Social Cooperation Research Program

Project Division of RNA Medical Science
RNA 医科学社会連携研究部門

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RNA no longer stands behind DNA or protein but stands in front of DNA and protein. Recent achievements and discovery in biological science clearly emphasize the importance of RNA in life: the discovery of RNA interference, molecular mimicry between protein and RNA, ribosome structure at atomic resolution, and RNA quality control triggered by aberrant mRNAs. Moreover, the completed human genome project revealed, to our great surprise, the existence of a large amount of protein-noncoding RNAs (ncRNAs). These ncRNAs can be classified into two types: one, like antisense and microRNA, those function with sequence complementarity to the target mRNA or DNA, while the other, like aptamer, those function independent of sequence complementarity. In our laboratory, we aim to create artificial aptamers to target proteins of therapeutic interest.

The concept of using single-stranded nucleic acids (aptamers) as affinity molecules for protein or compound binding was initially described in 1990. The concept is based on the ability of short oligonucleotides to fold, in the presence of a target, into unique three-dimensional (3D) structures that bind the target with high affinity and specificity. Aptamers are generated by a process known as systematic evolution of ligands by exponential enrichment (SELEX), which merges combinatorial chemistry with in vitro evolution from a complex library of randomized $10^{14\text{-}15}$ different sequences. Importantly, aptamer targets can be small (e.g., chemical compounds) or large (e.g., proteins), and simple (e.g., purified proteins) or complex (e.g., protein complexes or cell surface receptors). Therefore, aptamers can be used as therapeutic compounds or reagents for affinity purification or as biosensor elements.

1. Anti-TGF-β1 aptamer enhances therapeutic effect of tyrosine kinase inhibitor, gefitinib, on non-small cell lung cancer in xenograft model.

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Transforming growth factor β (TGF-β) is a multifunctional cytokine that plays crucial pathophysiological roles in various diseases, such as cancer and fibrosis. However, the disease modulation by targeting TGF-β1 isoform remains to be established, regardless of several studies employed with limited antibodies. Here, we developed an RNA aptamer to human active TGF-β1, named APT-β1, and characterized its properties in vitro and in vivo. APT-β1 bound to human and mouse active TGF-β1 proteins with high affinity and specificity and strongly inhibited TGF-β1-induced downstream signaling and cell morphology with 50% inhibition concentration (IC50) values at picomolar concentrations. In a xenograft mouse model of non-small cell lung cancer, APT-β1

alone showed no appreciable effect on tumor growth, while it greatly enhanced the anti-tumor effect of gefitinib, an approved tyrosine kinase inhibitor. These findings strongly suggest that the anti-TGF-β1 medication may be a promising cancer therapy to suppress repopulation of lung cancer in combination with certain anti-cancer drugs, such as gefitinib.

2. Nucleic acid aptamers emerging as modulators of G-protein coupled receptors: Challenge to difficult cell-surface proteins

Masaki Takahashi

G-protein-coupled receptors (GPCRs), among various cell surface proteins, are essential targets in the fields of basic science and drug discovery. The discovery and development of modulators for the receptors have provided deep insights into the mechanism of action of receptors and have led to a new therapeutic option for human diseases. Although various modulators against GPCRs have been developed to date, the identification of new modulators for GPCRs remains a challenge due to several technical problems and limitations. To overcome this situation, a variety of strategies have been developed by several modalities, including nucleic acid aptamers, which are emerging as unique molecules isolated by a repetitive selection process against various types of targets from an enormous combinatorial library. I reviewed and summarized the achievements in the development of aptamers targeting GPCRs, and discussed their isolation methods and the diverse functional features of aptamers against GPCRs.

Publications

1. Takahashi M, Hashimoto Y, Nakamura Y.: Anti-TGF-β1 aptamer enhances therapeutic effect of tyrosine kinase inhibitor, gefitinib, on non-small cell lung cancer in xenograft model." Mol Ther Nucleic Acids. 2022 June, 29; 29; 969-978.
2. Takahashi M.: Nucleic acid aptamers emerging as modulators of G-protein coupled receptors: Challenge to difficult cell-surface proteins. Cells. 2022 Jun, 2;11(11):1825
3. Iwano N, Adachi T, Aoki K, Nakamura Y, Hamada M.: Generative aptamer discovery using RaptGen. Nature Computational Science, 2022 June 2, 378-386.