Nuttkawee Thongthae 2014: Identification of Effective RNA Interference Target Sites within the Hepatitis B Post-transcriptional Regulatory Element. Master of Science (Biochemistry), Major Field: Biochemistry, Department of Biochemistry. Thesis Advisor: Assistant Professor Nattanan T-Thienprasert, Ph.D. 88 pages.

Hepatitis B virus (HBV) infection causes chronic diseases, including acute liver cirrhosis and hepatocellular carcinoma (HCC). More than two billion people worldwide have been infected, thus effective antiviral drugs or novel treatments are pressingly needed. This study was performed to identify the effective RNAi target sites within the highly conserved region of HBV RNA known as hepatitis B post-transcriptional regulatory element (HBV PRE) by using bioinformatics and experimental approaches. To identify potential siRNA target sites, eleven predicting siRNA targets were employed and the results were clearly demonstrated into nine clusters. Three potential siRNA target sites were selected from the major clusters and called 1317-1337, 1357-1377 and 1644-1664. The features of these selected siRNA target sites were then characterized. The feature analysis revealed that only siRNA target site 1317-1337 did not form actual hairpin structure of any tested genes and it contained the most preferential bases. Based on the results of luciferase assay and real-time PCR analysis, only the siRNA expression plasmids driven by H1 promoter (pShPRE1317-1337, pShH1-1357 and pShH1-1644) showed to have potent inhibitory effects on the expression of luciferase, core, surface and X transcripts. However, the siRNA 1317-1337 had the best inhibitory effect. Therefore, the results suggested that secondary structure and the base preference were the key features for being the effective siRNAs. In addition, only the siRNA 1357-1377 significantly inhibited level of cccDNA. Importantly, all three selected siRNA target sites did not induce the expression of STAT1 and OAS1 and did not cause cell cytotoxicity. Consequently, the study successfully identified effective siRNA target sites that may further develop as anti-viral drugs for inhibiting HBV replication.

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