【Grant-in-Aid for Scientific Research (S)】

Construction of Artificial Nucleic Acids with Highly Active Catalytic Target RNA Cleavage Ability and Development as a Therapeutic Platform

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Project Information	Project Number : 23H05465　　Project Period (FY) : 2023-2027 Keywords : Oligonucleotide Therapeutics, Catalytic Cleavage, RNase H, Improving Therapeutic Efficacy, Reducing Off-Target Effects

Purpose and Background of the Research

●Outline of the Research

We have proposed and demonstrated the novel design strategy to increase the catalytic turnover numbers of RNase H mediated catalytic target RNA cleavage reaction by chimeric artificial nucleic acids (CANAs), which are 5'-terminus modified phosphate anion backbones oligo-nucleotides, such as DNA, PS-DNA, LNA, and PS-LNA with neutral amide backbones oligo-artificial nucleic acids, such as peptide nucleic acids (PNAs) and peptide ribonucleic acids (PRNAs). In the CANA strategy, the target RNAs would be expected to undergo regioselective cleavage near the junction region between the DNA and the neutral amide backbone artificial nucleic acid induced by the regioselective binding of the DNA anion backbone of the CANAs to the cation channel of RNase H. This fundamental hypothesis of the increasing turnover numbers of catalytic cleavage behavior by CANAs has been demonstrated in various RNase H mediated target RNAs cleavage experiments with CANAs by gel electrophoresis and HPLC analysis results.

In this research, we try to elucidate the RNase H mediated catalytic target RNA cleavage mechanism with our original unique CANA medicines and then optimize the structures and sequences of CANAs to improve their pharmaceutical efficiency.

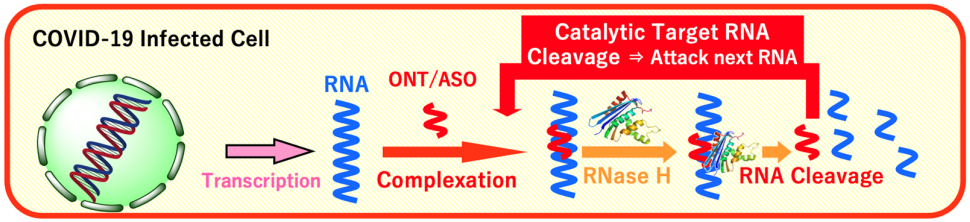

Figure 1. Schematic drawing of RNase H mediated catalytic target RNA cleavage antisense strategy of nucleic acid medicine for COVID-19 treatment.

●Background and Purpose of Research

Oligonucleotide therapeutics/nucleic acids medicines have received much attention as the next-generation modality of molecularly targeted therapy. However, in order to develop the nucleic acids medicines strategy into a general therapeutic modality, decreasing the "off-target effects" and improving the "low therapeutic potency mainly originated by extremely low intracellular concentrations" issues have to be required. In our group, the construction of a novel molecular system using artificial nucleic acids has been developed to improve these issues.

To improve the low therapeutic potency issues, catalytic antisense methods using RNase H have attracted attention. However, since the cytoplasmic RNase H concentration has been reported to be very low and RNase H is a family of non-sequence selective endonucleases, the catalytic turnover numbers of the target RNA cleavage reaction in the cytoplasm have generally been reported to be as low. Therefore, the improvements in the therapeutic efficacy of the RNase H strategy have been limited in some cases.

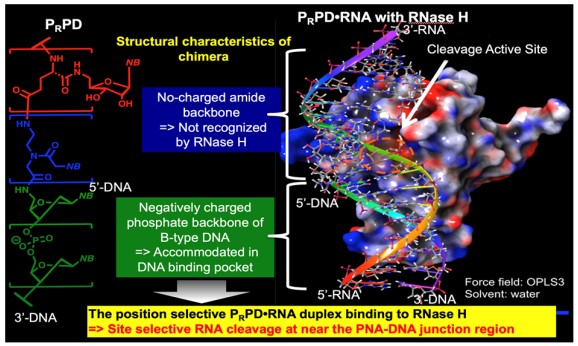

Figure 2 Schematic drawing of CANA/target RNA duplex and RNase H complex

We have proposed and demonstrated the novel design strategy for increasing the catalytic turnover numbers of RNaseH-mediated target functional RNA cleavage reaction by the chimeric artificial nucleic acids (CANA, Fig. 2) which are 5'-terminus modified DNA derivatives conjugated with non-ionic peptide backbone artificial nucleic acids such as PNA and/or Peptide Ribonucleic Acid (PRNA). The efficient RNase H-mediated catalytic target RNA cleavage capability of CANA and improvements in pharmaceutical efficacy have been demonstrated by the gel electrophoresis experiments and suppression of Renilla luciferase protein expression in cell experiments even at low concentrations.

In this study, we will elucidate the site-selective target RNA cleavage mechanism and catalytic turnover enhancement mechanism of the CANA strategy.

Expected Research Achievements

What the research will achieve

- **Elucidation of the RNase H mediated site-selective cleavage mechanism of target RNA by CANA**

  In this research project, we try to elucidate the complex structure of CANA/RNA with RNase H by Cryo-EM, NMR, and X-ray structure analyses and also investigate the site-specific cleavage mechanism and dynamics of target RNA in detail by the FRET method, SPR, and ITC.

- **Elucidation of the mechanism for increasing the turnover number of RNase H mediated target RNA cleavage reaction by CANA**

  In this research project, we try to elucidate the mechanism of catalyst turnover increase in detail through physical chemistry and structural biology investigations and aim to establish the CANA strategy as a general method.

- **Establishment of basic technology for the development of therapeutic treatments for COVID-19 utilizing CANA**

  In the AMED COVID-19 project, we have obtained primitive information on target RNA sequences for COVID-19 treatments. In this study, we try to design new CANA for these target RNA candidate sequences by the rational design strategy based on the basic studies on the mechanisms of site-selective cleavage and the increasing catalytic turnover number of the cleavage reaction and also establish an efficient synthesis method of the CANA.

Proceeding with this research project, we aim to propose and demonstrate an innovative therapeutic platform utilizing the CANA strategy as a general therapeutic method for COVID-19, cancers, and other non-targetable diseases.

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