Pharmaceutical seeds screening, Healthcare

Screening of nucleic acid-binding proteins based on profiling of molecular fingerprints

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Abstract

DNA carries the genetic information of living organisms, and therefore many nucleic acid-binding proteins need to directly access DNA. Hence, genetic deletions and mutations of such nucleic acid-binding proteins can cause various diseases. In this study, we developed a fingerprint-targeting enrichment that enables comprehensive search for proteins that bind to nucleic acids, and searched for proteins that bind to cytosine quadruplexes, a type of non-canonical DNA high-order structure. Characterization of the candidate proteins identified that nucleolin, a nucleolus-composing protein, binds to the cytosine quadruplex and relaxes its structure.

Background & Results

DNA is known to form a variety of non-canonical high-order structures, in addition to the canonical double-stranded helix structure. Non-canonical DNA structures occurring within the genome are involved in the regulation of diverse biological processes, such as transcription and replication. Protein factors that recognize these structures play an essential role in these processes, making their identification an important research topic.

Focusing on the binding specificity of nucleic acid-binding proteins and nucleic acids, we prepared probe DNA molecules that react with proteins in their proximity and introduce chemical modifications into proteins. Presence of the modifications on proteins can be considered as history of proximity to the probe DNA, i.e. a molecular fingerprint, and the proteins with the fingerprint are selectively enriched and analyzed by mass spectrometry. We name this method a fingerprint-targeting enrichment. By applying this method to a cytosine quadruplex (i-motif), one of non-canonical high-order DNA structures, we successfully obtained candidate proteins in addition to known i-motif-binding proteins.

Among the candidates, we focused on nucleolin, a nucleolus-organizing protein, and performed biochemical analysis using recombinant nucleolin. As a result, we found that nucleolin binds to i-motif DNA structures and further functions to relax these structures. We also identified that RNA-binding domains of nucleolin play key roles in this function.

Nucleoli, where nucleolin is localized, contain ribosomal DNA that encodes ribosomal genes responsible for protein synthesis. Ribosomal DNA is known to be a hotspot for the formation of non-canonical DNA high-order structures, suggesting that nucleolin may contribute to the control of ribosomal gene expression through the formation and relaxation of DNA high-order structures.

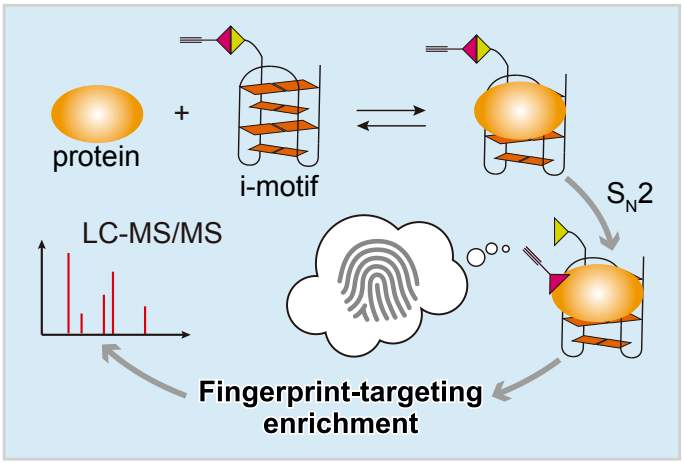

Fig.1 Searching for nucleic-acid binding proteins via fingerprint-targeting enrichment

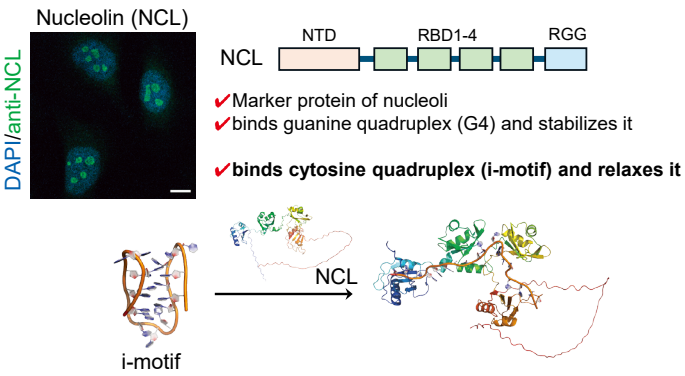

Fig.2 Characteristics of nucleolin newly-identified as an i-motif binding protein

Significance of the research and Future perspective

A major advantage of the “fingerprint-targeting enrichment” method is that it is applicable to a variety of nucleic acids, not only non-canonical DNA. The search for proteins that recognize special DNA structures that may cause disease will lead to the development of new drug discovery seeds. In addition, this method is also expected to be a powerful tool for analyzing the dynamics of nucleic acid drugs and intracellular interacting proteins.

Patent

Treatise Ban, Yuki; Ando, Yuka; Terai, Yuma et al. Profiling of i-motif-binding proteins reveals functional roles of nucleolin in regulation of high-order DNA structures. Nucleic Acids Research. 2024, 52(22), 13530-13543. doi: 10.1093/nar/gkae1001

URL

Keyword functional biological science, chemical biology, proteome analyses, nucleic acid-binding proteins