The Novel Design Strategy of DNA-Artificial Nucleic Acid Chimera Toward Enhancement of RNase H Mediated RNA Cleavage Activities: Application for COVID-19 Therapeutics

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Oligonucleotide therapeutics (OTS) strategies, such as antisense and siRNA technologies, are recognized as promising candidates for the next generation of molecularly targeted drugs. However, for the practical use of OTSs, there are some issues to be improved, 1) Inhibition/reducing *off-target effects*, 2) decreasing the dose amounts.[1] To overcome the issues 1) the *off-target effects* issue, we have proposed intracellular condition responsive artificial nucleic acid models, named peptide ribonucleic acids (PRNAs) for cancer and hypoxia cells selective OTSs.[2] Hypoxia cells selective OTSs utilizing PRNAs are received much attention for the safe and secure OTSs.

In this paper, for the decreasing the dose amount issue 2), we focused on the binding process of DNA/RNA hetero-duplex to the RNase H and tried to enhance the dissociation efficiency of cleaved RNA fragments from RNase H complex. Therefore, we designed, proposed, and demonstrated the novel design strategy of DNA-PRNA/peptide nucleic acid (PNA) chimeras for the purpose. The amounts of target RNAs, especially mRNAs, were reported as roughly a sub-μM order in a cell. In contrast, the typical internalize amounts of OTSs were reported as ca. an nM order, then, RNase H mediated catalytic antisense strategy would be required for the efficient therapeutics effects. To increase the turn number, one of the most promising approaches would be enhancing the releasing efficiency of the cleaved RNA fragments from the RNase H complex after the reaction. To achieve the purpose, we tried to cleave a target RNA at the middle position of the binding domain. Thus, we designed DNA-PRNA/PNA chimeras based on the reported crystal structure of DNA•RNA hetero-duplex with RNase H.[3] RNase H is a family of non-sequence-specific endonucleases that cleave RNA in DNA•RNA hetero-duplex *via* a hydrolytic mechanism. According to the crystal structure, the following a couple of the issues pointed out as potentially important. 1) RNase H recognizes negatively charged phosphate backbone of DNA by the positively charged amino acids channel of RNase H. 2) The 5–6 bases of DNA should be required for RNase H binding at the channel. Therefore, in the cases of DNA•RNA complexes with over 5-6 nucleobases are expect to bind and cleaved at multiple sites. Based on the information, we designed DNA-PRNA/PNA chimeras, in which both PRNA/PNA and DNA domains work as recognition sites for the complexation with target RNAs and PRNA moieties work as recognition control devices, while DNA-RNA hybrids formed in the DNA domains of the chimeras should be substrates of RNase H and then target RNAs cleaved by the enzyme (Fig. 1). In the design, the cleavage site of the target RNA should be restricted to the position of the junction site of the chimera, because the junction position between DNA's negative phosphate-furanose backbone and neutral amide backbone of PRNA/PNA should be located the nearby cleavage active site of RNase H. Fortunately, efficient and very enhanced cleavage of target RNAs compared with those with DNAs was observed for PRNA-DNA chimera/RNA complex by RNaseH. Regulation of protein synthesis by PRNA-DNA chimera was also evaluated by *in vitro* cell-free protein synthesis system, and effective regulation was observed.

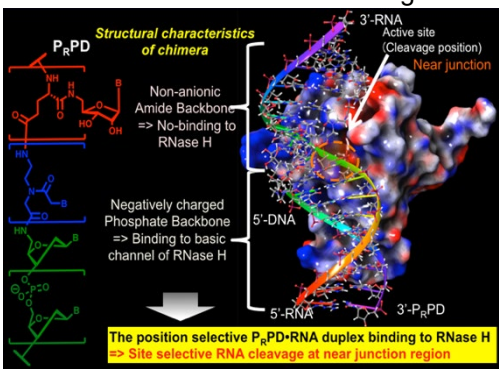

Fig. 1 Structure and DNA-PRNA/PNA chimera toward enhancement of RNase H cleavage activity.

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