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Expression of Hepatitis B Virus X Protein and Cellular Injury

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Hepatitis B virus (HBV) is closely associated with acute or chronic hepatitis as well as the development of hepatocellular carcinoma (HCC) [for review, see (Ganem & Varmus, 1987; Tiollais *et al.*, 1985)]. One of the HBV genes, X, encodes a basic protein of 154 amino acids, which has been implicated in the carcinogenicity of this virus as a potential causative factor because of its ability to induce transformation of rodent cells (Hohne *et al.*, 1990; Rakotomahanina *et al.*, 1994; Shirakata *et al.*, 1989) and to cause the development of HCC in transgenic mice (Kim *et al.*, 1991) . The X gene product is also known to transactivate a number of viral and cellular genes [(Spandau & Lee, 1988) , for review (Rossner, 1992)].

In recent years, the transactivation function of X protein has been extensively studied by a number of investigators and some of the functional roles of X protein have been revealed. However, the reported observations and proposed mechanisms have not always been consistent. Many reports have suggested that X protein may transactivate transcription via protein-protein interaction with cellular factors that bind to enhancer sequences. It was proposed that X protein might exert its effect by enhancing the binding of cellular factors to their recognition sequences (Maguire *et al.*, 1991; Natoli *et al.*, 1994a) . In addition, a Kunitz inhibitor (KI) domain-like region has been reported in X protein and was shown to be indispensable for the transactivation function of X protein (Takada *et al.*, 1994a; Takada & Koike, 1990) . Some reports have also suggested a possible role of X protein in the activation of a number of factors involved in the regulation of some signal transduction pathways (Benn & Schneider, 1994; Cross *et al.*, 1993; Kekule *et al.*, 1993; Natoli *et al.*, 1994b) . However, the in vivo interaction between X protein and these factors remains to be demonstrated.

In most human tumor cells studied, p53 gene abnormalities lead to loss of the p53 protein or to the expression of mutant forms of the protein without DNA binding activity (Baker *et al.*, 1990; Kern *et al.*, 1991; Takahashi *et al.*, 1989; Zambetti *et al.*, 1992). The state of the p53 gene has been studied

previously in human hepatoma cell lines, and its structure and expression in HepG2 and HuH7 cells were explored (Bressac *et al.*, 1990). Although the p53 protein in HepG2 cells was normal and the half-life was short, it was not normal in HuH7 cells, where the p53 protein half-life was markedly increased. In HuH7 cells, the p53 protein exhibits inability of DNA binding due to a point mutation in its DNA binding domain other than the carboxy-terminal region.

Disruption of p53 activity by interaction with viral oncoproteins or cellular proteins has been implicated in the development of some human cancers. It has been reported that HBV X protein physically associates with p53 protein and apparently blocks its normal function in vitro (Feitelson *et al.* 1993; Wang *et al.*, 1994). Subsequently, it has been indicated that liver tumor development in the X transgenic mouse is probably correlated with p53 binding to X protein in the cytoplasm and blockage of p53 entry into nucleus (Ueda *et al.*, 1995). However, there has been no evidence to indicate that transient expression of X gene is able to inhibit p53 entry into nucleus in the cell culture system. As it is known that normal or mutant p53 protein is located in the nucleus (Shaulsky *et al.*, 1991) and that X protein is found in the cytoplasm (Benn & Schneider, 1994; Siddiqui *et al.*, 1987).

To find out their connection, we examined that effect of X protein expression on the nuclear localization of p53 protein in human hepatoma cells by the immunofluorescent double-staining technique (Takada *et al.*, 1997). The location of transiently-expressed p53 protein was examined in X gene-transfected cells,where X protein was detected in the cytoplasm. The nuclear location of transiently-expressed p53 protein was changed to the cytoplasm by X protein co-expression. Endogenous p53 protein was also observed in the cytoplasm by X protein expression. The transcriptional activation domain of X protein and the carboxy-terminal region of p53 protein were found mutually responsible for the cytoplasmic retention of p53 protein in X gene-transfected cells. Therefore, the cytoplasmic retention of p53 protein may be closely correlated to the function of X protein expressed in the transfected cells.

We, then, examined the precise location of X protein in the cytoplasm using fluorescent double-staining and biochemical analyses. Our results demonstrate that X protein is associated with aggregated mitochondrial structures around the nuclear periphery. Consistent with our previous results, transiently expressed p53 protein was found co-localized with X protein in cells transfected with X gene. However, the aggregation of mitochondrial structures around the nuclear periphery was p53 independent. We carried out a series of cytochemical studies on the cell and mitochondrial structures in X-transfected cells. Results imply that the presence of X

protein may result in mitochondrial aggregation in cells in a p53-independent manner and in so doing, X protein may function as a cytopathic agent that leads to mitochondria-related cell death in the HBV-infected liver.

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