Functions of Hepatitis B Virus- X Gene product

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Hepatitis B virus (HBV)is a member of the Hepadna virus family whose members share a characteristic virion structure and genome size, around 3.2kb in a paritially double-stranded form. The genome of HBV contains four overlapping open reading frames designated as P(polymerase).
C(core),S(surface antigen)and X. The X gene has potential to encode 154 amino acids protein.

Knowledge about the functions of the X gene product has been accumulated in the past several years. The first established function of the X gene products is its role as the transcriptional activator. In the transient transfection experiments, overexpression of the X gene product can increase the expression of the reporter genes regulated by the various promoters including its own promoter. Generally, the enhancer element responsible for the X protein mediated transcriptional induction shares homology to the NF-kB binding or CRE-like sequences. Despite its role as the general transcriptional activator, X protein do not possess DNA-binding activity and the studies from several groups suggest that X protein performs its function via protein to protein interaction with the host cell DNA-binding transcription factors. The second established functions of the X protein is related to the mechanism of the hepatocellular carcinogenesis. In the recent transgenic mice study, the specific overexpression of the X protein in the transgenic liver resulted in the generation of the hepatocelular carcinoma providing the direct evidence that the X gene is indeed an oncogene. The third and most important function of the X gene which is still under debate is its possible relation to the replication of the hepatitis B virus. In the recent animal model study with the Woodchuck Hepatitis Virus(WHV), the virus defective of the X gene expression

displayed severe impairment in its ability to replicate following infection, estabilishing the importance of the X protein for WHV replication. In contrast to the above observation, the X protein, in case of HBV, dose not appear to be necessary for virus replication in cultured cells. The observed discrepancy is probably due to lack of adequate system to test the *in vivo* replication of HBV. Despite the discrepancy, it is believed that expression of the X protein is important to initiate or maintain the replication of HBV in highly differentiated hepatocytes of the natural host.

Insomuch as the X protein mediates its action via protein to protein interaction, we investigated the interaction of the X protein with the host cell proteins. The X protein was expressed in E.Coli as a fusion protein to Glutathione-S-transferase. The X protein in the inclusion body was renaturd, purified, and used as a probe to detect interacting interacting proteins in the liver cell extract immobilized on nitrocellulose blot. Approximately, ten to twelve bands with sizes ranging from 20kD to 150kD was specifically detected with the X protein probe but not with the control probe. The detection of various proteins in the liver cell nuclear extract provide an explanation for the pleiotropic effects of the X protein. Currently, the identity of these interacting proteins are being investigated. In addition, we studied the possible interaction of the X protein with the tumor suppressor gene products like RB or p53, insomuch as the transactivators of the several tumor viruses had been shown to interact with these tumor suppressor gene products. We found that the X protein interact with p53 but not with RB determined by the following criteria : 1)In far western blotting experiments, p53 immobilized on nitrocellulose filter was specifically detected by the [^{32}P]-labeled X protein. 2)*In vitro* translated X protein and p53 were coimmunoprecipitated by the antisera specific either to X protein or p53. Currently, the biological significance of these observations are under investigation.