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doi:10.3748/wjg.14.6915

World J Gastroenterol 2008 December 7; 14(45): 6915-6923
World Journal of Gastroenterology ISSN 1007-9327

TOPIC HIGHLIGHT

Harry HX Xia, PhD, MD, *Series Editor*

Mouse models in liver cancer research: A review of current literature

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Received: May 26, 2008 Revised: August 7, 2008
Accepted: August 14, 2008
Published online: December 7, 2008

Abstract

Primary liver cancer remains one of the most lethal malignancies worldwide. Due to differences in prevalence of etiological factors the incidence of primary liver cancer varies among the world, with a peak in East-Asia. As this disease is still lethal in most of the cases, research has to be done to improve our understanding of the disease, offering insights for possible treatment options. For this purpose, animal models are widely used, especially mouse models. In this review, we describe the different types of mouse models used in liver cancer research, with emphasis on genetically engineered mice used in this field. We focus on hepatocellular carcinoma (HCC), as this is by far the most common type of primary liver cancer, accounting for 70%-85% of cases.

Key words: Primary liver cancer; Hepatocellular carcinoma; Mouse model; Genetically engineered mice

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Leenders MWH, Nijkamp MW, Borel Rinkes IHM. Mouse models in liver cancer research: A review of current literature. *World J Gastroenterol* 2008; 14(45): 6915-6923 Available from: URL: http://www.wjgnet.com/1007-9327/14/6915.asp
DOI: http://dx.doi.org/10.3748/wjg.14.6915

INTRODUCTION

The American Cancer Society has estimated that, in 2007, there were over 700 000 new cases of primary liver cancer worldwide. It is the fifth most common malignancy in men and the eighth in women. Liver cancer is among the most lethal cancers (five-year survival rates under 11%), which makes it the third most frequent cause of cancer death in men and the sixth in women[1]. Liver cancer consists of several histologically different primary hepatic malignancies, such as cholangiocarcinoma, hepatoblastoma and haemangiosarcoma, but hepatocellular carcinoma (HCC) is by far the most common type, accounting for 70%-85% of cases[1,2].

Cirrhosis (due to for instance hemochromatosis), chronic hepatitis B and C viral infections, chronic alcohol consumption, aflatoxin-B1 intake (from contaminated food) are the most important of the well-defined risk factors for HCC. Variations in the prevalence of these etiological factors mirror the geographical distribution of the incidence of HCC. The worldwide (age-adjusted) incidence per 100 000 persons is 14.9/5.5 (men/women), varying from 2.6/1.3 in Northern Europe to 35.4/12.6 in East Asia[2,3].

Animal models for human HCC can be helpful to our understanding of the (molecular) mechanisms underlying the pathogenesis of HCC. The laboratory mouse remains one of the best models to study cancer *in vivo* due to various features, such as the small size, the similarities to humans and the entirely sequenced genome and the similarities to humans[4].

For instance, the risk of HCC in males is approximately 2-5 times greater than in females[5,6]. This gender difference is seen in mice as well. Recently, Naugler *et al*[5] attributed this disparity to a higher serum interleukin-6 (IL-6) concentration in male than in female mice after administration of the chemical hepatocarcinogen diethylnitrosamine. IL-6 is secreted by Kupffer cells in response to, for example, necrotic hepatocytes. They demonstrated that estrogen inhibits the secretion of IL-6.

Since the spontaneous incidence of liver tumors in

the most frequently used strains of mice is < 4%, mouse models have been developed to induce HCC-formation[7,8].

None of the currently available mouse models meet all criteria of the ideal animal model, which include biologic, genetic, etiologic and therapeutic criteria[9]. Therefore, the most appropriate model for a particular experimental question should be used to answer the specific research question.

This review highlights the currently used mouse models for HCC, with emphasis on genetically engineered models.

CARCINOGEN-INDUCED MOUSE MODELS OF HCC

An etiological role for external agents in contributing to human HCC, also referred to as hepatocarcinogens, has been established by primarily epidemiological studies[9]. Examples of known human hepatocarcinogens include aflatoxins, ethanol and combined oral contraceptives[10]. Despite the fact that some of these chemicals are carcinogenic to the mouse liver as well, this is not true for all human hepatocarcinogens. For example, cirrhosis and liver cancer have not been observed in mice subjected to ethanol solely[10-12]. At the same time, hardly any of the mouse hepatocarcinogens have shown to be carcinogenic for humans (aflatoxin B1 and oral contraceptives belong to the exceptions). This discrepancy in response to carcinogens can probably be explained by species differences[9-11]. Nonetheless, carcinogen-induced mouse models are still frequently used for HCC research.

The (hepato) carcinogens are subdivided into two classes, namely genotoxic and non-genotoxic (or epigenetic) carcinogens. The genotoxic carcinogens can presumably cause cancer by forming DNA adducts, which lead to genetic changes of the target cell. These changes can direct normal cells to a preneoplastic state (initiation). The non-genotoxic carcinogens do not modify DNA structure, but generally stimulate the preneoplastic or initiated cells to evolve into a malignant neoplasm by controlling cell proliferation, apoptosis and cell differentiation[10,11,13]. Because of the high incidence of altered (or preneoplastic) hepatocytes in certain mouse strains (especially C3H and B6C3F1 mice, the latter corresponding to the progeny of C3H mice coupled to C57BL/6 mice), epigenetic carcinogens alone can be used to induce HCC-formation in these mice[8,11].

Many chemicals have been shown to induce HCCs in the mouse liver[10,14]. To date, the most frequently used hepatocarcinogens in mice are diethylnitrosamine (DEN) and phenobarbital (PB), although the carcinogenic effect of PB is controversial[8]. Furthermore, attention has been paid to the hepatocarcinogenic effect of peroxisome proliferators such as clofibrate and the experimental Wy-14.643[13].

The single most frequently used chemical for induction of HCCs in mice is DEN, a genotoxic carcinogen. DEN is typically administered to mice between 12 and 15 d of age by a single intraperitoneal injection (5 μg/g body weight). Using this protocol, originally described by Vesselinovitch and Mihailovich, 100% of B6C3F1 male mice developed HCCs, on average, 44 wk after intraperitoneal injection of DEN[15-17]. Less frequently used protocols for DEN-administration include intraperitoneal injection of a higher dose of DEN (for example 80-90 μg/g body weight) to older mice (4-5 wk of age) and intraperitoneal DEN-injection 36 h after a partial hepatectomy (mice 5-6 wk of age). These protocols are less efficient in producing HCCs than those previously mentioned[8]. DEN-induced mouse models of HCC are predominantly used to study the molecular mechanisms of (chemical) hepatocarcinogenesis. Furthermore, the influence of (trans) genes and chemicals that might prevent HCC development can be evaluated by means of this model.

In the background of carcinogen-induced mouse models for HCC, identifying chemicals that might be carcinogenic to humans is the major application of laboratory mice. For this purpose, chemicals are either administered to newborn mice in order to determine genotoxicity, or compounds are administered for longer periods (usually 2 years) to assess epigenetic carcinogenity[6,14]. However, because of the significant inconsistencies between mouse and human carcinogens, extrapolating the results of these mouse studies to the human situation remains difficult[18].

Carcinogen-induced mouse models for HCC are useful for establishing a relationship between carcinogen exposure and specific genetic changes[19]. However, the influences of sex, age and genetic background of the mice on the predictability of HCC-development remain disadvantages of these models.

IMPLANTATION MODELS OF HCC

Implantation models are among the most widely used models to accomplish HCC formation in mice, because of their suitability in studies for preclinical evaluation of anticancer agents. In implantation models that are currently used to induce HCC formation in mice, HCC cell lines or tumor tissue fragments are implanted in recipient mice. Here, we will give an impression of the approaches that can be used to obtain HCCs in these mice.

The earliest implantation model is the syngeneic transplantable tumor model, in which a HCC cell line or tissue fragment is implanted in mice of the strain from which the implant originates. To date, this model is less frequently used, because of the discovery that human tumor cell lines and tissue fragments can be implanted into immunodeficient mice. Nevertheless, this model is still required when anticancer agents are tested that work by activating the immune system (immune therapy)[20-22]. The unpredictability of the effectiveness of studied anticancer agents in human HCC and the small number of available murine cell lines are the most important disadvantages of this implantation model[20].

As mentioned, nowadays, primary human HCC cell lines or tissue fragments are implanted into immunocompromised mice (xenograft models).

The most widely used mouse for this approach is the nude mouse (*nu* -/-). These mice are athymic, hairless and have a deficiency of T lymphocytes as well as an impaired T and B cell function[23]. Next to nude mice, severe combined immunodeficient (SCID) mice are frequently used in xenograft models of HCC. These mice have a deficiency in number and function of both T and B lymphocytes[24]. Since the establishment of the first human HCC cell line in 1963[25], many human HCC cell lines have been described, of which the HepG2, the Hep3B, the SMMC-7721 and the HuH7 cell lines are the most commonly used.

In allograft models, murine HCC cell lines or tumor fragments are implanted in (not necessarily syngeneic) mice. The HCC grafts have been derived from spontaneously occurring HCCs in mice, from carcinogen-induced tumors or from genetically engineered mice (GEM), which is discussed in the last part of this review.

The above mentioned xenograft or allograft HCC cell suspension or tumor tissue fragments can be implanted into recipient mice, either at ectopic or at orthotopic sites. Ectopic implantation of tumor cells or fragments usually occurs subcutaneously. Orthotopic implantation can be accomplished by subserosal injection of HCC cells or by surgical orthotopical implantation (SOI) of tumor fragments. These fragments, approximately 1 mm^3 in size, are derived from surgical specimens of human HCCs or from pieces of subcutaneously grown HCC cells, either from human or mouse origin.

Xenografts of human HCCs growing subcutaneously in mice are predominantly used in the preclinical evaluation of anticancer agents. The rapid formation of tumors, the minimal labor that is required, the relative inexpensiveness and the ability to measure tumor size non-invasively are the main advantages of the ectopic, subcutaneous, application of grafts from human HCCs[21,22]. However, many research groups have described the importance of the microenvironment on the biological behaviour of malignant cells. For example, many tumor cell lines do not spontaneously metastasize when they are subcutaneously implanted, while they do metastasize when they are orthotopically implanted. Hence, the interaction of organ-specific factors (such as fibroblasts, endothelial cells and inflammatory cells) with tumor cells, is important for HCC development[21,26]. For this reason, therapeutic results obtained by an ectopic implantation model of a HCC graft must always be verified in orthotopic models.

These orthotopic implantation models mimic human HCCs in a better way with respect to tumor morphology, microenvironment, metastatic potential and the response to anticancer agents[27,28]. Furthermore, processes involved in local invasion, like angiogenesis, can be examined in their normal microenvironment[20]. Nonetheless, disadvantages of orthotopic implants of HCC xenografts include a more difficult surgical implantation procedure and more expensive procedures. Furthermore, tumor growth and response cannot be determined as easily as in ectopic transplantation models.

Despite the fact that (ectopic and orthotopic) xenograft implantation models are among the most widely used models for preclinical evaluation of anticancer agents, it has been demonstrated excessively that these models have a poor predictive value for the anti-tumor effects in patients. This can possibly be explained by the fact that the injected tumor cells are often cultured for a long period. Due to selection pressures in culture, these tumor cells have no longer maintained the original molecular characteristics and the heterogeneity of the patients' tumor[4,20,28,29]. By implanting a tumor fragment of a patient, the morphological and molecular characteristics of a patient's tumor are preserved better. However, establishing a parallel in vitro cell line from a patient's tumor is often very difficult.

The abovementioned features of implantation models are but a few arguments why implantation models are not ideal for the preclinical evaluation of anticancer agents. However, because of the suitability of these models, implantation models are still frequently used for this purpose.

GENETICALLY ENGINEERED MOUSE MODELS FOR HCC

The introduction of transgenic mouse models in the early 1980's made it possible to study the molecular features of human malignancies *in vivo*[30,31]. Since then, much progress has been made in techniques of producing GEM[4].

In studying the molecular mechanisms involved in hepatocarcinogenesis, GEM are particularly used to explore the role of a specific gene and to explore the interaction of different genes (e.g. oncogenes and tumor-suppressor genes) in the development of HCC. GEM is also suitable to investigate the role of specific genes in combination with a liver-specific carcinogen.

As is the case in other types of cancer, genetic alterations in various cellular pathways (including pathways involved in growth, apoptosis, proliferation and angiogenesis) are needed for the development of HCC. Although the exact genetic events in hepatocarcinogenesis are not entirely clear, there is evidence that the p53, Rb and Wnt/β-catenin pathways are involved[32,33]. Several transgenic mouse lineages that are nowadays used to induce formation of HCCs are transgenic in one of these pathways (Table 1). The most commonly used models will be discussed here.

Since the late 1980's, transgenic SV40 T-Ag (Simian Virus 40 T-antigen) mice have been studied extensively. The genome of the simian virus 40 (a DNA tumor virus) encodes two oncogenic proteins, the large and small T antigen (T-Ag and tAg, respectively, herein together referred to as T-Ag). After infection, large T-Ag can cause malignant transformation of the host cell primarily by inactivating the tumor-suppressor genes *p53* and *Rb*[34,35].

Research groups have reported the production of transgenic mice expressing SV40 T-Ag directed to the liver by the promoter/enhancer antithrombin-Ⅲ (AT Ⅲ)[36], albumin (Alb)[37] and α-1-antitrypsin (AAT)[38]. For

Table 1 Transgenic mouse models for HCC

Transgene	Promoter	Mouse strain	Percentage HCCs	Reference
c-myc	Alb	C57BL/6 × CBA/J	65% in males at 20 mo	40
TGF-α	MT	CD1	50% in males > 12 mo	77, 78
c-myc/TGF-α	Alb, MT	C57BL/6 × CBA/J × CD1	100% in males at 8 mo	40
SV40 T-Ag	ATⅢ	C57BL/6 × DBA2	100% at 8 mo	36
E2F-1	Alb	C57BL/6 × CBA/J	33%-60% at 12 mo	79, 80
c-myc/E2F-1	Alb	C57BL/6 × CBA/J	100% at 9 mo	80

example, Dubois *et al*[36] produced transgenic mice by putting the SV40 T-Ag under the control of the human ATⅢ promoter. In mouse lineages that expressed the highest level of the transgene, by the age of 8 mo, 100% of mice had developed HCCs and 10% had developed lung metastases.

Another commonly used transgenic mouse model was described by Murakami *et al*[39]. They generated double transgenic mice overexpressing c-myc and TGF-α in the liver (Alb-c-myc/MT-TGF-α mice) by crossing Alb/c-myc mice (transgenic mice overexpressing c-myc, directed by the albumin promoter) with MT/TGF-α mice (transgenic mice overexpressing TGF-α, directed by the metallothionein 1 promoter). Santoni-Rugiu *et al*[40] demonstrated that these mice developed HCCs substantially earlier and at a higher rate than single transgenic mice, overexpressing either c-myc or TGF-α. Within 8 mo after birth, 100% of male and 30% of female Alb-c-myc/MT-TGF-α mice had developed HCCs.

Although these conventional transgenic mouse models have been very useful to study the role of particular genes in hepatocarcinogenesis and to study the multistep nature of HCC development, one limitation of these models is the fact that the transgene is expressed in all hepatocytes, including the tumor microenvironment. Furthermore, the mutations are already present during embryogenesis and thus, might activate compensatory (molecular) pathways[26]. To overcome these limitations, mouse models have recently been developed in which the genetic alterations are induced in a tissue-specific and time-controlled fashion (conditional mouse models).

For instance, Lewis *et al*[41] used a retroviral transduction strategy to deliver oncogenes to hepatocytes *in situ*. They made use of the fact that mice do not express the TVA receptor, which is the receptor for the avian leukosis sarcoma virus subgroup A (ALSV-A). Lewis *et al*[41] generated TVA transgenic mice, in which TVA was specifically expressed within the liver. Delivery of ALSV-A viruses encoding PyMT (mouse polyoma virus middle T antigen, an oncogene) to these mice at the age of 2-3 d, subsequently led to tumor formation by the age of 4-6 mo (in 17 of 26 mice). They also exposed TVA transgenic mice that were deficient for p53 to PyMT-bearing ALSV-A viruses. Interestingly, the tumor incidence in these mice was not increased, but 6 of 16 p53 null mice that had developed HCCs, showed lung metastases (in contrast with 1 of 17 p53 wild-type mice). Consequently, this mouse model might be of value as a metastatic HCC model. Moreover, this model can be easily used to study the effect of other oncogenes in hepatocarcinogenesis, through the delivery of other oncogene-bearing ALSV-A viruses to TVA transgenic mice.

In addition, Lou *et al*[42] created mice with a regulated expression of liver-specific SV40 T-Ag. The SV40 T-Ag in these mice is preceded by a stop signal flanked by *loxP sites*. Hence, the SV40 T-Ag is expressed upon Cre-mediated excision, either by adenoviral expression of Cre recombinase or by administration of tamoxifen to mice that are transgenic for a liver-specific tamoxifen-inducible Cre. HCCs were observed in mice 5 mo after administration of adenoviral Cre recombinase or tamoxifen.

Several research groups employed alternative recombinase-mediated conditional gene-mutation strategies[32,33,43]. Colnot *et al* generated a mouse strain in which exon 14 of both *Apc* (adenomatous polyposis coli) alleles were flanked by *loxP* sites. The *Apc* alleles become invalidated (leading to β-catenin signaling) upon liver-targeted expression of Cre recombinase. Of these mice, 67% develop HCCs 8-9 mo after Cre recombinase administration[32,33].

Promising results have been published with these and other conditional mouse models to induce HCC-formation. Nonetheless, to date, these models are mainly used to study the effect of genetic alterations (mutation, deletion, or overexpression of a certain gene) on hepatocarcinogenesis and not to induce HCCs.

VIRAL HEPATOCARCINOGENESIS

More than 80% of HCCs in humans are attributable to infection with either hepatitis B virus (HBV) or hepatitis C virus (HCV) or both[44]. HBV- and HCV-related HCC are characteristically preceded by liver cirrhosis, though this is not always the case[45]. It may take more than 20 years for HCC to develop in HBV or HCV infected persons. For this reason, hepatocarcinogenesis due to viral hepatitis probably requires multiple steps of genetic alterations.

Finding the molecular mechanisms that drive these multiple steps by using cell-culture and non-genetic animal models is difficult. Therefore, in the past decades various animal models for investigation of viral hepatitis were developed. One problem in establishing such a model is that HBV and HCV require human hepatocytes to induce hepatitis, due to the stringent human tropism of these viruses[46,47].

In HBV research the finding of HBV-related viruses, e.g. the woodchuck hepatitis virus (WHV) and the ground squirrel hepatitis virus (GSHV), has provided opportunities for *in vivo* studies[46,48]. Another approach

for studying hepatitis B and C infection is the use of immunocompromised mice or rats. Recently, several animal models have been developed in which human hepatocytes or human liver tissue are transplanted into these animals. The transplanted hepatocytes in these animals can be infected with HBV or HCV *in vivo* or *ex vivo*. Alternatively, an already intrinsically infected specimen is transplanted[46,47]. These models are promising for the evaluation of therapeutics and prophylactics against hepatitis due to HBV or HCV, but are not useful to study HBV- or HCV-associated HCC. For that purpose, transgenic mice expressing HBV or HCV proteins represent a better model. In this section, the most frequently used transgenic mouse models for studying HBV- and HCV-associated HCC will be discussed.

By means of these models, two pathways have been proposed that might participate in the hepatocarcinogenic effect of chronic viral hepatitis. First, it is considered that chronic inflammation of the liver, continuous cell death and subsequent chronic hepatocyte regeneration due to viral hepatitis might increase the incidence of genetic alterations[48-53]. The second pathway encompasses a direct oncogenic effect of HBV or HCV on the infected hepatocyte. In the case of HBV (a DNA virus), this carcinogenic effect is believed to be accomplished through cis-activation or trans-activation of cellular genes. In cis-activation, genomic instability is a result of integration of HBV DNA into the host genome. In trans-activation, HBV proteins activate transcription of the HBV genome and host genes by binding to cellular sequences[48,50,51]. For HCV, a direct cytopathic effect has also been reported. As HCV is a RNA virus, it cannot integrate into the host genome. Therefore, other pathways must be of importance[52,53].

As a consequence of the chronic inflammatory state of the infected liver and the direct oncogenic effects of the hepatic viruses (as mentioned before), genetic alterations occur in various cellular pathways, which might eventually lead to the development of HCC. HBV proteins have been shown to manipulate the *p53*-, *Rb*-, *cyclinD1*- and *p21*-genes[51]. HCV is frequently associated with mutations of p53 and β-catenin[52,53].

HEPATITIS B VIRUS-ASSOCIATED HCC

Approximately 380 million people are chronically infected with HBV. These chronic HBV infected people have a 100-fold greater lifetime risk of developing HCC in comparison with non-carriers[3]. For this reason, HBV infection is the leading risk factor for the development of HCC. Worldwide, over 50% of HCC cases are associated with chronic HBV infection and the highest incidence of HCC is in South East Asia and sub-Saharan Africa, regions with a high prevalence of HBV infection[44,54].

As early as 1985, the first transgenic mouse models for investigating HBV infection were developed[55,56]. HBV transgenic mice have been created with the full HBV genome and with every single HBV gene, namely those encoding for the surface envelope proteins (large, middle and small), X protein (HBx), core and precore proteins.

It did not take long before the first transgenic mouse models for evaluation of HBV-associated hepatocarcinogenesis appeared. Until now, merely the large envelope protein and the HBx protein have displayed a carcinogenic role[48,57].

Chisari *et al*[58] described a mouse model in which transgenic mice were generated that carried an integrated HBV DNA fragment coding for the HBV large envelope polypeptides on a C57BL/6 genetic background. As a result, non-secretable hepatitis B surface antigen (HBsAg) particles formed that accumulated in the endoplasmic reticulum of the hepatocyte. In mice with 100% of the hepatocytes expressing HBsAg (lineage 50-4), liver injury begins at 2-3 mo of age; at 6 mo regenerative nodules appear and from the age of 15 mo HCCs develop.

Another HBV gene that has been extensively studied is the HBx gene. Though several research groups could not find evidence for a hepatocarcinogenic role of HBx in HBx transgenic mice[54,59], Kim *et al*[60] did report such a role in 1991. They produced HBx transgenic mice by injection of HBV DNA containing the HBx gene into single-cell embryos from CD1-mice. In these mice, liver tumors began to emerge after 8-10 mo. Both male and female transgenic mice died early in comparison with control CD1 mice, at the age of 11-15 mo *vs* 17-21 mo, respectively. On autopsy, 80%-91% of male transgenic mice and 60%-67% of female transgenic mice showed one or multiple HCCs. Yu *et al*[61] generated transgenic HBx mice using the same technique as Kim *et al*[60], but in a C57BL/6 genetic background and with a much weaker HBx expression in the liver. They reported an incidence of grossly identified HCCs and small neoplastic nodules, without signs of cirrhosis or inflammation, in 86% of 11-18 mo old HBx transgenic mice.

Possible explanations for the different outcome in transgenic mouse models for the hepatocarcinogenic role of HBx, may include a difference in mouse strains that were used. Male mice of the CD-1 strain develop spontaneous HCC in 5.7%[62], an incidence that is somewhat higher than the rate in for instance C57BL/6J mice (< 4.0%)[61]. In addition, the expression level of HBx-mRNA in the livers of transgenic mice and the type of HBx used may be different in the various studies. Finally, the integration site of HBx in the genome of the mice might influence the hepatocarcinogenic effect of HBx[57,61,63].

Efforts have been made to accomplish a model in which complete HBV genome transgenic mice demonstrate HCCs. Thus far, this has not been successful[63,64].

Nowadays, models based on the HBsAg transgenic mouse model of Chisari *et al* and the HBx transgenic mouse model of Kim *et al*[60] are commonly used to study mechanisms involved in hepatocarcinogenesis. These models are also applied to study possible synergistic relations between chemical carcinogens (such as aflatoxin B1 or diethyl nitrosamine) and HBV-infection[65-67]. Another application is the use of bitransgenic mouse models, in which mice are produced that are transgenic for a gene

of interest (such as *c-myc* or *TGF*-α) in conjunction with HBsAg or HBx[48,68,69]. For instance, Jakubczak *et al*[68] produced bitransgenic mice by pairing HBsAg transgenic mice described by Chisari *et al* to TGF-α transgenic mice. At 8 mo of age, 76% (13 of 17) of male bitransgenic mice developed HCCs, while TGF-α transgenic control mice showed HCC in only 6% (1 of 17) and HBsAg transgenic control mice in 0%. These bitransgenic mouse models can be used to investigate the effect of a particular gene on HBV-induced hepatocarcinogenesis.

HEPATITIS C VIRUS-ASSOCIATED HCC

Worldwide, approximately 30% of HCC cases are related to chronic HCV infection, making HCV the second most frequent cause of HCC[44]. In some areas, like Southern Europe and Japan, HCV infection is the strongest predisposing factor for HCC[70]. Patients infected with HCV have a risk of up to 35% for developing liver cirrhosis[47,70]. Thereafter, the cumulative risk of developing HCC in these cirrhotic patients is 1%-7% per year. HCC is the most frequent cause of death in HCV infected persons[47,70,71].

Various HCV proteins have been expressed in transgenic mice to study the pathogenesis of HCV-associated HCC, particularly the HCV polyprotein, the core protein and the core protein in combination with E1 and E2 envelope proteins. Interestingly, the expression of the core protein of HCV seems to be the major factor contributing to the hepatocarcinogenic effect of HCV infection, as transgenic mice that do not express this protein, no HCCs arise[47,52].

Moriya *et al*[72] were the first to describe such a transgenic mouse model. They generated transgenic mice that carried the HCV core gene. These mice showed histological features of steatosis in the liver, without inflammation, from the age of 3 mo and showed HCCs with close histological resemblance of HCCs in human chronic HCV infection, by the time they were 16 mo old. The incidence of HCC in 16-19 mo old male transgenic mice was 26% to 31%, in contrast to a low incidence in the female transgenic mice, which is in accordance with the human situation[73]. By means of such transgenic mouse models numerous molecular and pathogenetic pathways have been investigated that have led to a better understanding of HCV-associated hepatocarcinogenesis.

To study the role of HCV proteins other than the HCV core protein in hepatocarcinogenesis, Lerat *et al*[74] developed full-length HCV polyprotein transgenic mice and compared them with transgenic mice encoding merely the structural HCV proteins (including the core and the E1 and E2 envelope proteins). HCCs occurred (exclusively in males) in 5 of 38 transgenic mice expressing the full HCV polyprotein and in 1 of 43 transgenic mice expressing the structural HCV proteins. These findings suggest that HCV proteins, other than the HCV core protein, may endorse development of HCC as well, because in these mice the HCV protein levels are much lower in the first group[74].

The HCCs that develop in the mouse models described by Moriya *et al* and Lerat *et al* show proper (histological) resemblance to the corresponding lesions in patients with HCV-associated HCC. In the model described by Lerat *et al*[74], tumors develop regardless of the absence of detectable levels of the expression of HCV proteins, mimicking the situation in HCV infected patients. Furthermore, constitutive HCV gene expression results in immunological tolerance to the HCV genes, allowing the study of the direct hepatocarcinogenic effect of HCV proteins in the absence of an immune response to the viral proteins[52,74]. Disadvantages of these models are the possible significance of the genetic background of the mice and the relative unpredictability of HCC formation.

As described above for HBV, the HCV transgenic mouse models of Moriya *et al*, Lerat *et al* and comparable models, are presently used to study carcinogenic mechanisms in HCV-related HCC. In addition, these models are applied to study relations between carcinogens (like DEN) and HCV-infection in inducing HCC[75,76]. Furthermore, bitransgenic mouse models have been developed to investigate the effect of a particular gene on HCV-induced hepatocarcinogenesis.

CONCLUSION AND FUTURE PERSPECTIVES

Mouse models in cancer research are developed to imitate human carcinogenesis. Although the ideal animal model does not (yet) exist, mouse models can imitate parts of human carcinogenesis. To date, different types of mouse models are available to induce HCC, varying in complexity. The most appropriate model for a particular research question should be chosen to answer that specific question. Each approach has its own advantages and disadvantages, which are discussed in this review.

First, carcinogen-induced models are used to identify chemicals that might be carcinogenic to humans. Furthermore, these models are used for establishing a relationship between carcinogen exposure and specific genetic changes. In HCC, DEN is especially used to induce HCC. Major disadvantages remain the influence of sex, age and genetic background of the mice on the predictability of HCC-development. Moreover, there is a species difference in the response to hepatocarcinogens between humans and mice.

Next, because of their suitability, implantation models are still frequently used for the screening of different types of anticancer drugs. Nonetheless, these models have a poor predictive value for the anti-tumor effects in patients. This is probably the consequence of culturing the tumor cells for a long period, which alters the molecular characteristics and the heterogeneity of the original tumor.

Agents tested in mice with subcutaneously implanted tumors, should always be tested in orthotopic models as well, because of the importance of the microenvironment on the biological behaviour of malignant cells.

HCC is a result of different genetic mutations. Con-

ventional transgenic mouse models have been developed to study the role of different genes in HCC formation and to study molecular features involved in hepatocarcinogenesis. Limitations of these models include the expression of the transgene in all hepatocytes (hence, the tumor microenvironment as well) and the presence of the genetic alterations during embryogenesis (which might activate compensatory pathways). To overcome these problems, conditional mouse models have been developed recently. To date, these models are mainly used to study the effect of genetic alterations and, unfortunately, not to induce HCCs. Genetically engineered mouse models are also used in studying the role of viral hepatitis in HCC formation, as HBV and HCV require human hepatocytes to induce hepatitis and, consequently, hepatitis-induced hepatocarcinogenesis.

Highly sophisticated genetically engineered mouse models will become increasingly available and will help to answer a variety of research questions. Nevertheless, significant differences between mice and humans have to be taken into account when interpreting the (molecular) mechanisms of hepatocarcinogenesis. The most familiar of these (interspecies) differences are the much longer telomeres in mice, due to persistent telomerase expression in mice (as opposed to limited or absent telomerase expression in humans). Humans also differ from mice in respect to, for instance, their metabolism and immune system. To extrapolate the results from cancer studies in mice to humans, humanized mice should be generated and used in (genetically engineered) mouse models in the future.

This will bring us one step closer to the ideal animal model for cancer research.

As mentioned above, the adequate mouse model will be used depending on the research question. However, whether the impact of a possible carcinogen is investigated, the development of anticancer drugs or the genetic background of HCC formation is studied, all experiments have the goal to reduce the prevalence of HCC.

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