have some degree of fibrosis or injury. This issue is now under further investigation in our laboratory.

Marra et al[28] reported that MCP-1 enhances the migration of HSC in culture. Moreover, it has been reported that HSC themselves produce MCP-1[29,30] and that TGF-β induces the secretion of MCP-1.[29] Together with our present study, it is likely that MCP-1 stimulates liver fibrogenesis by 2 mechanisms: (1) MCP-1 induces macrophage infiltration, and macrophages secrete an activating factor(s) for HSC; (2) MCP-1 acts directly on activated HSC to modulate their function. In favor of this notion, it has been reported that in cultured skin fibroblasts, MCP-1 increases the gene expressions of α_1 (I) procollagen and TGF-β[31] and of matrix metalloproteinase 1 and 2 (and of their inhibitor, tissue inhibitor of metalloproteinase 1)[32] and that MCP-1 stimulates the proliferation of cultured vascular smooth muscle cells.[33] Collectively, these observations suggest that MCP-1 secreted from macrophages and from HSC themselves may facilitate the production of TGF-β, as well as of matrix metalloproteinases and their inhibitors, thereby enhancing inflammation and tissue remodeling (fibrogenesis).

We expressed 7ND in livers expecting that it would inhibit MCP-1 as a dominant-negative mutant.[16] It has been reported that a 75:1 molar ratio of 7ND/wild-type MCP-1 is needed for a 50% inhibition of monocyte chemotaxis in vitro.[16] In our setting, the amount of 7ND was substantially higher than that of rat MCP-1 in both sera and livers for at least 2–2.5 weeks under DMN treatment (Figure 2). We have not yet determined how much 7ND is required to inhibit the actions of MCP-1 in vivo, specifically in the case of the DMN-injured rat liver. Thus, it is not certain whether the actions of MCP-1 were indeed inhibited in our experiments or whether the observed inhibition of macrophage infiltration was indeed achieved via a suppression of MCP-1 by 7ND. We would like to add that numerous reports (13, to our knowledge) have been published in which the same 7ND construct as that used in this study was introduced (either by direct injection or by electroporation with an expression plasmid) into various animal models, and suppression of macrophage infiltration and some biological effects were seen in every one of these reports. In 3 of these 13 studies, both 7ND and endogenous MCP-1 proteins in serum were measured, and the values obtained (7ND/MCP-1) were 220/71 pg/mL,[23] 226/85 pg/mL,[24] and 124/92 pg/mL[25] (all in mice). We detected a peak value of 528 ± 182 pg/mL for 7ND and 62 ± 12 pg/mL for endogenous rat MCP-1. Both this peak value for 7ND and the ratio between 7ND and endogenous MCP-1 are the highest among the values reported in the literature so far.

In summary, we have shown that the macrophages that infiltrate into livers immediately after an initial injury are critical both for HSC activation and for the subsequent fibrogenesis, and we also showed that TGF-β, which is required for activated HSC to survive, is not an activating factor for HSC (at least in this situation). Macrophages may themselves secrete an activating factor(s) for HSC.

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EDITORIAL

Integration of hepatitis B virus DNA and hepatocellular carcinoma

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Hepatocellular carcinoma (HCC) is one of the most common malignant human tumors worldwide.[1] Hepatitis B virus (HBV) and the hepatitis C virus (HCV) infections are etiologically linked to this cancer. HCC often emerges after the development of cirrhosis in patients with chronic HCV infection. In contrast, in patients with the HBV infection, HCC sometimes develop in non-cirrhotic patients, although the incidence is much lower than in patients with cirrhosis.[2] The HBV is a DNA virus that integrates into the chromosome of the host. In contrast, the HCV is a RNA virus that does not integrate into the host genome. It seems that the integration of the HBV into host chromosome(s) plays an important role in the development of HCC, especially in non-cirrhotic patients with HBV infection.

There are two major possible carcinogenic effects of HBV on the development of HCC. One is the direct effect of viral proteins such as HBx. In fact, this protein has been proven to be a transactivator that activates a variety of viral and cellular promoters. It has also been reported that HBx binds to p53 and inhibits p53-mediated cellular processes, including DNA binding, transcriptional transactivation and apoptosis. The viral proteins that might be produced as a result of integration and truncation of preS2/S and hepatitis B spliced proteins have also been reported to function as transcriptional modulators.[3] These proteins have been shown to modulate the transcriptional activation of cellular growth-regulating genes, to modify apoptosis and to inhibit nucleotide excision and the repair of damaged cellular DNA. The other possible carcinogenic effect of HBV is the integration of HBV-DNA into a host chromosome. This might interrupt cellular tumor suppressor genes, or cause alterations in the expression of cellular growth factors and/or apoptosis regulating factors. Although frequent activation of the N-myc oncogene has been reported in woodchuck hepatitis virus integration,[4] no apparent favored locus of human oncogene/tumor suppressor gene has been found. However, there are many reports that describe the alteration of cellular proliferation pathways (important in the control of cell signaling, proliferation and viability) as a result of the insertion of HBV-DNA. Recent reports have shown that in at least some hepatocellular carcinomas, the human telomerase reverse transcriptase (hTERT) gene is a non-random integration site of the HBV genome, which activates the hTERT transcription *in cis*.[5]

In this issue of the Journal, Huang *et al.*[6] reported a high incidence of HBV-DNA integration in tumor and non-tumor tissues of children with chronic HBV infection. They detected a higher incidence of the HBV integration using a sensitive inverse polymerase chain reaction (IPCR). This method was developed to amplify unknown sequences that flank a region of a known sequence (Fig. 1)[7,8]. This technique is very sensitive because a nested polymerase chain reaction is used for amplification of DNA. However, in some instances, it fails to detect integrated HBV-DNA. For example, integrants that do not contain a full four site of primer escape detection. Alternatively, the flanking region that does not contain an employed restriction enzyme digestion site near the integration portion fails to be amplified. The latter possibility is less likely because the employed restriction enzymes are four base recognizing cutters, the restriction site often appearing in common nucleotide sequences. However, it sometimes the restriction enzyme site is too near to the integration site and consequently, the amplified flanking sequences are too short to analyze. This could be avoided if one were to use several different pairs of enzymes in order to analyze the samples. It is, thus, highly likely that the incidence of integration of HBV-DNA in a host chromosome is higher than that detected.

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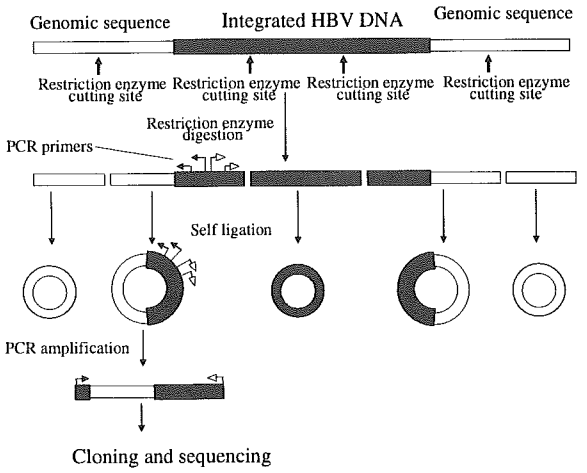

Figure 1 Alu-polymerase chain reaction to detect the integrated hepatitis B virus DNA in host chromosomes.

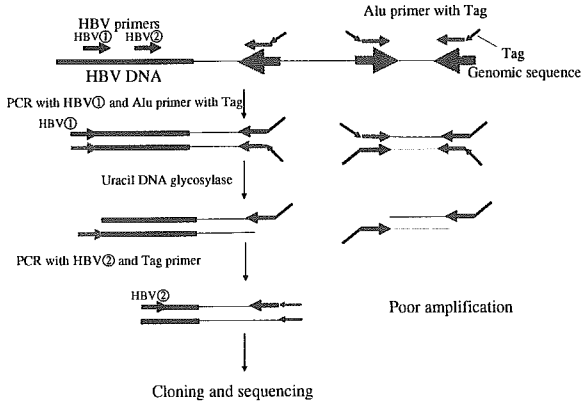

Figure 2 Cellular DNA was amplified using primer HBV1 and Alu specific primer with 5′-Tag sequence. Primers were synthesized using dUTP. The amplified products were then digested with uracil DNA glycosilase. Primers in the amplified DNA were digested with this enzyme leaving complementary sequences. The second round PCR was then carried out using HBV2 and a Tag primer. Only sequences that contain HBV-DNA and Alu sequence were effectively amplified.

An alternative method used to detect integration of HBV-DNA is Alu PCR (Fig. 2).[9] A recent study showed that this method is very sensitive, detecting more than 70% of HCC related to HBV containing integrated HBV-DNA.[10] However, this approach might fail to detect integrated HBV-DNA if the site of integration is too far from the Alu sequences. Also, it is possible that the flanking sequence contains a PCR resistant secondary structure or guanine–cytosine rich regions. Furthermore, similarly to IPCR, if the integrated sequence does not contain a sequence for primers to anneal, the examined integration rate might be underestimated.

The high integration rate (related or unrelated to carcinogenesis) and the fact that the related hepadna virus, the duck hepatitis B virus (DHBV), integrates into a host genome very early after infection, show us that almost all infected cells contain integrated HBV-DNA.[11] However, previous studies have shown that a greater proportion of HBV-DNA integrations are not related to cancer development. Although it is obvious that chronic inflammation and fibrosis are main factors for accelerated cell proliferation and transformation, there are many steps to the development of HCC. Although it remains unclear if the integration of HBV plays an important role in hepatocarcinogenesis, it might open up new avenues of research for hepatocarcinogenesis, for example, to improve the detection of HBV-DNA integration (see Huang *et al.*[6]), and to continue the analysis of the effect of integrated HBV-DNA to intracellular biological phenomenon. Tissues obtained from HCC in children might be good candidates for such analysis because they might cause a serious alteration in chromosomal genetic functions.

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