ABSTRACT

Hepatitis B virus (HBV) infection is endemic in Africa. As many as 98% of black Africans are infected during their lives and about 10% (65 million) have chronic HBV infection, which is the cause of 70-80% of all hepatocellular carcinoma (HCC) cases. Despite this high prevalence of HBV and the high incidence of HCC in Africa, relatively few complete HBV genomes from African HCC cases have been deposited in international data bases. In order to gain a clearer understanding of the role of genetic variants and mutants in the development of HCC, the complete genomes of HBV isolated from southern African HCC patients were amplified and molecularly characterized. HBV DNA was extracted from the serum forty HBsAg-positive HCC patients. Twenty six complete genomes were successfully amplified, cloned and sequenced from nine HCC patients.

Phylogenetic analyses of the complete genomes and the individual open reading frames of HBV isolates from the HCC patients, led to the classification of all the isolates within subgenotype A1. No isolates belonging to subgenotype A2 and genotype D were identified, even though these genotypes/subgenotypes have been shown to circulate in South Africa. Three patients contained the uncommon combination of serological subtype *ayw*1 in the subgenotype A1 strain. This combination has been found previously in South Africa and the Phillipines.

Seventy-eight percent of the patients carried HBV strains with the double basic core promoter (BCP) mutation (1762T/1764A), previously shown to reduce HBeAg expression. Furthermore, complete genome sequence analysis has revealed a complex combination of mutations, which include at least three or five of these residues 1753C1762T1764A1766T1768A1809T1812T occurring as the dominant

HBV strains isolated from 5/9 HCC patients. These mutations have previously been shown to regulate gene expression at various levels, to enhance viral replication and simultaneously decrease HBeAg expression.

All five HBV genomes isolated from one patient contained novel complex BCP rearrangements, which introduced 2 HNF1 and 1 putative HNF3 transcription factor binding sites. These mutations can enhance viral replication and simultaneously abolish HBeAg expression at a transcriptional level. Furthermore, truncated core proteins would be expressed from 4/5 isolates and none would express wild-type HBx. Several mutations were identified in the pre-S/S genes of 2/5 isolates, which would result in the expression of novel 3' truncated medium surface proteins ($MHBs^t$) and large surface proteins ($LHBs^t$). The majority of the mutations would contribute to hepatocyte pathogenesis and transformation by activating cell proliferating pathways.

Two patients also contained rare HBV variants not previously identified in HBV strains from southern Africa. These included an HBV splice variant and a poly (dA) variant from patient 10 and patient 6, respectively. These variants occurred in combination with other isolates within the respective patients.

The envelope genes were characterised in a total of 18 HCC patients, the pre-S gene of HBV contained deletions in 72% of the patients. Deletions across pre-S1/pre-S2, pre-S2 initiation codon mutations with internal deletions, and S gene nonsense mutations were prevalent. Mutated envelope proteins have been shown to accumulate within the hepatocyte endoplasmic reticulum (ER) and are a characteristic histopathological hallmark of HCC known as ground glass

hepatocytes. HBV induced ER stress has been shown to dysregulate several cell cycle regulatory pathways, which contribute to HCC.

In addition several novel LHBs^{t} and MHBs^{t} have been described. These potential transactivators require further investigation. The HBV mutations described in this study have been associated with increased risk for HCC.

Despite the obvious heterogeneity HBV displays within and between patients, there are common characteristics shared between the HBV variants which emerge during the development of HCC. These include the BCP and pre-C (1753C1762T1764A1766T1768A1809T1812T) mutations and the pre-S/S mutations. These mutations are able to affect HBV replication and gene expression, and may work synergistically to promote liver dysfunction and HCC.