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Application of Nucleic Acids for Inhibition of Viral Replication

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Nucleic acids provide an enormous potential as a therapeutic reagent against viral diseases. Here I show an attempt to develop two types of therapeutic nucleic acids; oligodeoxyribozyme and RNA aptamer, targeting hepatitis C virus and SARS coronavirus, respectively. A class of antisense oligodeoxyribozymes, known as the 10-23 DNAzyme, has been shown to efficiently cleave target RNA at purine-pyrimidine junctions *in vitro*. I have utilized a strategy to identify accessible cleavage sites for DNAzyme in the target RNA, the hepatitis C virus nonstructural gene 3 (HCV NS3) RNA that encodes viral helicase and protease, from a pool of random DNAzyme library. The screening procedure identified 18 potential cleavage sites in the target RNA. Corresponding DNAzymes were constructed for the selected target sites and were tested for RNA-cleavage *in vitro*. The selected DNAzymes, when transfected to the human hepatoma cells harboring the HCV subgenomic replicon RNA, efficiently inhibited HCV RNA replication in cells by reducing expression of HCV NS3 RNA and protein. Thus, the selected oligonucleotides as well as the selection strategy can be applicable for a new class of anti-HCV drugs as antisense oligonucleotides-based therapy. Severe Acute Respiratory Syndrome (SARS) that caused almost 800 victims requires a development of efficient inhibitor against SARS coronavirus (SCV). We have isolated RNA aptamers against SCV NTPase/Helicase from RNA library containing random sequences of 40 nt using *in vitro* selection technique. Nucleotide sequences of enriched RNA aptamer pool (SE15 RNA) contain AG-rich conserved sequence of 10 ~ 11 nucleotides [AAAGGR(G)GAAG; R, purine base] and/or additional sequence of 5 nucleotides [GAAAG], which mainly reside at the loop region in all the predicted secondary structures. The isolated RNAs were observed to efficiently inhibit double-stranded DNA unwinding activity of the helicase by up to ~85% with an IC_{50} value of 1.2 nM. These results suggest that the pool of selected aptamers might be potentially useful as anti-SCV reagents. **This work was supported by a grant (20080401034026) from BioGreen 21 Program, Rural Development Administration, Republic of Korea.

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