

## CLINICAL IMPLICATIONS OF BASIC RESEARCH

## Hepatocellular Carcinoma and Sex

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Hepatocellular carcinoma is an important disease worldwide, with an increasing incidence in the United States. The large majority of cases occur in patients with chronic infection with hepatitis B virus (HBV) or hepatitis C virus (HCV). The relative risk of hepatocellular carcinoma in patients with chronic HBV or HCV infection is about 25 to 30 times that of those without infection. The inflammatory immune response of the host to viral antigens induces hepatocyte damage, which is followed by the regeneration of hepatocytes and the development of fibrosis and cirrhosis — important features in the pathogenesis of hepatocellular carcinoma.

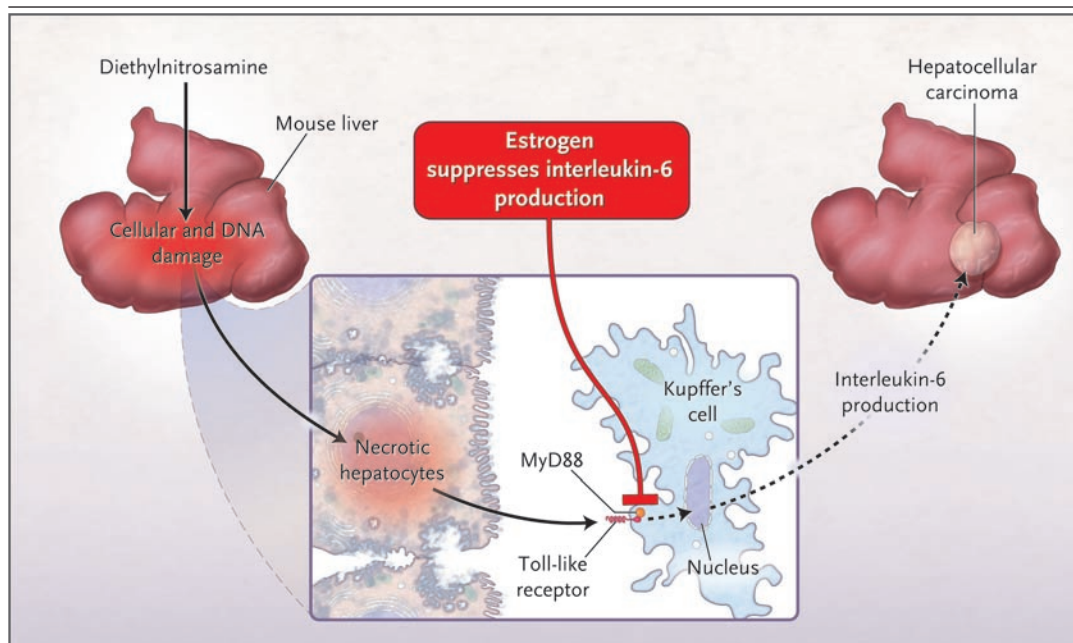
Men have a higher prevalence of hepatocellular carcinoma than women; the ratio of affected men to affected women varies between 2:1 and 4:1, depending on the geographic region. For example, the prevalence among men and the ratio of affected men to affected women is higher in the Asian Pacific region, where chronic HBV infection is endemic, than in other regions.<sup>1</sup> The reasons for the disparity between men and women are obscure, but they may include environmental factors such as a higher prevalence of persistent HBV or HCV infection, alcohol abuse, and smoking in men than in women. Genetic and hormonal factors may also be important, as has been underscored in a recent study by Naugler et al.<sup>2</sup>

In mouse models of hepatocellular carcinoma, in which the cancer is induced by exposure to diethylnitrosamine, the disparity between the sexes is even more striking than that observed in humans. When diethylnitrosamine is given parenterally in a large dose, almost all male mice and approximately 30% of female mice will subsequently have hepatocellular carcinoma. Diethylnitrosamine is converted by enzymes in the liver to an electrophilic form, which damages DNA, causing cell death and regeneration of liver tissue, which subsequently lead to the formation of hepatocellular carcinoma (Fig. 1). Studies have

shown that hormonal factors may be critical to diethylnitrosamine-mediated carcinogenesis; castration or administration of estrogens reduces the rate of formation of hepatocellular carcinoma in male mice.<sup>3</sup>

Naugler et al. have delineated the molecular mechanism that underlies this protective effect of estrogen. Key to this mechanism is the level of the proinflammatory cytokine interleukin-6 after the administration of diethylnitrosamine. The authors found that male mice had greater elevations in the alanine aminotransferase level (indicative of damage to hepatocytes), higher rates of hepatocyte apoptosis, and accelerated hepatocyte proliferation after exposure to diethylnitrosamine as compared with before exposure. But the administration of estrogen to males lowered the alanine aminotransferase levels to those of females, and ovariectomized mice had the same alanine aminotransferase levels as male mice — indicating a striking effect of estrogen on liver injury. And in interleukin-6–knockout male mice, the administration of diethylnitrosamine resulted in a lower incidence of hepatocellular carcinoma and much higher odds of survival.

Naugler et al. showed that expression of interleukin-6 depends on the small adaptor protein myeloid differentiation factor 88 (MyD88), which is involved in signaling by toll-like receptors (Fig. 1). Male mice deficient in MyD88 did not have hepatocellular carcinoma after exposure to diethylnitrosamine. Finally, the investigators showed that the treatment with estrogen of Kupffer's cells (a type of macrophage) isolated from the liver resulted in reduced transcription of the interleukin-6 gene after exposure to necrotic hepatocytes — suggesting that estrogen-mediated reduction of interleukin-6 production by Kupffer's cells reduces the risk of cancer in female mice.<sup>2</sup> Therefore, on a cellular level, the Kupffer's cell seems to be pivotal. It detects necrotic debris by means of toll-like receptors, which convey the



**Figure 1. Sex and Susceptibility to Hepatocellular Carcinoma.**

The administration of diethylnitrosamine to mice at a dose of 100 mg per kilogram of body weight results in hepatic injury, as manifested by elevations in the alanine aminotransferase level, apoptosis, and cell death, followed by hepatocyte proliferation. Several months later, 100% of males and 30% of females will have hepatocellular carcinoma. A recent study by Naugler et al.<sup>2</sup> indicates that modulation by estrogen of interleukin-6 synthesis in the Kupffer's cell explains this difference in susceptibility. Estrogen blunts the interleukin-6 response of the Kupffer's cell to necrotic hepatocytes. In this context, the expression of interleukin-6 messenger RNA in Kupffer's cells appears to be dependent on signaling by myeloid differentiation factor 88 (MyD88) through toll-like receptors. Therefore, estrogen at levels found in female mice but not male mice inhibit interleukin-6 production and suppress the diethylnitrosamine-induced formation of hepatic tumors.

signal to the nucleus through the messenger molecule MyD88. This up-regulates interleukin-6 production — an event that is countered by estrogen. Elevated levels of interleukin-6 contribute to the development of hepatocellular carcinoma.

How do these findings relate to biologic characteristics and disease in humans? Nitrosamines may be found in certain foods, such as meat, after the addition of nitrates or nitrites as preservatives, but their levels are far lower than those required to cause hepatocellular carcinoma in mouse models. Perhaps the best rodent model for hepatocellular carcinoma in humans is the Eastern American woodchuck (*Marmota monax*).<sup>4</sup> This species has been particularly useful for the development of nucleoside analogs that inhibit viral replication and thereby greatly reduce the risk of hepatocellular carcinoma. The infection of newborn pups with the woodchuck hepatitis virus, which has high nucleic-acid homology to HBV,

causes persistent viral infection and chronic hepatitis, as well as the subsequent integration of the viral DNA into the host's cellular DNA, followed by the development of hepatocellular carcinoma in almost 100% of the animals within 4 years.<sup>4</sup> However, after neonatal exposure to the woodchuck hepatitis virus, male and female woodchucks have similar odds of becoming chronic carriers, of having hepatocellular carcinoma, and of surviving. It would be worth testing whether interleukin-6 levels are similar in male and female woodchucks, as might be predicted from the study by Naugler et al.<sup>2</sup> Also worth testing is the existence of a disparity between the sexes in serum interleukin-6 levels or levels of expression of interleukin-6 messenger RNA (mRNA) in the hepatocytes of persons with chronic HBV or HCV infection who have cirrhosis and in those who do not have cirrhosis.

If high-risk men have elevated interleukin-6

mRNA and protein levels as compared with women, would estrogens or estrogen-mimetic compounds be useful for prophylaxis in humans? Although attractive, such an approach in persons with a lifelong risk of hepatocellular carcinoma raises issues of potentially adverse side effects on the hepatic, cardiovascular, and endocrine systems that can be evaluated only by clinical studies. In the meantime, antiviral approaches that reduce replication or eradicate persistent HBV or HCV infection are likely to have a substantial effect on the prevention of hepatocellular carcinoma and should be pursued.

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