Functional Restoration of p53 Tumor Suppressor Protein by Adriamycin in HBx Oncoprotein-expressing Liver Cells

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**Background** p53 tumor suppressor protein is a common target site in molecular pathogenesis of DNA tumor virus. Hepatitis B virus X (HBx) oncoprotein implicated in the development of liver cancer may inhibit the function of p53 tumor suppressor protein through cytoplasmic retention of p53 protein. Here, we attempt to investigate whether the functional inhibition of p53 protein by HBx oncoprotein is reversible and what the mechanism would be.

**Methods** We have employed ChangX-34 cells that express HBx protein in a doxycycline-inducible manner. The association of p53 protein with HBx was determined by co-immunoprecipitation and immunofluorescence staining. Transcriptional activity of p53 was analyzed by using chloramphenicolacetyl transferase reporter system and the levels of HBx, p53, p21\textsuperscript{WAF1} and MDM2 were determined by Northern blotting and Western blotting.

**Results** We first provide the evidence for the association of endogenous p53 protein with HBx by co-immunoprecipitation in ChangX-34 cells. By immunofluorescence microscopy, the major location of p53 protein of ChangX-34 cells was confirmed at the nuclear periphery as well as in the cytoplasm where HBx protein is mainly expressed. Interestingly, treatment with adriamycin, a chemotherapeutic drug, not only increases the level of p53 protein but also triggers the nuclear translocation of p53 protein sequestered in the cytoplasm. This change is accompanied by the restoration of p53 activity, which results in increased transcriptional activity at the p53-responsive DNA elements as well as increase of p21\textsuperscript{WAF1} mRNA expression. We then attempted to investigate the mechanism underlying the restoration of p53 activity. We found that adriamycin dramatically reduced the level of MDM2 protein, thereby resulting in accumulation of p53 protein. In addition, co-immunoprecipitation assay showed that adriamycin may release some of p53 protein from the HBx/p53 complex, which efficiently elevates the p53-mediated transcriptional activity.

**Conclusion** We demonstrate that functional inhibition of p53 protein through its cytoplasmic retention by HBx protein can be restored by adriamycin treatment. These results may be extended into other tumors of which p53 activity is modulated by viral oncoproteins.