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MINIREVIEWS

Recent advances in mouse models of obesity- and nonalcoholic steatohepatitis-associated hepatocarcinogenesis

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Abstract

Hepatocellular carcinoma (HCC) is the fifth most common cancer, and obesity has been established as a risk factor for HCC development. Nonalcoholic steatohepatitis (NASH) is apparently the key link between obesity and hepatocarcinogenesis, and obesity also accelerates HCC development synergistically with other risk factors, such as hepatitis virus infection and alcohol consumption. As an explanation for the pathogenesis of NASH, the so-called "two-hit" theory has been widely accepted, but recently, a better model, the so-called "multiple-hits hypothesis" was proposed, which states that many disease-promoting factors may occur in parallel, rather than consecutively. However, the overall mechanism remains largely unknown. Various cell-cell and organ-organ interactions are involved in the pathogenesis of NASH, and thus appropriate *in vivo* disease models are essential for a deeper understanding. However, replicating the full spectrum of human NASH has been difficult, as NASH involves obesity, insulin resistance, steatohepatitis, fibrosis, and ultimately HCC, and the lack of an appropriate mouse model has been a considerable barrier to determining the missing links among obesity, NASH, and HCC. In recent years, several innovative mouse models presenting obesity- and NASH-associated HCC have been established by modified diets, chemotoxic agents, genetic manipulation, or a combination of these factors, shedding some light on this complex network and providing new therapeutic strategies. Thus, in this paper, I review the mouse models of obesity- and NASH-associated HCC, especially focusing on recent advances and their clinical relevance.

Key words: Obesity; Metabolic syndrome; Nonalcoholic steatohepatitis; Hepatocellular carcinoma; Mouse model

Core tip: Obesity is a recognized risk factor for the development of hepatocellular carcinoma (HCC) and nonalcoholic steatohepatitis (NASH), which in turn can

trigger hepatocarcinogenesis. Once, no appropriate mouse model allowed exploration of the associations among obesity, NASH, and HCC, but several innovative mouse models have become established in recent years. These models have afforded new insights into the mechanisms of disease and have suggested new therapeutic strategies. Therefore, this paper reviews mouse models of obesity- and NASH-associated HCC, focusing on recent advances and clinical relevance thereof.

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INTRODUCTION

Hepatocellular carcinoma (HCC) is the fifth most common cancer and a leading cause of cancer-related death[1]. Although the short-term prognosis of patients with HCC has improved due to advances in early diagnosis and treatment, the long-term prognosis remains unsatisfactory, with a low overall survival of 22%-35% at 10 years[2,3]. More than 90% of HCC develops in the context of chronic liver damage and inflammation[4], and obesity has recently been established as a risk factor for HCC development, with a 1.5-4-fold increased risk[5,6]. Accumulating evidence indicates that nonalcoholic fatty liver disease (NAFLD) is the key link between obesity and hepatocarcinogenesis, with evidence indicating that obesity accelerates HCC development synergistically with other risk factors, such as chronic viral hepatitis and alcohol consumption[7,8]. Because the prevalence of obesity has been increasing worldwide, its possible association with hepatocarcinogenesis has attracted considerable attention in recent years.

NAFLD, the most common chronic liver disease in developed countries, has been recognized as a hepatic manifestation of metabolic syndrome. NAFLD encompasses a wide range of pathological conditions, ranging from "simple" steatosis to the more aggressive form "nonalcoholic steatohepatitis (NASH)," which is accompanied by inflammation, cell death, and scarring (fibrosis) that eventually results in cirrhosis and/or HCC. Histologically, NASH is characterized by the presence of ballooning hepatocytes and lobular inflammation with or without perisinusoidal fibrosis in addition to steatosis[9]. To explain the pathogenesis of NASH, the so-called "two-hit" theory proposed by Day *et al*[10] in 1998 has been widely accepted. They suggested that after a first hit (hepatic steatosis), another hit is needed for NASH to develop. Since then, various factors such as pro-inflammatory cytokines, dysregulation of adipokines, gut-derived endotoxins, oxidative stress, endoplasmic reticulum (ER) stress, lipotoxicity, altered gut microbiota, and activation of intracellular signaling pathways have been suggested to be the second hit, and which factor(s) is (are) the true driving force of disease progression from simple steatosis to NASH has been debated[11-15]. Recently, a better model, the so-called "multiple-hits hypothesis" was proposed by Tilg *et al*[16], which states that many of the events described above may take place in parallel, rather than consecutively. However, the overall mechanism is very complex and remains largely unknown. Thus, no specific established treatment exists to prevent NASH progression and subsequent HCC development.

A good mouse model is indispensable for understanding such a complicated liver disease, involving interactions with various other organs, such as the gut, brain, and adipose tissue, because mice are readily amenable to genetic modifications and easy to handle. Although various NASH mouse models have been reported, most of the existing models do not replicate the full spectrum of human NASH, which includes obesity, insulin resistance, steatohepatitis, fibrosis, and ultimately HCC[17]. In particular, mimicking hepatocarcinogenesis is difficult, and the lack of appropriate mouse model(s) has been a considerable barrier for understanding the underlying pathogenesis behind the link(s) among obesity, NASH, and HCC.

Several innovative mouse models presenting obesity- and NASH-associated HCC have been established in recent years using modified diets, chemotoxic agents, genetic manipulation, or a combination of these factors. They have provided new insights into mechanisms as to how obesity and NASH promote HCC and have also resulted in the suggestion of new therapeutic strategies. Although several review articles on mouse models of NASH have been recently published[17-21], to our knowledge, no review focusing on mouse models of obesity- and NASH-associated hepatocarcinogenesis has been published. Thus, in this paper, I review the mouse models of obesity- and NASH-associated HCC, especially focusing on recent advances and their clinical relevance.

MOUSE MODELS OF OBESITY- AND NASH-ASSOCIATED HCC

Obesity- and NASH-associated HCC mouse models have been created using modified diets, chemotoxic agents, genetic manipulation, or a combination of these elements. Here, we classify current mouse models into four groups and discuss their characteristics: dietary models, diet in combination with chemotoxic agent models, genetically engineered models, and genetic manipulation in combination with dietary models (Table 1).

DIETARY MODELS

Long-term high-fat diet

A high-fat diet (HFD) is widely used to cause obesity and hepatic steatosis in mice, and long-term feeding

Table 1 Mouse models of obesity- and nonalcoholic steatohepatitis - associated hepatocellular carcinoma

	Obesity	Insulin resistance	Steatosis	Steatohepatitis	Fibrosis	HCC
Dietary models						
Long-term HFD	Yes	Yes	Yes	Yes	Yes	Yes
CD-HFD	Yes	Yes	Yes	Yes	Yes	Yes
High fat and fructose diet	Yes	Yes	Yes	Yes	Yes	Yes
Dietary in combination with chemotoxic agent models						
DEN with HFD	Yes	Yes	Yes	No	No	Yes
STZ with HFD	No	Yes	Yes	Yes	Yes	Yes
DMBA with HFD	Yes	N/A	Yes	No	No	Yes
Genetically engineered models						
Liver-specific PTEN knockout mice and p110α transgenic mice	No	No	Yes	Yes	Yes	Yes
Liver-specific NEMO knockout mice	No	No	Yes	Yes	Yes	Yes
miR-122 knockout mice	No	Yes	Yes	Yes	Yes	Yes
FXR knockout mice	No	Yes	Yes (mild)	Yes (mild)	Yes	Yes
AOX knockout mice	No	N/A	Yes	Yes	No	Yes
MAT1A knockout mice	No	No	Yes	Yes	Yes	Yes
FLS mice crossed with *ob/ob* mice	Yes	Yes	Yes	Yes	Yes	Yes
Dominant negative form of RARα transgenic mice	No	No	Yes	Yes	No	Yes
Genetic manipulation in combination with dietary models						
MUP-uPA transgenic mice with HFD	Yes	Yes	Yes	Yes	Yes	Yes
Adiponectin knockout mice with HFD	Yes	Yes	Yes	Yes	Yes	No (adenoma)
AIM knockout mice with HFD	Yes	Yes	Yes	No	No	Yes
MC4R knockout mice with HFD	Yes	Yes	Yes	Yes	Yes	Yes

N/A: Not assessed; HCC: Hepatocellular carcinoma; HFD: High-fat diet; CD: Choline-deficient; DEN: Diethylnitrosamine; STZ: Streptozotocin; DMBA: Dimethylbenz(a)anthracene; NEMO: Nuclear factor κB essential modulator; miR: MicroRNA; FXR: Farnesoid X receptor; AOX: Acetyl CoA oxidase; MAT1A: Methionine adenosyl transferase 1A; FLS: Fatty liver Shionogi; RAR: Retinoid acid receptor; AIM: Apoptosis inhibitor of macrophage; MC4R: Melanocortin 4 receptor.

of HFD also induces insulin resistance. Although HFD-induced fatty liver has been considered to represent "simple" steatosis, some recent studies have shown that an extended period of HFD feeding (*e.g.*, 60 wk) in C57/BL6J mice could induce steatohepatitis with weak perisinusoidal fibrosis, and also neoplastic lesions, including HCC, in approximately 50% of mice[22,23], which suggests that excess intake of dietary fat can be a causal factor in HCC development. However, the phenotypes induced by HFD are variable according to mouse strains, fat content in the diet, and the composition of the dietary fat. Importantly, disruption of IRS-1, a mediator of insulin and IGF signals in C57/BL6J mice, was found to dramatically protect against long-term HFD-induced liver tumorigenesis despite the presence of severe insulin resistance and marked postprandial hyperglycemia[23]. This finding suggests that hyperglycemia itself may not play a role in NASH or NASH-associated hepatocarcinogenesis.

Choline-deficient high-fat diet

A methionine- and choline-deficient (MCD) diet is a classic and widely adopted model for studying NASH. Because methionine and choline are essential for hepatic β-oxidation and the production of very low-density lipoprotein (VLDL), their deficiency leads to extensive hepatic lipid accumulation, and steatohepatitis subsequently develops, which resembles the pathology of human NASH[17]. However, the MCD diet does not cause obesity, insulin resistance, or metabolic syndrome; rather, it induces weight loss and even cachexia. To overcome these limitations, Wolf *et al*[24] combined choline deficiency with an HFD on the basis of clinical observations of choline deficiency in patients with NASH. CD-HFD-fed C57/BL6 mice revealed obesity and insulin resistance as well as a human NASH-like pathology, with mild pericellular fibrosis. Furthermore, long-term feeding (12 mo) resulted in spontaneous HCC development in 25% of mice, including classical trabecular HCC. In contrast, only 2.5% of HFD-fed mice developed a liver tumor over the same time period. In this model, hypernutrition and choline deficiency activated intrahepatic natural killer T (NKT) cells, which in turn enhanced hepatocyte lipid uptake and aggravated liver steatosis *via* secretion of LIGHT [a member of the tumor necrosis factor (TNF) superfamily]. Also, $CD8^+$ T cells, NKT cells, and associated inflammatory cytokines cooperatively cause liver damage and nuclear factor κB (NF-κB) activation, which facilitates the NASH-to-HCC transition. Thus, hepatocyte-lymphocyte cross talk may be a promising therapeutic target for NASH and NASH-associated HCC.

High-fat and fructose diet

Recently, long-term feeding (12 mo) of an HFD in combination with fructose syrup has also been reported to cause the development of liver tumors, including HCC, as well as steatohepatitis and mild fibrosis[25]. This model exemplifies the clinical setting, the so-called "American lifestyle-induced obesity syndrome." However, the incidence of macroscopically visible nodules was not very high, and characterization of the tumors and

analysis of the mechanism were not sufficient due to the small number of occurrences. Further studies are needed with this promising mouse model.

DIET IN COMBINATION WITH CHEMOTOXIC AGENT MODELS

Diethylnitrosamine with HFD

Diethylnitrosamine (DEN) is the most commonly used genotoxic chemical carcinogen to develop HCC because inducing HCC is easy, and DEN-induced HCC shows histology and gene expression similar to human HCC, especially a poor prognosis[4]. A single intraperitoneal injection of DEN to 2-wk-old male mice is sufficient to induce HCC[26,27], and HFD feeding to DEN-injected mice strongly enhances HCC development[28]. The greatest benefit of this model is that HCC is easy to induce and its incidence rate is almost 100% at 8 mo of age. However, the initiation step of HCC development basically depends on artificial, toxic DNA damage, and non-tumor liver tissue corresponds to simple steatosis, lacking inflammatory cell infiltration and fibrosis. However, this model is suitable for analyzing obesity-associated promotion and progression processes in HCC. In fact, HFD feeding resulted in systemic low-grade inflammation, and ablation of interleukin-6 (IL-6) and the TNF receptor 1 abrogated their tumor-promoting effects, suggesting that IL-6 and TNFα play important roles in the promotion of obesity-associated HCC[28].

Streptozotocin with HFD

Streptozotocin (STZ), a drug particularly toxic to β-cells in the pancreas, is widely used to induce diabetes in mice[29]. Recently, STZ in combination with an HFD has been reported to induce NASH and spontaneous HCC development[30]. In this model, low-dose STZ was injected subcutaneously at 2 d after birth, and then HFD feeding was started at 4 wk of age. Steatohepatitis occurred at 8 wk of age along with pericellular fibrosis, and all male mice developed well-differentiated-type HCC at 20 wk. These findings lead to a revolutionary hypothesis that insufficient insulin signaling, rather than hyperinsulinemia, plays a key role in NASH-associated HCC. The advantage of the STZ with HFD model is that it can replicate human NASH-like pathology and can also induce spontaneous HCC in a relatively short time. However, these mice do not show obesity or insulin resistance. In addition, whether the cancer initiation process depends on NASH-induced chronic inflammation or STZ administration remains unclear because STZ is known as a carcinogen, and administration of STZ alone can induce HCC in Syrian golden hamsters[31].

Dimethylbenz(a)anthracene with HFD

Dimethylbenz(a)anthracene (DMBA), a chemical carcinogen that causes an oncogenic Ras mutation, is widely used to induce skin and breast cancer[32]. Recently, Yoshimoto *et al*[33] reported that neonatal treatment with DMBA on the dorsal surface at day 4-5 followed by an HFD for 30 wk induced HCC in all male mice, whereas none of the normal diet-fed DMBA-treated mice developed HCC. This paper elegantly showed that increased deoxycholic acid (DCA) by an obesity-induced alteration in the gut microbiota provoked a senescence-associated secretory phenotype (SASP) in hepatic stellate cells (HSCs), which in turn secreted various inflammatory and tumor-promoting factors, eventually resulting in the malignant transformation of initiated hepatocytes by DMBA. In addition, lowering the DCA concentration by treatment with ursodeoxycholic acid (UDCA) or antibiotics inhibited HCC development significantly. Thus, although UDCA failed to improve the histology in patients with NASH compared with placebo in some clinical trials[34,35], a possibility exists that long-term treatment with UDCA may prevent HCC development in obese patients with NASH, independent of NASH disease status. Also, the gut microbiota may be a future therapeutic target candidate. However, DMBA treatment can induce oncogenic mutations in various cell types. In fact, lung cancer also developed with DMBA treatment regardless of diet in this study. Thus, although they showed the absence of the hot spot mutation of the H-Ras gene in HSCs, whether SASP of HSCs is specific to this model or a universal phenomenon using other obesity- and NASH-associated HCC models should be examined.

GENETICALLY ENGINEERED MODELS

Liver-specific PTEN knockout mice and p110α transgenic mice

A tumor suppressor PTEN negatively regulates the phosphatidylinositol 3-kinase (PI3K)/Akt/mTOR signaling pathways by its lipid phosphatase activity. Liver-specific PTEN knockout mice generated by crossing PTEN flox/flox mice with Albumin-Cre transgenic mice spontaneously develop steatohepatitis with marked triglyceride accumulation *via* the activation of Akt signaling and upregulation of PPARγ and SREBP1[36]. Additionally, all mice developed hepatocellular adenomas, and 66% developed HCC by 74-78 wk of age. This mouse model is one of the most well-known genetically engineered models of NASH-associated HCC. Hepatocyte-specific transgenic mice of p110α, which is a catalytic subunit of PI3K, also showed similar phenotypes[37]. Although pathological features of these mice are similar to human NASH-associated HCC, these models do not show obesity or metabolic syndrome, but instead are hypersensitive to insulin.

Liver-specific NF-κB essential modulator knockout mice

NF-κB essential modulator (NEMO), also known as inhibitor of NF-κB subunit γ (IKKγ), controls NF-κB activation through its interaction with ubiquitin chains[4]. Liver-specific NEMO knockout mice exhibit spontaneous liver damage, hepatosteatosis, fibrosis, and HCC

development[38]. Although the mechanism as to how NEMO deletion causes liver steatosis remains unclear, death receptor-mediated and oxidative stress-dependent hepatocyte death are triggers of liver damage and inflammation. The disease process in this model is similar to human HCC, which is a consequence of chronic inflammation, hepatocyte death, and compensatory proliferation. However, because this model does not show obesity or metabolic syndrome, it is not suitable for studying the metabolic consequences of NAFLD.

MicroRNA-122 knockout mice

MicroRNA(miR)-122 is a predominant liver microRNA, accounting for 70% of the total miRNAs in the liver. Both mice with germline knockout and liver-specific knockout of miR-122 revealed steatohepatitis and fibrosis, and also developed HCC, including metastasizing aggressive cases, at 12-17 mo of age[39,40]. Enhanced lipogenesis and suppressed lipid secretion through loss of miR-122 cause liver steatosis in these mice. Loss of miR-122 promotes HCC development not only indirectly through chronic inflammation, but also directly through induction of the epithelial-mesenchymal transition (EMT) by E-cadherin downregulation[40], which plays an important role in EMT in HCC[41]. These findings are pathophysiologically important and clinically relevant because the expression of miR-122 is significantly decreased in patients with NASH[42]. Furthermore, adeno-associated virus-mediated delivery of miR-122 suppressed Myc-driven HCC[39], suggesting the potential utility of miR122 delivery for patients with HCC.

Farnesoid X receptor knockout mice

The nuclear bile acid receptor farnesoid X receptor (FXR) is highly expressed in the liver and intestine, and cross talk between these two organs plays a key role in maintaining bile acid homeostasis[43]. FXR also plays important roles in lipid and glucose metabolism and regulation of insulin sensitivity by regulating the expression of various metabolic genes. FXR knockout mice exhibit chronic liver damage with mild steatosis and fibrosis, and aged mice develop spontaneous HCC by the age of 12-16 mo[44]. Although steatosis in FXR knockout mice is mild, the combination of LDL receptor knockout and HFD induces significant steatosis and ballooning degeneration of hepatocytes[45]. Currently, FXR is an attractive therapeutic target in the clinical setting because the FXR agonist obeticholic acid was found to show significant improvements in histological features of NASH in a recent multicenter placebo-controlled randomized trial[46]. However, treatment with obeticholic acid was associated with some disadvantageous effects, such as increases in serum cholesterol and low-density lipoprotein cholesterol concentrations, a decrease in serum high-density lipoprotein cholesterol concentrations, and increased insulin resistance. Furthermore, recent experimental studies using tissue-specific FXR knockout mice indicated complicated cross talk between liver and intestine FXR, and even an apparently opposite function of FXR signaling for NASH progression between the liver and intestine[47-49]. Further studies are needed to clarify the roles of this signaling process in NASH and NASH-associated HCC.

Other genetically engineered models

Acetyl CoA oxidase (AOX) is the rate-limiting enzyme of the peroxisomal β-oxidation of long-chain fatty acids. AOX knockout mice have defective peroxisomal β-oxidation and exhibit steatohepatitis without fibrosis[50]. AOX knockout mice also exhibit hepatocellular adenoma and HCC by 15 mo of age. However, hepatic steatosis is reversed by a compensatory increase in fatty acid oxidation by 6-7 mo of age.

Methionine adenosyltransferase 1A (MAT1A) is the rate-limiting enzyme of methionine metabolism in the liver. MAT1A knockout mice develop spontaneous steatohepatitis and HCC *via* abnormal expression of genes involved in lipid and carbohydrate metabolism[51]. However, MAT1A knockout mice do not show obesity or metabolic syndrome, except hyperglycemia.

The "fatty liver Shionogi" (FLS) mouse strain shows lipid deposition in hepatocytes from the neonatal stage, and the degree of hepatic lipid accumulation increases as the mouse grows without obesity. Additionally, crossing FLS mice with leptin mutant *ob/ob* mice induces obesity, metabolic syndrome, NASH, and spontaneous HCC development[52]. Although the mechanism of this phenotype in FLS mice is not fully understood, it is believed to be caused by a complex polygenic trait.

Transgenic mice expressing the dominant-negative form of the retinoid acid receptor (RAR)α in hepatocytes display microvesicular steatosis and spotty necrosis at 4 mo of age, and aged mice develop spontaneous HCC at the age of 12-18 mo[53]. In dominant-negative RARα transgenic mice, mitochondrial β-oxidation of fatty acids is decreased, but peroxisomal β-oxidation and microsomal ω-oxidation are increased, resulting in an enhanced accumulation of reactive oxygen species.

GENETIC MANIPULATION IN COMBINATION WITH DIETARY MODELS

Major urinary protein-urokinase-type plasminogen activator transgenic mice with HFD

Major urinary protein (MUP)-urokinase-type plasminogen activator (uPA) mice are uPA transgenic mice under the control of the mature hepatocyte-specific promoter for MUP[54]. MUP-uPA mice express the uPA protein in mature hepatocytes, where it accumulates in the ER, leading to chronic ER stress in the hepatocytes. We recently reported that feeding an HFD to *MUP-uPA* mice resulted in steatohepatitis that closely resembles the pathology of human NASH, with ballooning degeneration, hepatocyte death, and pericellular/bridging fibrosis, eventually leading to spontaneous development of HCC, including classic trabecular HCC and steatohepatitic HCC, by 40 wk of

age[55]. In this mouse model, the vicious cycle of ER stress and hypernutrition synergistically aggravates lipid accumulation in the liver *via* sterol regulatory element binding protein (SREBP) activation, which leads to excess oxidative stress, ballooning degeneration, and susceptibility to lipotoxic cell death. In parallel, increased TNFα expression during this process further accelerates NASH and HCC development in a TNF receptor 1-IKK-NF-κB-dependent manner. Reducing ER stress using chemical chaperones significantly improved liver pathology, suggesting that interrupting this vicious cycle might be a promising therapeutic target for NASH and HCC.

Isolated premalignant HCC progenitor cells (HcPC) from DEN-injected mice can be transplanted into MUP-uPA mice[56], and the NASH-like microenvironment created by HFD feeding significantly promotes the progression from HcPC to HCC[55]. This approach may allow us to separate cell-autonomous effects of genetic manipulation as well as dietary conditions within pre-neoplastic cells from effects exerted within the surrounding liver parenchyma, and furthermore, allow us to separate the effects of NASH on the tumor progression process from the tumor initiation process. In this regard, this is a unique mouse model to analyze the mechanisms of NASH-associated HCC.

Adiponectin knockout mice with HFD

Adiponectin, one of the major adipokines, possesses anti-inflammatory and insulin-sensitizing properties, and levels typically decline with increasing body weight[57]. Hypoadiponectinemia is seen in patients with NASH, and reduced adiponectin levels are associated with more extensive liver steatosis and necroinflammation[58]. Adiponectin knockout mice have insulin resistance and glucose intolerance on a normal diet[59]. HFD-fed adiponectin knockout mice exhibit NASH-like pathology, including hepatocyte ballooning, spotty necrosis, and pericellular fibrosis *via* increased hepatic expression of TNFα and SREBP1c at 24 wk, and furthermore, some HFD-fed adiponectin knockout mice (12.5%) develop hepatocellular adenoma at 48 wk[60]. This experimental study showed that too little adiponectin can be a causal factor of obesity-associated liver tumorigenesis. However, several recent epidemiological studies have shown that a higher serum adiponectin level is associated with an increased risk of future HCC development[61-63]. The effects of too much adiponectin on hepatocarcinogenesis remains poorly understood, and some reports have shown cancer-promoting effects of adiponectin[64,65]. Because adiponectin signaling is considered to be a promising target of NASH, further basic and clinical studies should be conducted.

Apoptosis inhibitor of macrophage knockout mice with HFD

Circulating apoptosis inhibitor of macrophage (AIM) is incorporated into adipocytes and hepatocytes, and inactivates cytoplasmic fatty acid synthase *via* direct binding. Thus, AIM knockout mice show increased steatosis and lipid accumulation in the liver after HFD feeding. Furthermore, all HFD-fed AIM knockout mice spontaneously develop HCC without apparent liver inflammation or fibrosis by 55 wk of age[66]. AIM accumulates on the HCC cell surface and activates the complement cascade, provoking HCC cell necrosis. Administration of recombinant AIM was found to prevent HCC development in HFD-fed AIM knockout mice. These findings suggest that delivery of AIM to HCC cells may be a novel therapeutic strategy against obesity-driven HCC.

Melanocortin 4 receptor knockout mice with HFD

Melanocortin 4 receptor (MC4R) is expressed in the hypothalamic nuclei and has been implicated in the regulation of food intake and body weight. Several pathogenic mutations in the *MC4R* gene have been reported, especially in early-onset obesity[67]. MC4R knockout mice in combination with an HFD exhibit steatohepatitis, which is associated with obesity, insulin resistance, and dyslipidemia. In addition, all HFD-fed MC4R knockout mice developed HCC after 1 year of HFD feeding[68]. Although the detailed mechanism remains unclear, it seems likely that the hepatic phenotype in MC4R knockout mice results from loss of its function in the brain because the expression of MC4R mRNA is basically restricted to the brain.

CONCLUSION

Table 1 lists the key features of each mouse model described in this review. The dietary models most closely mimic the human condition. However, HCC development is slow and its incidence relatively low. The combination of DEN and HFD affords a model superior in terms of certainty and ease of use. The STZ/HFD combination triggers HCC development relatively quickly. The MUP-uPA/HFD model is a unique in that the effects of NASH on tumor progression can be separated from the effects on tumor initiation. Although the histopathological characteristics of miR-122- and FXR-knockout mouse livers are not identical to those of human NASH patients (weak steatosis and lack of ballooning hepatocytes), the phenotypes of mice so affected are important pathophysiologically and clinically relevant, as discussed above. It is important to understand the advantages and disadvantages of each mouse model and to choose the model that is optimal for the experimental purpose.

The tumor-promoting effects of obesity and NASH are caused by not only changes in the hepatic microenvironment, such as excess lipid accumulation, oxidative stress, ER stress, and inflammatory cytokines secreted by immune cells and fibroblasts, but also by changes in the extrahepatic environment, such as visceral fat accumulation, altered gut microbiota, and hypothalamic appetite dysregulation. Such a complex situation, composed of various cell-cell and organ-organ interactions, cannot be reproduced *in vitro*, and

appropriate *in vivo* disease models are essential to fully understand it. Recent advances in mouse models shed some light on this complicated network and have suggested several new therapeutic targets. However, we are still far from a complete understanding and no specific established treatment exists to prevent NASH or NASH-associated HCC. Thus, further studies and novel strategies clarifying the entire picture of this complex disease are still needed to translate the findings obtained from experimental research into clinical practice.

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