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Name of the Scholar: Masarrat Afroz

Name of the Supervisor: Dr. Syed Naqui Kazim

Name of the Centre: Centre for Interdisciplinary Research in Basic Sciences

Title of the Thesis: *In Vitro* Study of Hepatitis B Virus Replication with the Help of Lamivudine Associated Mutant Plasmid Constructs Harboring Basal Core Promoter/Precore (BCP/PC) Mutations

Hepatitis B virus infection is an important health problem worldwide. It is a major cause of chronic hepatitis, cirrhosis as well as hepatocellular carcinoma. To stop or delay disease progression, Several therapeutic options are possible based on drugs approved by the US Food and Drug Administration, i.e. alpha interferon and four nucleos(t)ide analogues: lamivudine, adefovirdipivoxil, entecavir and telbivudine. These drugs are effective in decreasing HBV replication. However, the long term efficacy of drugs may become limited by the emergence of anti drug resistant mutants. Mutations in the YMDD motif of HBV genome is known to be associated with lamivudine resistance, which have been proven to lower down and alter the viral DNA replication.

Basal Core Promoter (BCP) mutations and Precore (PC) mutations possibly increase HBV DNA replication levels *in vitro*. Despite the good amount of work done on the issue, there are still controversies which exist regarding the effect of BCP/PC mutations on HBV DNA replication. *In vitro* studies have shown that mutations in the BCP or PC region restore the replication inefficiency of the lamivudine resistant mutants. Based on the conflicting findings and knowledge on one or the other aspect of relationship of BCP and PC mutations with most commonly existing lamivudine resistant mutants in terms of response to therapy, biochemical consequences and the viral life cycle (particularly in *in vitro* cell culture), an extensive study was needed to understand the underlying realities on molecular basis of the mentioned aspect.

Therefore, the present study was undertaken to address the issues related to underlying mechanisms that influence the viral life cycle of lamivudine resistant mutant in presence of mutations of Basal Core Promoter and Precore. We tried to unravel the mechanisms with the help of different recombinant constructs of wild type as well as mutant types of HBV by single and cotransfection experiments in a couple of hepatoma cell lines HepG2 and Huh7. The two most important antigens (HBsAg and HBeAg) in cell culture supernatant were assessed qualitatively and quantitatively. Quantitative real time PCR of extracellular DNA, viral replicative intermediates including covalently closed circular DNA (cccDNA) were performed with standard protocols.

The results obtained are as follows:

- Both single as well as double mutations of Basal Core Promoter (BCP) and Precore (PC) regions of Hepatitis B Virus (HBV) result into reduced level of HBsAg and HBeAg secretion in HepG2 cells.
- Lamivudine resistant mutations that do not employ any substitution in overlapping surface gene encoding HBsAg, have nearly equal amount of HBsAg synthesis and secretion as wild type in HepG2 cells. On the other hand, lamivudine resistant mutant with overlapping creation of surface gene stop codon, results into complete loss of HBsAg in HepG2 cells.
- Unlike HBsAg, HBeAg secretion in HepG2 cells by lamivudine resistant HBV is relatively reduced than the wild type virus.
- DNA of replicative intermediates are much affected compared to extracellular DNA by BCP mutations in HepG2 cells.
- Single mutation of Precore has reduced level of viral DNA, but double mutations of PC (G1896A and G1899A) restore the viral replication upto the level of wild type virus replication.
- In absence of lamivudine, lamivudine resistant mutants replicate as wild type in HepG2 cells, rather rtM204I can replicate even more than wild type.
- BCP wild as well as mutant constructs in pcDNA3 backbone affect the production of HBsAg and HBeAg in cotransfection with wild type HBV genome.
- Double mutations of BCP or PC, if reduce the secretion of antigens in cotransfection settings, they result into simultaneouly enhanced production of viral DNA, especially intracellular DNA in HepG2 cells.
- Lamivudine resistant mutants face more pronounced effect in reduction of HBeAg in cotransfection with BCP mutant construct in pcDNA3.
- Extracellular viral DNA does not show much variation in lamivudine resistant construct, cotransfected with BCP wild or mutant constructs. On the other hand, other forms of viral DNA appear at the increased level (as equal to as wild type) compared to the extracellular DNA.
- Lamivudine resistant mutant showing reduced level of HBeAg production under the influence of BCP mutations, does not do the same at the level of replicative intermediates and cccDNA. This is possibly the underlying molecular mechanism which makes the lamivudine resistant mutant Hepatitis B virus more escapist and protected from the effectiveness of host immune mechanism involved in viral clearance and persistence.