学 位 論 文 の 要 旨

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学 位 論 文 名　*In Silico* Analysis Predicts That Mir-6770-5p Can Target the X Gene of All Hepatitis B Virus Genotypes
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論 文 内 容 の 要 旨

INTRODUCTION

Hepatitis B virus (HBV) is a DNA virus around 3.2 kb in size that infects the human liver and causes cirrhosis, liver failure, and hepatocellular carcinoma. Of the 240 million people with chronic HBV infection, 75% live in Asia. Approximately 600,000 people die every year due to HBV-related diseases. To date, effective medication against HBV has not been developed.

MicroRNAs (miRNAs) have been explored as a potential therapeutic approach to inhibit the replication of viruses such as hepatitis C virus (HCV), primate foamy virus type 1 (PFV-1), and vesicular stomatitis virus (VSV). Some miRNAs related to HBV have also been discovered, such as miR-192-3p, which can promote HBV replication by inhibiting Akt/mTOR signaling.

MiRNAs comprise short, non-coding RNAs of 20–24 nucleotides that function as gene expression regulators. They are derived from pri-miRNA, which is transcribed from promoter genes and then undergoes an enzymatic reaction to become pre-miRNA. The pre-miRNA is then exported to the cytoplasm by exportin-5 and cleaved by dicer, forming miRNA duplexes that eventually separate into 5p and 3p single mature miRNAs. MiRNAs inhibit human or viral mRNA by repressing translation. MiRNAs can target both the 3' untranslated region (3' UTR) and the coding region of mRNA. DNA methyltransferase 3b (DNMT3b) expression is inhibited by miR-148 through the targeting of its coding sequences.

Mir-3145 is an example of a miRNA candidate targeting influenza A viruses identified through *in silico* analysis whose real effectiveness was confirmed in a laboratory in vitro test. In this study, *in silico* analysis was used to predict the potency of miRNA candidates in targeting HBV mRNA.

MATERIALS AND METHODS

The nucleotide sequence of HBV genotype B (accession number LC456112) was

downloaded from the National Center for Biotechnology Information. In this study, a modified *in silico* analysis protocol adapted from Khongnomnan was used. The HBV sequence was divided into smaller segments of 50 nucleotides, with an overlap of 25 nucleotides between each segment. Each of the small segments was inputted into the miRBase website, which contains 1917 datapoints of human miRNA (https://mirbase.org/search.shtml), using the SSEARCH tool. This tool identified miRNAs that were homologous to the DNA sequence and its complement. However, homology did not always indicate the actual candidate miRNA, which could be the 5p or 3p variant. Whether the miRNA exhibits effective hybridization with the mRNA target or not, RNA Hybrid (https://bibiserv.cebitec.uni-bielefeld.de/rnahybrid) was used to check this indicator. The first essential parameter was the pairing pattern between the miRNA and mRNA. For effective binding, one of three pairing patterns was required: 5' seed, 5' canonical, and 3' complementary. The 5' seed pattern required a perfect match between the 2nd and 8th nucleotides of the miRNA and the target, although another study suggested that a match in the 2nd–7th nucleotides was sufficient. The 5' canonical pairing pattern required nucleotide matches in the 3' part in addition to a 5' seed. The 3' complementary pattern indicated no perfect match in the 5' seed part but required matches of more than half the miRNA at the end of the 3' part.

The second essential requirement was low pairing energy between the miRNA and the target to stabilize the binding. A minimum free energy (mfe) of less than –10 kcal/mol was required to conclude that the miRNA candidate had good potency.

RESULTS AND DISCUSSION

1. Viral nucleotide sequences

The HBV genome is circular, has a length of 3215 bp, and contains four main genes, the surface (S), polymerase (P), core (C), and X genes. The four genes have different lengths, with some overlapping nucleotides and were divided into short segments consisting of 50 nucleotides each. Between these short segments, 25 nucleotides overlapped. They were inputted into the miRBase website containing 1917 datapoints of human miRNA to identify miRNAs that are homologs of the short segments and their complements. To identify homologs or candidates for human miRNAs, alternative software and databases can be used instead of miRBase, including DIANA-microT, PITA, TargetScan, and miRWalk. Multiple tools should be used to obtain a comprehensive list of candidate miRNAs, as each database may have different lists of miRNAs.

2. Thirty-nine miRNAs were homologs of HBV DNA segments and their complements

A total of 39 miRNAs out of the 1917 miRNAs listed on the miRBase website were found to be homologs of HBV genotype B, with 21 homologs corresponding to the DNA sequence and 18 to the complementary sequence. These miRNAs were classified based on the gene types. Of the 39 homologs, five, seven, eight, and 19 homologs were identified for the C, X, S, and P genes, respectively. HBV has some overlapping genes, wherein the P gene completely overlaps

the S gene and some parts of the C gene.

3. Four miRNAs qualified based on the requirements

The effective hybridization of the miRNAs with the mRNA targets was assessed using RNA Hybrid. Both the 3p and 5p versions of the 39 homologs were subjected to RNA Hybrid analysis to assess their pairing patterns and mfe values. Four miRNAs met the abovementioned criteria and were considered potential candidates. Specifically, miR-6793-3p targeted the C gene (2057–2089), while miR-6770-5p, miR-6770-3p, and miR-6888-5p targeted the X gene at positions 1538–1566, 1552–1573, and 1829–1848, respectively. Notably, miR-6770-5p and miR-6770-3p were derived from the same precursor and could target the X gene at nearby locations. All the identified miRNAs had low mfe values, which suggested stable binding. Among the miRNAs, miR-6793-3p, miR-6770-5p, and miR-6888-5p displayed strong pairing patterns, namely 5' canonical 7mer-m8, 8mer, and 7mer-m8, respectively. Although miR-6770-3p had a 5' canonical 6mer, it was still regarded as a potential candidate since it was found to target mRNA.

4. MiR-6770-5p could target all HBV genotypes

The four miRNAs could target HBV genotype B. The DNA target of HBV genotype B was inputted into NCBI BLAST to determine whether other genotypes also had homologous DNA sequences. The other genotypes were found to have varying homologies from 0 to 100% compared to HBV genotype B.

MiR-6770-5p could target all genotypes with remarkable potency based on the pairing pattern of miRNA-mRNA 5' canonical 8mer, which is a strong bond with the lowest mfe. MiR-6770-3p placed second and could target most genotypes except E and F. The pairing pattern was weaker than that of miR-6770-5p, being only 5' canonical 6mer. MiR-6888-5p placed third and could target HBV genotypes A, B, D, F, G, H, and J. It had the effective pairing pattern 5' canonical 7mer-m8 and a low mfe. Some genotypes showed a difference in the 5' seed region in the last five DNA nucleotides which could affect the binding. However, because G can also match with U (wobble pair), no effect was observed. The fourth miR-6793-3p, could only target genotype B due to its long sequence of 33 nucleotides.

CONCLUSION

Bioinformatics was used to predict the potential for miRNAs to target HBV based on the miRBase database. We showed four qualifying miRNAs that can target the HBV gene. MiR-6793-3p can target the C gene, and the three other miRNAs, miR-6770-5p, miR-6770-3p, and miR-6888-5p, can target the X gene. MiR-6770-5p is the best candidate because it can target all HBV genotypes at the X gene, which is essential for efficient virus replication. All candidates can target HBV genotype B, which is common in the Asia-Pacific region. However, in vitro analysis is still required to prove the inhibitory effect of all candidates on HBV.