In vivo evidences that Pre-S deletion mutants of hepatitis B virus is more likely to retain in the endoplasmic reticulum than the wild type

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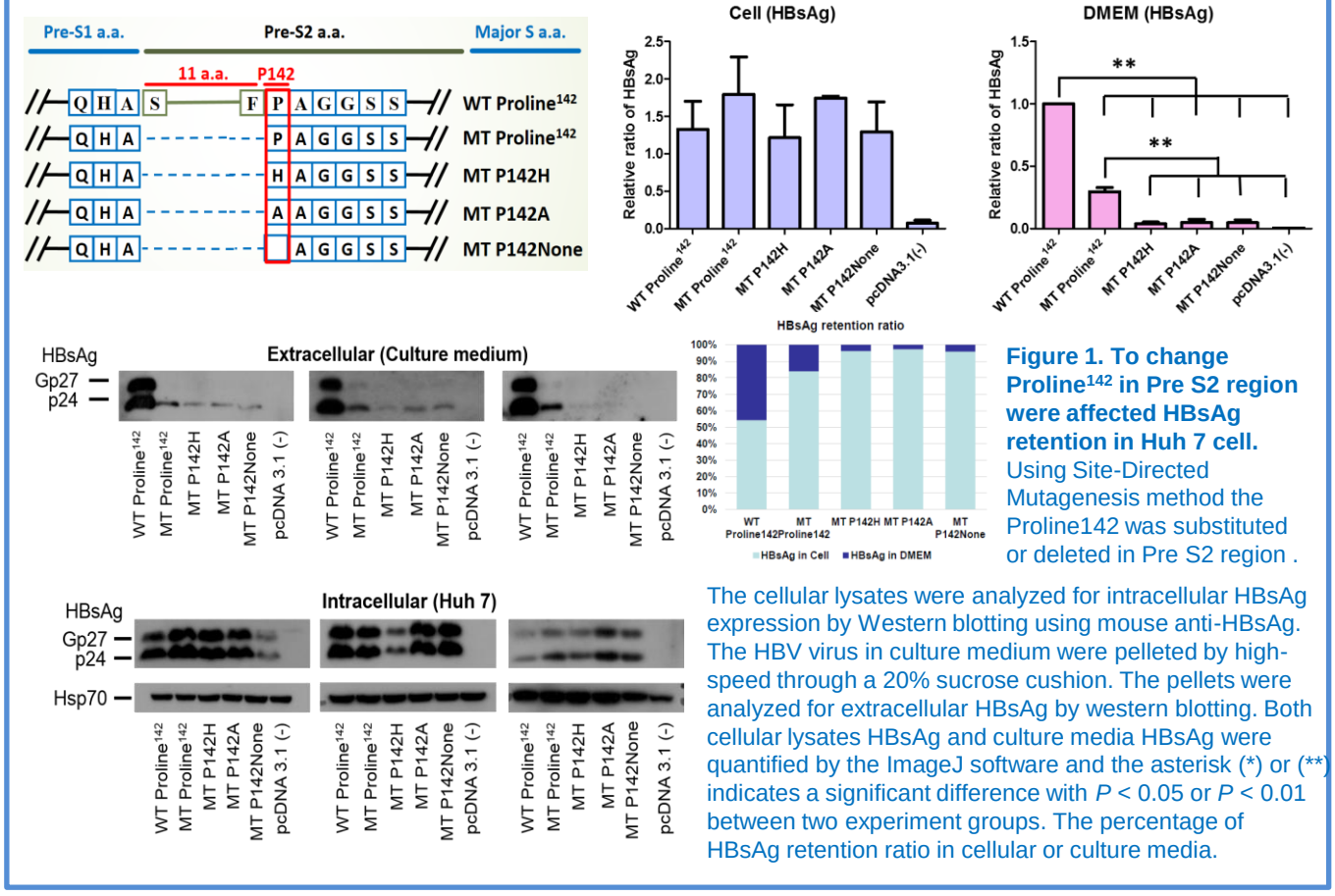


Figure 1. To change Proline[142] in Pre S2 region were affected HBsAg retention in Huh 7 cell. Using Site-Directed Mutagenesis method the Proline142 was substituted or deleted in Pre S2 region .

The cellular lysates were analyzed for intracellular HBsAg expression by Western blotting using mouse anti-HBsAg. The HBV virus in culture medium were pelleted by high-speed through a 20% sucrose cushion. The pellets were analyzed for extracellular HBsAg by western blotting. Both cellular lysates HBsAg and culture media HBsAg were quantified by the ImageJ software and the asterisk (*) or (**) indicates a significant difference with $P < 0.05$ or $P < 0.01$ between two experiment groups. The percentage of HBsAg retention ratio in cellular or culture media.

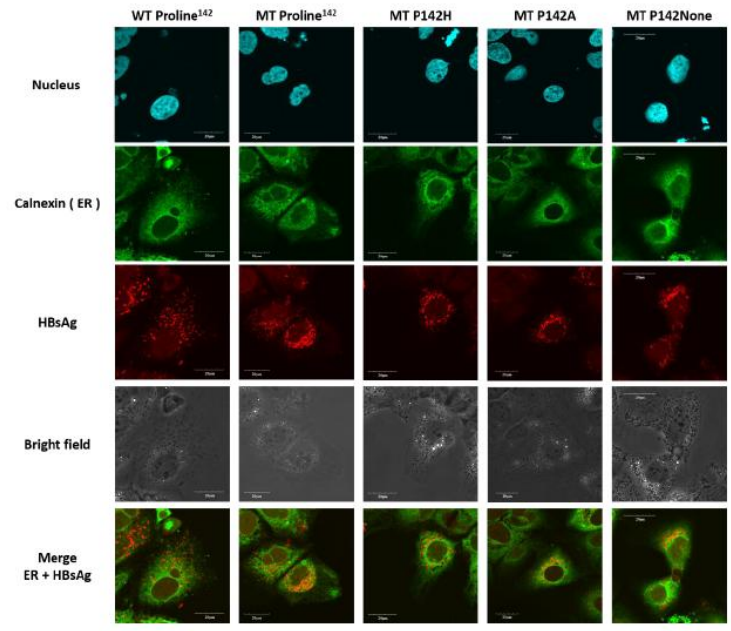


Figure 2. The influence of short segment pre-S deletion and single amino acid Proline[142] deletion at pre-S2 on the expression and ER localization of HBsAg. Immunofluorescence staining of HBsAg and ER in transfected Huh 7 cells. In the WT HBV transfected cells, the HBsAg showed diffuse fine spotty distribution in cell cytoplasm. However, the MT group transfected cells HBsAg was stained coarse granular clustering in peri-nuclear area of ER. This data suggest that the substitution or deletion of Proline142 result in HBV and HBsAg retention in the ER of cells

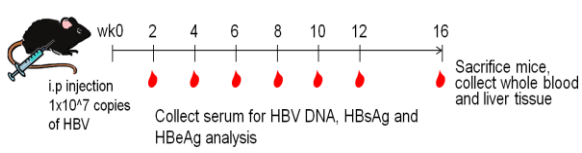
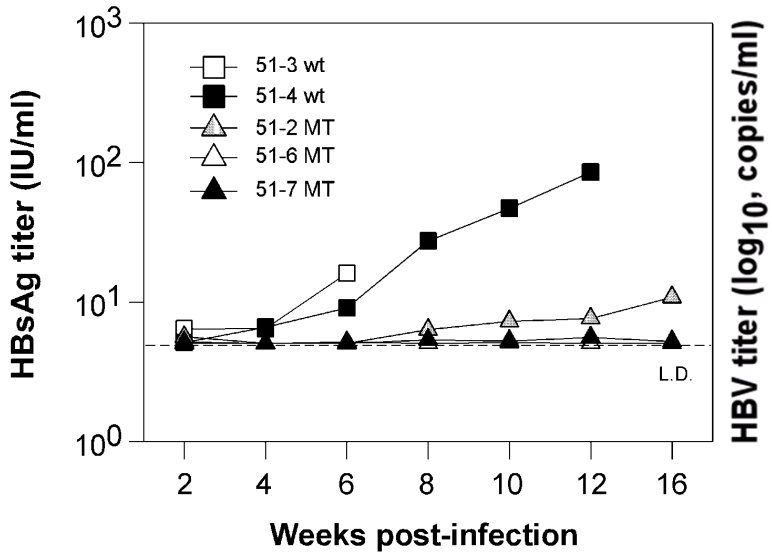
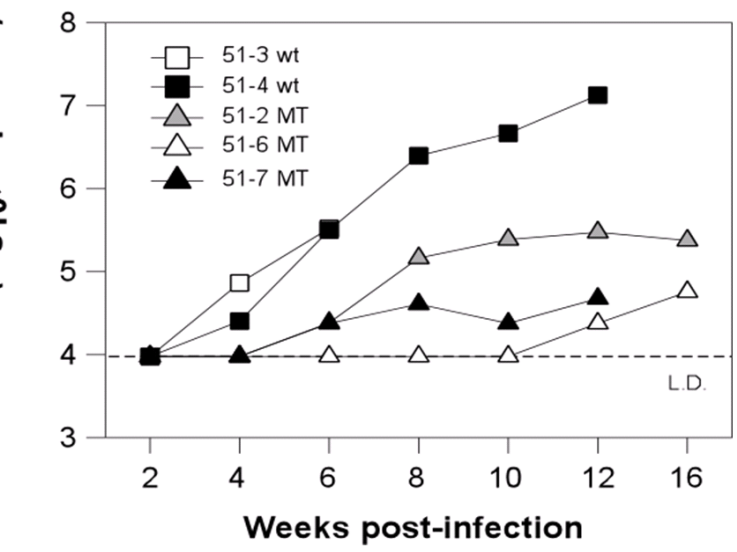


Figure 3. WT and MT infection in huFRG mice. Mice serum were collected for HBV DNA, HBsAg and HBeAg analysis. The serum HBV DNA levels of HBV pre-S2 deletion mutants were about 2 logs lower than those of the wild type due to secretion defect





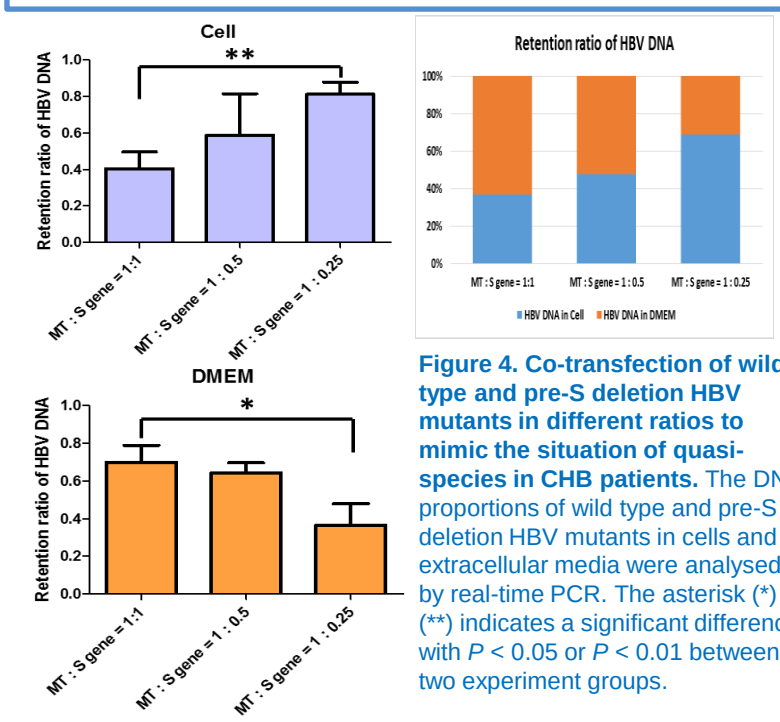
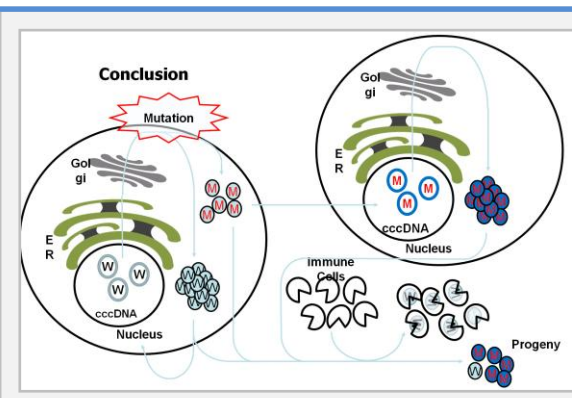
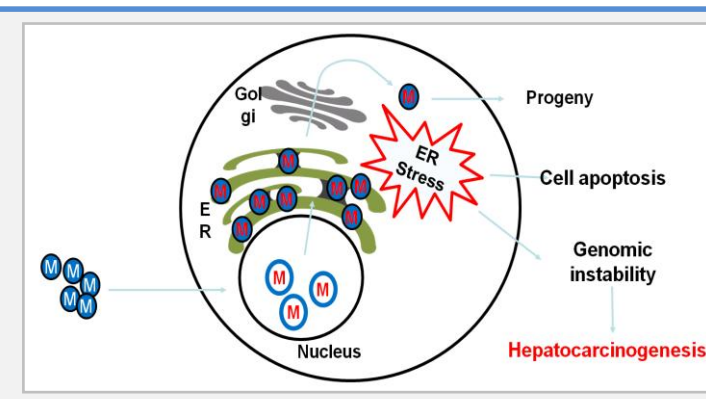


Figure 4. Co-transfection of wild type and pre-S deletion HBV mutants in different ratios to mimic the situation of quasi-species in CHB patients. The DNA proportions of wild type and pre-S deletion HBV mutants in cells and extracellular media were analysed by real-time PCR. The asterisk (*) or (**) indicates a significant difference with $P < 0.05$ or $P < 0.01$ between two experiment groups.





Conclusion: MT group of HBV core particle could compete with WT group of HBV core particle for normal surface protein and the MT group of HBV could secret or infect hepatocytes using envelopes of wild type HBsAg. However the normal envelope proteins might lead HBV under immune attack easier than envelope protein with deletion segments containing T cell or B cell immune epitopes. If immune cells eliminate most of WT HBV with WT envelope proteins, the ratio of MT envelope proteins was increased. Although the MT envelope proteins have deletion mutations in immune epitopes and help HBV escapes immune attacks, it reduces HBV secretion and result in retention of HBsAg in ER and then induces ER stress contributing to hepatocarcinogenesis