# Molecular mechanism of hepatoma development in Hepatitis B surface antigen (HBs) transgenic (Tg) mice

C. Fujii, Y. Nakamoto, P. Lu, and N. Mukaida

Hepatocellular carcinoma (HCC) is a common complication of chronic hepatitis B virus (HBV) infection, although HBV does not harbor any oncogenes. Thus, it is presumed that persistent inflammatory reactions are responsible for HCC development. In order to elucidate the molecular and cellular mechanism, one of us (YN) have established a mouse model of HCC by using HBV s antigen (HBs) transgenic mice. In this model, bone marrow cells and splenocytes were obtained from syngeneic wild-type mice, which were immunized with HBs antigen and were transplanted into HBs transgenic mice, which were myeloablated beforehand. At about 15 months after the transplantation, the transgenic mice developed multiple foci of HCC. We obtained samples from non-tumor sites as pre-malignant lesions. Total RNAs were extracted from control liver and pre-malignant lesions for the analysis by fluorescence differential display method (FDD).

FDD using 60 distinct sets of primers demonstrated that 38 bands were up-regulated in pre-malignant lesions while 56 bands were down-regulated in pre-malignant lesions, compared with control liver. The determination of the nucleotide sequence of each band revealed that the expression of 6 unreported genes and 19 known genes was enhanced in the pre-malignant lesions. Moreover, the expression of 19 known genes was diminished in pre-malignant lesions, compared with control liver. A semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR) further demonstrated that the mRNA expression of 5 out of 6 unreported genes was enhanced actually in pre-malignant lesions. We are under the way to identify the molecular and functional characteristics of these 5 unreported genes.

Among 19 known genes whose expression was enhanced in pre-malignant lesion, we focused on pim-3. Pim-3 belongs to a family of proto-oncogenes such as pim-1 and -2, which exhibit serine threonine kinase activitiy, but previous studies reported a selective expression of pim-3 in neuronal system, but not in the course of any types of carcinogenesis. We observed that the expression of pim-3 was also augmented in liver in the course of diethylnitrosamine-induced hepatocarcinogenesis. Moreover, we observed that pim-3 mRNA was constitutively expressed in all human hepatoma cell lines that we examined. These observations suggest the potential involvement of pim-3 in hepatocarcinogenesis.