Name of Scholar : Sabihur Rahman Farooqui
Name of Supervisior : Dr. Syed Naqui Kazim
Centre : Centre for Interdisciplinary Research in Basic Sciences
Title of the Thesis : Molecular mechanism of life cycle in antiviral resistant mutants of Hepatitis B Virus

Abstract

Among some of the most serious and persistent health problems of human being is the infection with Hepatitis B Virus. Despite the availability of an effective vaccine against it since 1980s, it still affects over 2 billion people worldwide. Out of them 350 million are chronic carriers and 75% of them resides in the Asia Pacific region. A study conducted by Global Burden of Disease which was published in 2010, ranked infection with HBV as the major health priorities of the World causing 786000 deaths per year.

The goal of antiviral agents used in the treatment of HBV infection is to prevent viral replication and disease progression. Currently two immune modulators, interferon-α and pegylated-interferon-α and five nucleoside/nucleotide analogues, lamivudine, adefovir, entacavir, telbivudine and tenofovir are approved for the treatment. Treatment with these nucleoside/nucleotide analogues results in emergence of various antivitral resistant mutations of Hepatitis B Virus, altering the course of treatment.

Antiviral therapy associated surface mutations are mainly found in the ‘a’ determinant region. However, reports of mutations downstream of ‘a’ determinant region selected during antiviral therapy are also present. Other antiviral associated surface mutations are premature stop codon mutations. Due to these mutations a carboxyl end truncated surface protein is generated. These stop codon mutations are clinically relevant as they are associated with development of hepatocellular carcinoma in chronically infected patients receiving antiviral therapy.

The present study has been aimed to systematically investigate the molecular mechanisms of viral life cycle in cell culture systems, for the antiviral resistant mutant viruses. Prime focus was on the antiviral resistant mutations of polymerase resulting into premature truncation of overlapping surface protein due to creation of a stop codon. The present study was designed to understand the influence of such mutations in their sole nature of presence as well as their presence in combination with other antiviral resistant mutations.

To achieve this goal six replication competent plasmids were constructed harboring various clinically relevant and novel antiviral resistant mutations. The mutations studied were rtV191I/sW182Stop, rtM204V/sI195M, rtM204I/sW196F, rtM204I/sW196Stop. These

mutations were either studied alone or in combination. They were transiently transfected in HepG2 cells to analyze and compare the replication phenotype of the various antiviral resistant mutants. Replication competence was measured by analyzing the levels of various replicative intermediates of HBV. HBV specific serological markers were also analysed.

The mutations rtM204V and rtM204I were found to have no effect on the levels of HBsAg. Though these mutations caused change in the overlapping surface gene but they do not altered the surface protein conformation. In case of pHBV VI the mutation rtV191I in the polymerase protein produces a stop codon at 182 amino acid position of surface protein. This mutation of surface protein leads to the premature truncation of surface protein. This premature truncation prevents the protein from folding properly. Moreover the improper folding leads to loss of antigenicity of epitopes causing lower levels of detection of surface protein in culture supernatant.

The secreted HBsAg levels for single (rtV191I/sW182Stop) and double (rtV191I+rtM204I/sW182Stop+sW196Stop) stop codon mutations in surface protein were determined respectively. It led to the conclusion that presence of a single stop codon mutation in the surface protein significantly lowers the level of its production causing lower virion production. While presence of double stop codon mutations in the surface protein, completely abolishes the secretion of surface protein.

The results of replication competence of the rtM191I, rtM204V and rtM204I showed significantly reduced replication phenotype when compared to replication phenotype of wild type *in vitro*.

In this study, we reported for the first time the replication efficiency of HBV harboring stop codon mutations at 182 and 196 amino acids of surface protein. Replication efficiency of such mutation harboring constructs was found to about 85% in comparison with wild type.

To conclude, our study for the first time showed comparison of mutations rtVI19I, rtM204V and rtM204I. They all presented similar replication phenotype. Further, prematurely truncated surface protein produced by mutation rtV191I/sW182Stop caused retention of surface proteins intracellularly and possibly leads to decrease in levels of HBV DNA.