Novel Mouse Models of Hepatocarcinogenesis with Stepwise Accumulation of Genetic Alterations

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Key Words

Hepatocellular carcinoma · Activation-induced cytidine deaminase · Chronic inflammation · Hepatocarcinogenesis

Abstract

Hepatocellular carcinoma (HCC) is one of the most common cancers worldwide. Various risk factors are involved in hepatocarcinogenesis. Among them, chronic inflammation, including chronic hepatitis and cirrhosis mainly caused by hepatitis B virus and/or hepatitis C virus infection, plays an important role in HCC development. On the other hand, comprehensive genetic analyses of HCC using whole genome and exome sequencing revealed that cancer cells possess a large number of somatic mutations, suggesting that a wide variety of genetic alterations and the resultant dysregulated molecular pathways contribute to the development of HCC. Activation-induced cytidine deaminase (AID) is a nucleotide-editing enzyme, and aberrant expression of AID induced by inflammatory responses contributes to hepatocarcinogenesis via the accumulation of genetic alterations in various tumor-related genes. Constitutive expression of AID in hepatocyte-lineage cells provides novel mouse models that recapitulate the tumorigenesis of human HCC through stepwise accumulation of genetic alterations.

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Introduction

Hepatocellular carcinoma (HCC) is the fifth most common cancer in men, the seventh most common cancer in women and the third most common cause of cancer-related death worldwide [1]. The risk factors for HCC vary by region and race, but hepatitis B virus (HBV) infection, hepatitis C virus (HCV) infection, alcoholic liver disease and nonalcoholic fatty liver disease/nonalcoholic steatohepatitis are considered to be major causes worldwide [2, 3]. Most of these risk factors are closely related with inflammation-associated chronic liver damage and thus the majority of HCCs develop under the background of chronic hepatitis or cirrhosis. Here we review the mechanisms underlying the contribution of chronic inflammation to the development of HCC.

Chronic Inflammation and HCC

HCV infection is a major risk factor for human HCC. The prevalence of HCV infection is 80–90% in patients with HCC in Japan, 30–50% in the USA and 44–66% in Italy [4]. The estimated risk for HCC is 15–20 times higher in HCV-infected patients compared with those who are negative for HCV infection. Epidemiologic

studies have clearly revealed that most human HCCs arise in the setting of chronic hepatic inflammation associated with liver cirrhosis or chronic hepatitis caused by HCV infection [5, 6], and the estimated annual rate of HCC development in those with chronic hepatitis and liver cirrhosis is 0.5–1.0% and 5–8%, respectively. We previously revealed that HCC developed in 237 out of 846 patients (28.0%) with chronic HCV infection with a mean follow-up of 4.66 years [7]. The cumulative incidence rate of HCC among those patients was 18.1% at 5 years and 45.6% at 10 years. Based on the classification of chronic liver disease at study entry, 94 out of 576 patients with hepatitis (16.3%) and 143 out of 270 patients with cirrhosis (53.0%) developed HCC over a 10-year period. In addition, the incidence rate of HCC in interferon-treated patients with chronic HCV infection was 15.6% (35/224) over 10 years, much lower than that of HCV-infected patients without interferon therapy. These findings indicate that chronic inflammation in the liver is closely related with HCC development, and suppression of HCV-related chronic inflammation by interferon therapy successfully contributes to reduce the incidence of HCC.

The number of people with HBV-chronic hepatitis is approximately 300 million worldwide and HBV is the most frequent cause of HCC worldwide, accounting for more than 50% of HCC cases and 70–80% of HCC cases in highly HBV endemic regions [1, 4]. Although HBV infection sometimes causes HCC in the liver in the absence of hepatic inflammation, most HBV-related HCCs are found in patients with chronic hepatitis and cirrhosis. Previous meta-analyses revealed that HBsAg-positive individuals have an approximately 15- to 20-fold greater risk for HCC than HBsAg-negative individuals, similar to those with chronic HCV infection. Furthermore, patients with chronic HBV infection under the condition of cirrhosis have a greater risk of developing HCC. Indeed, the annual incidence rate of HCC in HBV-infected patients with and without cirrhosis is 2.0–6.6% and 0–4%, respectively [8]. In addition, another study reported that the incidence rate of HCC development in individuals with HBV-related cirrhosis was 820–2,247 (per 100,000 person years), whereas in those without cirrhosis the incidence rate was much lower, at 280–474 [9].

Taken together, these findings indicate that chronic inflammation caused by HCV or HBV infection plays an important role in the development of HCC. How chronic inflammation contributes to HCC development, however, has not been fully elucidated.

Fig. 1. Linkage between chronic inflammation and hepatocarcinogenesis.

Mechanism of Chronic Inflammation-Induced Genetic Aberrations in Hepatocytes

Cancer cells possess various genetic alterations, including somatic mutations, chromosomal rearrangements, copy number alterations and epigenetic changes [10, 11]. Recent dramatic advancements in genome-wide analysis methods and progress in technology have allowed for whole genome and exome sequencing of various types of cancers, which has unveiled the overall genetic landscape of human cancers, including HCC [12]. Several comprehensive genetic analyses of HCC with various etiologies, including HBV-HCC, HCV-HCC and alcohol-related HCC, have been performed [13–15]. Approximately tens of thousands of somatic mutations on average are detected by whole-genome sequencing in each HCC tissue, and generally ~100 mutations are detected throughout the whole exons per tumor. These findings indicate that normal liver cells acquire a large number of genetic alterations during the process of tumorigenesis and a wide variety of genetic alterations and resultant dysregulated molecular pathways might contribute to the development of HCC. When and how the accumulation of so many somatic mutations are generated under the background of inflamed liver tissues, however, remains unclear.

We recently found that a nucleotide-editing enzyme, activation-induced cytidine deaminase (AID), plays a critical role in chronic inflammation-associated hepatocarcinogenesis by inducing genetic alterations [6, 11]. AID possesses a nucleotide-editing capacity that induces DNA mutations in the human genome by deaminating

Fig. 2. Schema of genetically engineered HCC mice models. **a** Wild-type mouse. **b** Transgenic mouse model with overexpression of a specific oncogene. **c** Knock-out mouse model with disruption of a specific tumor-suppressor gene. **d** AID-expression mouse model: stepwise accumulation of genetic alterations in oncogenes and/or tumor-suppressor genes are induced by mutagenic activity of AID, which leads to tumorigenesis.

cytidine. AID is expressed only in activated B cells under physiologic conditions and is essential for two important molecular mechanisms' immune diversity, somatic hypermutation and class switch recombination [16]. Aberrant AID expression is induced by the stimulation of pro-inflammatory cytokines such as tumor necrosis factor- α (TNF- α) or interleukin-1 β in various epithelial cells through the nuclear factor- κ B (NF- κ B) pathway [17–19]. TNF- α is upregulated in the liver of patients with chronic viral hepatitis or cirrhosis due to HCV infection. We demonstrated that AID expression is induced in response to TNF- α stimulation. In addition, AID expression is also upregulated in cultured human hepatocytes expressing HCV core protein via activation of the NF- κ B pathway [17]. Enhanced AID protein expression is widely detectable in both human HCC and the surrounding inflamed hepatocytes, whereas no AID expression is detected in normal liver tissues lacking an inflammatory response [20]. Importantly, aberrant expression of AID in hepatocytes leads to the accumulation of genetic alterations in tumor-related genes, such as TP53. These findings suggest that in adult hepatocytes, both the response to HCV infection itself and the resultant chronic inflammation trigger aberrant AID upregulation, and due to the mutagenic activity of AID, nucleotide alterations gradually accumulate in tumor-related genes in chronically inflamed liver tissues (fig. 1).

Novel Mouse Models with Stepwise Accumulation of Genetic Alterations Leading to Hepatocarcinogenesis

To gain a better understanding of human hepatocarcinogenesis, various animal models mimicking the process of human HCC development have been established [21]. Mouse models of HCC are divided into two types: chemical induced and genetically engineered. Chemical-induced models of HCC include mice exposed to diethylnitrosamine [22], aflatoxin B1 [23] and thioacetamide [24], but these models mainly reflect chemical-induced carcinogenesis, although chronic inflammation might be related to cancer development to some extent. Genetically engineered mouse models of HCC include *c-myc*/TGF- α transgenic mice [25], Trp53 knockout mice [26] and others. Certainly, HCC develops in these models via overexpression of the specific oncogene or disruption of the specific tumor-suppressor gene. These models, however, do not necessarily mimic the process of chronic inflammation-associated human hepatocarcinogenesis (fig. 2), because hepatocarcinogenesis might not result from an abnormality of only one or two specific tumor-related genes or pathways, but rather from stepwise accumulation of genetic alterations.

Several novel mouse models have been established by taking advantage of AID-mediated stepwise genotoxicity

(fig. 2). Okazaki et al. [27] and Morisawa et al. [28] generated AID transgenic (Tg) mice with constitutive and ubiquitous AID expression and found that AID Tg mice developed malignant T cell lymphomas as well as various epithelial tumors, including liver, lung and gastric cancers, indicating that normal epithelial cells with constitutive AID expression could transform into tumor cells via the accumulation of genetic alterations. Unfortunately, however, AID Tg mice tend to die young because of lethal malignant lymphomas, and thus detailed analyses of epithelial tumors, including HCC, in AID Tg mice are difficult. Therefore, to examine the effect of constitutive AID expression in mainly hepatic-lineage cells, we established two mouse models: (i) tissue nonspecific alkaline phosphatase (TNAP)-AID mice and (ii) a transplantation model of hepatic progenitor cells derived from AID Tg mice.

AID conditional Tg mice were established by insertion of the gene encoding GFP flanked by two loxP sites into the CAG promoter-driven AID transgene, which allows for AID expression only after Cre-mediated deletion of the GFP cDNA from the transgene [29]. On the other hand, TNAP is a member of the alkaline phosphatase family and is also a marker of embryonic stem cells and immature stem cells, especially in murine hepatocytes. TNAP-Cre mice were generated by inserting the coding sequence of Cre recombinase into the locus of the TNAP gene. To obtain double-transgenic mice in which AID is expressed only in cells that produce TNAP (TNAP-AID mice), we crossed AID conditional Tg mice with TNAP-Cre mice [30]. HCC developed spontaneously in 4 out of 15 (27%) TNAP-AID mice at approximately 90 weeks of age, whereas no tumors were detected in the littermate control mice [30]. Furthermore, a high accumulation of *Trp53* gene mutations was observed in both HCC tissues and noncancerous liver tissues in TNAP-AID mice. These findings suggest that the TNAP-AID mouse is a useful model of HCC that shares genetic and phenotypic features with human HCC.

Next, to investigate whether liver cancer originates from fetal hepatic progenitor cells with constitutive AID expression, we constructed a transplantation model of hepatic progenitor cells derived from AID Tg mice. We enriched hepatic progenitor cells from the E13.5 fetal liver of AID Tg mice and transplanted those cells, which have enhanced liver regeneration activity, into recipient mice. HCC developed in 7 out of 11 (63.6%) recipient mice receiving enriched hepatic progenitor cells from AID Tg mice, whereas no tumorigenesis was observed in recipient mice transplanted with hepatic progenitor cells

from wild-type mice (control) [31]. Furthermore, whole exome sequencing of tumor cells allowed us to determine the landscape of the accumulated genetic alterations during tumorigenesis and clarified that several dozen genes acquired single nucleotide variants in tumor tissues originating from the transplanted hepatic progenitor cells of AID Tg mice. Pathway analysis using a KEGG database revealed that the acquired genetic aberrations affected several important signaling pathways, thereby disturbing normal homeostasis [31]. These results indicate that accumulation of genetic alterations in fetal hepatic progenitor cells could lead to HCC development.

Conclusion

Human hepatocarcinogenesis is closely related with chronic inflammation, and aberrant AID expression in the inflamed liver could play an important role in inflammation-associated HCC development by inducing accumulation of genetic alterations in various genes. Taking advantage of the mutagenic activity of AID, we established novel mouse models of hepatocarcinogenesis.

Many mechanisms underlying chronic inflammation-associated hepatocarcinogenesis, such as the contribution of epigenetic modulations to HCC formation, however, remain unsolved. In addition, the detailed relevance of chronic inflammation for the development of NASH-related HCC is unknown. Further genetic and molecular investigations are required to understand the actual state of chronic inflammation-related HCC.

Disclosure Statement

The authors have no conflicts of interest to declare.

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