COMBINING APTAMERS AND IN SILICO INTERACTION STUDIES TO DECIPHER THE FUNCTION OF HYPOTHETICAL PROTEINS

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We present the potential role of aptamers in elucidating the function of hypothetical proteins, as well as the possibilities provided by bioinformatics for establishing a benchmark for aptamer-protein prediction methods. With these future perspectives, the role of hypothetical proteins as target molecules for diagnostics and therapies could prove to be very useful in development of medical technology.

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Introduction

Aptamers are single stranded DNA or RNA or small peptide molecules designed to bind target molecules with high affinity and selectivity. Aptamers have been developed to specifically bind small organic molecules and cellular toxins, viruses, ligands to important proteins enabling biomarker discovery and early treatment of diseases, with the latter subjected to using developed cell lines with defined genetic elements.1 In the recent past, DNA/RNA aptamers have been widely employed as a novel tool for elucidation of protein and/or nucleic acid interactions,2,3 detection applications, regulation of gene expression, as well as purification of target molecules for diagnostics and therapies.4-6 The global market for aptamers is expected to make a turnover in excess of $1200 million showing a vast growth of the market from the $10 million. An overall 120 % Compound Annual Growth Rate (CAGR) suggests that aptamers are emerging and will play a major role in all biotechnological, pharmaceutical and diagnostic applications.7

Discussion

On the technical side, when a protein or other target of interest is presented to an aptamer library, any unbound aptamers are washed away and aptamers that survive multiple bind/wash cycles are enriched. While aptamers undergo in vitro selection process, production of antibodies requires biological systems. In comparison with aptamers, the limitations of antibodies include inadequate supply, high costs and heterogeneity. To produce antibodies, the induction of an immune response is necessary. This procedure may discriminate target proteins that have a similar structure in comparison with endogenous proteins. Moreover, toxic compounds used as antigens or epitopes in a bioconjugated construct may lead to severe systemic effects and be ultimately lethal to the host organism. By isolating aptamers in vitro using chemical modification, they can be easily produced for any target molecule. Moreover, they are known to be stable at elevated temperatures and can be regenerated easily. Due to this, the structural features determined by the functional mers can be retained despite temporary denaturation during experimental procedures. Theoretically, as for antibodies, all proteins are targets for aptamers, making it difficult to predict which aptamers would be better than others. However, several researchers are working on generation of aptamers with high specificity for chosen target proteins.8

While aptamers are inexpensive compared to antibodies, the fundamental science of aptamers needs to mature to identify conditions/applications where they would be most suitable. To find more specific aptamers for a target protein, machine learning methods can help increase the likelihood in determining whether or not an aptamer can recognize the protein with high specificity. A recent report suggests that an improved understanding of the interactions between nucleic acid aptamers and their targets – the molecular recognition properties help improving design of aptamers.9

A pull-down assay uses a small-scale affinity tag to an antibody similar to immunoprecipitation. The affinity system consists of a glutathione S-transferase (GST)-, polyHis- or streptavidin bead which is then immobilized and can be cleaved only by thrombin. In the recent years, single-molecule pull-down (SiMPull) assay was introduced, facilitating probing of single macromolecular complexes directly in cell or tissue extracts.10 In the case of proteins, whose existence, function and even interacting partner have been theoretically (hypothetically) predicted but never experimentally demonstrated, pull-down assays can have a significant role. The use of biological data along with Gene Ontology functional dependencies specific to organelles could be of immense interest for deducing functions of uncharacterized proteins. However, based on the
conventional usage of antibodies, such pull-down assays would be highly expensive, counteracting the feasibility of the required experimentation. Hence, such hypothetical proteins (HP) and their interacting partners remain uncharacterized due to lack of feasible screening methods. Although the methods to identify the functional contexts of activity of the interacting protein have been presented, the necessary experimental boundary to characterize them does not exist. Therefore, we envisage the use of aptamers for pull-down assays or label-free detection to ascertain function of some classes of proteins such as HPs. Application of aptamers in this research area would have immense potentials as only few analytical techniques are known to be capable of detecting minute changes with a sensitivity matching that of antibodies. Targeting whole proteins and selection of specific residual sequences as epitopes is needed for functional characterization of HPs, such as Twinkle helicase, also known as Progressive External Ophthalmoplegia (PEO) in humans, encoded by the gene C10orf2 which is similar to the GP4 helicase structure and an interacting partner of the DNA mismatch repair protein, MLH1.

The ability to predict aptamer binding sites for known proteins, aided by bioinformatics predictions, could allow researchers to develop new diagnostic markers and procedures beyond the traditional medical diagnostics, as well as design new vaccines. Development of such bioinformatics prediction tools could also provide the fundamental basis and standard applicable for elucidation of functions and interacting partners of hypothetical proteins, lessening the scale of needed experimentation. Existing experimental data could be utilized as input for computational methods, to establish a benchmark for aptamer-protein prediction methods. We anticipate that aptamers can make good candidates for use in diagnostics and therefore can be tailored to address the role of hypothetical proteins in therapeutics, drug discovery and clinical applications in the future.

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References


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