most frequent cause in humans. Among viral models, we must highlight the transgenic animals that express the hepatitis B virus related to HBx genes. They cause the development of HCC after 52–104 weeks[37-39]. In mice models that express hepatitis C virus the animals developing HCC after 60 weeks by insertion of structural protein from the core E1 and E2[40,41].

Other models of HCC are made with mice that express oncogenes, such as *c-Myc*, β-*catenin* or *PTEN* Mice *PTEN* deficient show specific chromosomic changes of liver cells, that induce the development of NASH and HCC after[42-45].

Xenografts models

Cancer cells cultured in vitro can form tumors when implanted subcutaneously in an immunocompromised mouse[46]. This xenograft model has advantages for preclinical studies of antineoplastic drugs because the tumors are quickly and easily induced and their subcutaneous location allows direct measurement of tumor growth. Tumors from other animals (xenografts) have the ability of growing fast, due to cancer cells replication, collagen deposition, and neo-angiogenesis[47,48].

In the orthotopic xenografts models, the cancer human cells are injected in mice liver allowing a better understanding of metastatic spread of the tumor and screening chemotherapies drugs[49-51]. Using this model, the authors were able to demonstrated a rapid tumor development, with higher capability of generating metastases and satellites nodules[52]. The major advantage of this model is related to the small period of time between the implant and the development of the tumor. Its disadvantage relies on the fact that this model is not similar with the main changes observed in humans. In another model for cancer in vivo, "hollow fiber assay" (HFA)[53], human tumor cell lines are loaded into biocompatible polyvinylidene fluoride (PVDF) has an advantage comparing with other xenografts models, the possibility of testing several lines of tumor cells, and several drugs in a single rat[54,55].

Carcinogenesis model of cholangiocarcinoma (CCA)

• CAA induced by chemotoxic agents

Carcinogenic compounds as dimethylnitrosamine (DMN), DEN and TAA, can be used to increase CCA tumor formation through the expansion of pre-neoplastic cells.

• CAA induced by DMN and DEN

DMN promotes an increase in oxidative stress with damage to DNA, proteins and lipids. Conse-

quently, DMN induces not only CCA, but also other gastrointestinal tumors, as well as skin, lung and hematopoietic tumors. The most commonly used animal model of CAA is based on DMN association combined with bile duct ligation in hamsters, resulting in the development of CAA in approximately 40% of animals. Administration of DEN combined with pentachlorophenol results in the formation of multifocal biliary cystic lesions in mice and induces CCA[56]. Another interesting models are transgenic mice associated with DEN or phenobarbital inducing CCA[57].

• Thioacetamide (TAA)

TAA oral administration is frequently used for hepatic fibrosis, cirrhosis[50], biliary dysplasia and CAA[58,59]. TAA is administered with drinking water at a dose of 0.03% induces progressive weight loss, liver damage and there is a stimulus for proliferation of foci of cholangiocytes and liver cancer, including CCA[58,59]. Evident tumors are observed after the 16th week of administration, and after the 24th week almost 100% of the animals presents larger and invasive tumors, regardless of the kind of rat used[59,60]. The mortality is almost null in experiment with 48 weeks of observation. Some experiments attempt to modify the protocol above, increasing the daily dose of TAA, however higher doses of TAA (0.15%) have shown high mortality, before the CCA development[61].

The advantage of the TAA model is that it does not require any abdominal surgery or manipulation, and it induces a consistent development of CCA nodules. It has been largely employed for pre-clinic studies, to test therapeutic and diagnostic approaches for CCA[60].

• CCA induced by CCA cells implant

The CCA model proposed by Sirica et al.[62] consists on the intra-hepatic implant of cell lines derivate from rats' CCA (BDEneu) in rats Fisher 344. Cells BDEneu express activated p185^{neu}, which is an indicator of cholangyocites progression. This model presents formation of tumor in 100% of animals, with high level of consistency of tumor mass after 20–22 day of bile duct inoculation. The development of the tumor has an exponential growth tendency, and important increase of bilirubin levels. The growth of intra-hepatic tumor was accompanied by hepatic ducts obstruction, peritoneal metastasis and progressive loss of weight. This model has the advantage of having tumor nodules developing consistently within a short period of time and uses cells that presents similar biological features as the ones observed in the human disease, such as TRAIL expression, *COX-2* over expression and *ERK1/2* hyper-phosphorylation[62-64]. It's also associated with biliary obstruction, which increases the tumor development and progressive loss of weight. Those characteristics are important use this model in pre-clinic studies. Blechacz et al. showed that Sorafenib is able to reduce CCA growth, using this model. The treatment with Sorafenib resulted in a significant tumor suppression and complete tumor regression in 22% of the animals tested[65]. The disadvantage of this model is the lack of development of de novo CCA, and the absence of chronic biliary and hepatic lesion, which are both present in the human disease. Other disadvantage relies in the abdominal and biliary manipulation, which could cause different expression of inflammatory agents within the liver. It was also developed only in rats, limiting the possibility of pathophysiological studies using transgenic animals.

CCA induced by genetic manipulation

• *Smad4 - PTEN* knockout

Smad4 e PTEN are genes that when inactivated are related with carcinogenesis of several tumors, including CCA. Smad4 is one of the most frequently altered tumor suppressor genes. Its inactivation in the early phases of carcinogenesis has been related to aggressive growth and worst prognosis[66,67]. Xu et al. proposed the model "Smad4-PTEN knockout", in which they caused a targeted disruption of tumor suppressors *smad4* and *PTEN*, using the Cre-loxP approach[66]. This transgenic animal was crossed with an Alb-Cre–transgenic mouse, resulting in several different genotypes. Hyperplastic foci emerge exclusively from bile ducts of mice Smad4$^{Co/Co}$Pten$^{Co/Co}$Alb-Cre at 2 months of age and continue to grow, leading to tumor formation. All animals at 4–7 months of age presented the sustained development of CCA, followed by progressive intrahepatic nodules growth. This model has a great importance on the

comprehension of genetic and molecular mechanisms related to the disease development because presents consistent tumor growth after 4–5 months, without extra manipulation. The disadvantages are the lack of chronic hepatic lesion resulting of inflammation, the absence of metastasis even in older animals and the simultaneous development of salivary glands' tumor (only in a limited number of animals).

• p53 knockout and CCl4

Mutations of the p53 gene are frequent genomic alterations observed in human intra-hepatic CCA (IH-CCA). Farazi et al. proposed a model that consists in CCl_4 administration three times a week for 4 months to p53 knockout mice[68]. Only the mice with p53 -/- presented early foci of CCA. After the administration of CCl_4, hepatic progressive nodules with genomic features similar to human IH-CCA, hepatic fibrosis and bile duct proliferation were observed in 54% of the animals p53 -/- after 29 weeks, and in 18% of the animals p53 ± after 52 weeks[68-70].

Therefore, the p53 genotype had a big impact in IH-CCA development. Pathophysiological, the advantage of this model resides in the combination of genetic susceptibility with a chronic and toxic liver lesion, a similar condition that evolves to CCA in humans, with malignant cholangyocites positive to *iNOS*, *COX-2*, *c-Met* and *cErbB2*[69,70]. The disadvantage includes the long period needed to established the tumors (29–52 weeks) and the lack of consistent tumor development.

• Xenograft models

Hudd et al. developed a CCA model injecting a cell line originated from a human CCA metastasis subcutaneously into the flank of nude mice[71]. Nude mice present immunologic deficiency characterized by lack of T cells, which mark those animals for no rejection of tissues and tumors implanted, and higher susceptibility for infections. Tumor can be noticed after 2 weeks of cells' implant, and its growth is progressive. Important pharmacodynamics discrepancy was observed in this model due to the fact that the cells lines are in a micro-environment different from the liver, beside the species-specific differences[72]. Concerned in solving the micro-environment issue, Yokomuro et al. implanted CCA cells directly in nude mice liver. This model also presented a progressive growth of the tumor; However, it has a disadvantage: the need of an abdominal incision[73].

• The "DEN and left median bile duct ligation" model

Yang et al. recently proposed a combined model of weekly injections of DEN followed by rubber band ligation and oral DEN in young adult Balb/c mice. The animals developed from multifocal cysts, to biliary adenomas and ACC after 28 weeks of experiment. Overall survival was around 70% after 28 weeks of experiments[74]. The advantage of this model is that it can be performed in unmodified mice, which have developmental characteristics similar to primary human liver cancers, in a short period of time. Its disadvantage is how much cellular proliferation it can be both in cholangiocytes, hepatocytes and inflammatory cells, since the stimulus is via include the overexpression of C-Myc[74].

CONCLUSION

In conclusion, each liver carcinogenesis model features, its advantages and disadvantages, and similarities/differences with the corresponding human disease, to properly choose the better model to each situation, while the "ideal" model isn't developed yet.

Authors' contribution

Galvão FHF and Oliveira CP: conception, administrative support, and design. Traldi MCC and Araújo RSS: provision of study materials or patients and collection and assembly of data. Galvão FHF, Stefano JT, Oliveira CP: data analysis and interpretation. Galvão FHF, Traldi MCC, Araújo RSS, Stefano JT, D'Albuquerque LAC and Oliveira CP: manuscript writing and final approval of manuscript.

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Galvão FHF, Traldi MCC, Araújo RSS, Stefano JT, D'Albuquerque LAC, Oliveira CP. Modelos pré-clínicos de câncer hepático. Arq Gastroenterol. 2023;60(3):383-92.

RESUMO – Contexto – Este manuscrito fornece uma visão geral da carcinogênese hepática em modelos murinos de carcinoma hepatocelular (CHC) e colangiocarcinoma (CCA). **Objetivo –** Realizar uma revisão de artigos científicos até agosto de 2022 utilizando as bases de dados MEDLINE e EMBASE. **Métodos –** A busca foi realizada em todas as bases de dados eletrônicas e as palavras-chave usadas foram CHC, CCA, carcinogenesis, modelos animais e fígado. A exclusão dos artigos baseou-se na falta de estreita relação com o assunto. Os modelos de carcinogênese do CHC incluíram: CHC induzido por senescência em animais transgênicos, CHC induzido por dieta, CHC induzido por agentes quimiotóxicos, xenoenxerto, oncogenes e CHC em animais transgênicos inoculados com vírus B e C. Os modelos de CCA incluíram: o uso de dimetilnitrosamina (DMN), dietilnitrosamina (DEN), tioacetamida (TAA) e tetracloreto de carbono (CCl4). Os modelos murinos de CCA induzidos por incluir: células de CCA, manipulação genética, animais nocaute para Smad4, PTEN e p53, xenoenxerto e ligadura do ducto biliar mediano esquerdo. **Resultados –** Nesta revisão, descrevemos diferentes modelos murinos de carcinogênese que reproduzem os pontos-chave para a gênese do CHC e do CCA, permitindo uma melhor compreensão de suas anormalidades genéticas, fisiopatológicas e ambientais. **Conclusão –** Cada modelo tem suas vantagens, desvantagens, semelhanças e diferenças com a doença humana correspondente e deve ser escolhido de acordo com a especificidade do estudo. Em última análise, esses modelos também podem ser utilizados para testar novas abordagens terapêuticas anticancerígenas.

Palavras-chave – Modelos animais; carcinogênese; colangiocarcinoma; carcinoma hepatocelular e fígado.

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