ANALYSING THE EFFECTS OF HBV SPLICE VARIANTS ON WILDTYPE HBV REPLICATION

Authors: McCoullough L[1,2] & Sozzi V[1], Sadauskas T[1], Mason H[1], Mak KY[2], Littlejohn M[1], Revill P[1]

[1]Victorian Infectious Diseases Reference Laboratory (VIDRL), Royal Melbourne Hospital at the Peter Doherty Institute for Infection and Immunity
[2]Department of Microbiology and Immunology, The University of Melbourne

Background: Chronic hepatitis B virus (HBV) affects over 257 million people worldwide and an infection can lead to liver cirrhosis and hepatocellular carcinoma (HCC). Prior to nuclear export, the pregenomic RNA (pgRNA) can be spliced by the host cell spliceosome to form shorter RNA sequences, known as splice variants. This splicing is not essential for HBV replication; however, their role still remains largely unknown. To date, 21 splice variants have been identified and an increased proportion of splice variants in patient sera has been associated with the development of HCC. Furthermore, different splice variants have been shown to have different effects on wildtype HBV replication. Sp1, Sp10 and Sp13 have been shown to decrease wildtype replication, whereas Sp7 has been shown to increase replication. It remains unknown how other common splice variants, Sp3 and Sp9, affect wildtype replication.

Methods: 1.3mer Sp1, Sp3 and Sp9 DNA clones were co-transfected with a wildtype 1.3mer HBV DNA clone into Huh7 and HepG2 hepatoma cells. WT DNA and RNA production were measured via Southern blotting and northern blotting respectively. Intracellular core, S and X protein production were measured via western blotting and intracellular and secreted E and S antigen were measured via the Elecsys quantitative assay.

Results: Co-transfection of Sp1 with WT DNA resulted in a reduction of intracellular WT DNA. Co-transfection of Sp9 with WT DNA inhibited WT DNA production and reduced pgRNA, core, S and HBeAg production. Sp3 did not affect HBV replication.

Conclusion: Sp1 was found to decrease replication. Sp9 was also found to decrease replication to a greater extent than Sp1. On the other hand, Sp3 had no effect on replication. Taken together, these findings suggest that different splice variants have different effects on wildtype HBV replication, perhaps as a mechanism to promote HBV pathogenesis.

Disclosure of Interest Statement: Professor Revill has received research funding from Gilead Sciences and is on the Scientific Advisory Board of Enochian Biosciences.