Aptamer

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Aptamers are oligonucleotides, such as ribonucleic acid (RNA) and single-strand deoxyribonucleic acid (ssDNA) or peptide molecules that can bind to their targets with high affinity and specificity due to their specific three-dimensional structures.

Concept

The concept of joining nucleic acids with proteins began to emerge in the 1980s from research on human immunodeficiency virus (HIV) and adenovirus. Viruses encode a number of small structured RNAs that bind to viral or cellular proteins with high affinity and specificity . In the case of HIV, a short RNA ligand called the trans-activation response (TAR) element promotes trans-activation and virus replication by binding with the viral Tat protein . The adenovirus also has a short RNA aptamer, virus-associated (VA)-RNA, that regulates translation. A Universal DNA Aptamer that Recognizes Spike Proteins of Diverse SARS-CoV-2 Variants of Concern

Applications: Theranostic

Therapeutics – Neutralization of virus, protein, cancer cells, toxin etc

Diagnostic – Labelled with fluorescence tag.

Advantages of Aptamers Vs Antibodies

1. High stability of aptamers: It is well known that proteins are easily denatured and lose their tertiary structure at high temperatures, while oligonucleotides are more thermally stable and maintain their structures over repeated cycles of denaturation/renaturation. Hence, the greatest advantage of oligonucleotide-based aptamers over protein-based antibodies is their stability at elevated temperatures.

Aptamers recover their native conformation and can bind to targets after re-annealing, whereas antibodies easily undergo irreversible denaturation .

2. Production of aptamers (synthesis/modification):

Identification and production of monoclonal antibodies are laborious and very expensive. Immunoassays are required to confirm the activity of the antibodies in each new batch.

However, aptamers, once selected, can be synthesized in quantity with great accuracy and reproducibility via chemical reactions. These chemical processes are more cost effective than the production of antibodies.

Aptamers can be easily modified by various chemical reactions to increase their stability and nuclease resistance. Additionally, it is possible to introduce signal moieties, such as fluorophores and quenchers, that greatly facilitate the fabrication of biosensors.

3. Low immunogenicity of aptamers

Aptamers -low-immunogenic and low-toxic molecules, because nucleic acids are not typically recognized by the human immune system as foreign agents.

However, antibodies are significantly immunogenic, which precludes repeat dosing.

e.g., The Eyetech Study Group demonstrated that a VEGF-specific aptamer displayed little immunogenicity when given to monkeys in 1,000-fold higher doses.

4. Variety of target:

In instances of toxins or molecules that do not elicit strong immune responses, it is difficult to identify and produce antibodies, but aptamers can be generated in sufficient numbers.

Aptamers show a high affinity and specificity for some ligands that cannot be recognized by antibodies, such as ions or small molecules, indicating that employing aptamers as the recognition components may markedly broaden the applications of the corresponding biosensors.

5. unlike antibodies, aptamers are very sensitive and capable of distinguishing between isoforms of the same target hence they are regarded as good candidates for diagnostics and can be applied in therapeutics.

Selection of Aptamers

In vitro selection process called **Systematic Evolution of Ligands by Exponential enrichment** (SELEX) was first reported by both Gold's group and Szostak's group in 1990.

Due to the development of SELEX, which is now a basic technique for the isolation of aptamers, many aptamers could be directly selected in vitro against various targets, from small biomolecules to proteins and even cells. Short DNA or RNA sequences are selected through the SELEX process

Conventional Genomic SELEX
Affinity chromatography and magnetic bead-based SELEX,
Nitrocellulose membrane filtration-based SELEX,
Capillary electrophoresis-based SELEX

The initial ssDNA or RNA pool is incubated with targets to form aptamer-target complexes. The target-bound aptamers are separated from unbound oligonucleotides and then amplified by PCR (DNA aptamers) or RT-PCR (RNA aptamers). This enriched pool of aptamers is used in the next round of selection. After the final round of selection, the enriched aptamer sequences are identified by cloning and sequencing individual clones.

Aptamers targeting bacteria can be classified into the following two groups:

(a) targeting predefined bacteria cell surface antigens or bacterial virulence factors; and
(b) targeting whole cells with known or unknown molecular targets.

Studies on antibacterial aptamers have been mostly focused on :

Mycobacterium tuberculosis (*M. tb*),
Salmonella,
Listeria monocytogenes (*L. monocytogenes*),
Staphylococcus aureus (*S. aureus*) and
Escherichia coli (*E. coli*)

Aptamers targeting *M.tb* surface lipoglycan, proteins and whole bacterial cells have been generated as potential diagnostic tools. ssDNA aptamer T9, which binds to mannose-capped lipoarabinomannan (ManLAM) from the predominant clinical epidemic *M.tb* strains of the Beijing genotype. ManLAM is the major surface lipoglycan of *M. tb* and has immunomodulatory activity. Aptamer T9 detects ManLAM antigens in serum and sputum samples from patients with active pulmonary TB (aPTB) and patients with extrapulmonary TB (EPTB). The 6 kDa early secreted antigenic target (ESAT6) and 10 kDa culture filtrate protein (CFP10) are secreted early by virulent *M. tb* and are not present in nonvirulent BCG. Rotherham et al. have reported ssDNA aptamers against ESAT6 and CFP10 (CE protein). These aptamers can detect ESAT6 and CFP10 antigens in serum samples from patients with aPTB or EPTB or in sputum samples.
MPT64, which is a 24 kDa protein secreted by *M. tb* during bacterial growth, is also used as a diagnostic target for aptamers

Many scientists chose outer-membrane proteins of Salmonella Typhimurium (S. Typhimurium) as the selection target and generated aptamers 33 and 45.25 .
Aptamer 33 was conjugated to magnetic beads and used to capture S. Typhimurium seeded in whole-carcass chicken rinse samples, followed by detection via quantitative RT-PCR. They reported that 10–100 colony-forming units (CFUs) were successfully detected in 9 mL of the chicken rinsate in a pull-down assay.

HIV accessory proteins include the trans-activator of transcription (Tat) protein, which is important for the transcription of viral RNA and enhances the amount of protein produced by attaching itself to the viral RNA. The Tat protein is one of the most promising candidates for HIV screening because this protein is released in the body at an early stage of infection. Yamamoto *et al*. first generated an RNA aptamer specific for the HIV-1 Tat protein. The aptamer was then used to develop biosensors detecting the Tat protein. Another research conducted by Rahim Ruslinda et al. yielded a split RNA aptamer as the detection probe for Tat.

Although aptamers are promising candidates for diagnostic and therapeutic applications, some **challenges** still remain.

1. **Nuclease degradation**
   Unmodified aptamers (especially RNA aptamers) are generally susceptible to digestion by cellular nucleases present in body fluids or cells. Therefore, chemical modifications are often introduced into the oligonucleotides to increase in vivo stability of aptamers.
   These chemical modifications include capping the 3-end with inverted thymidine/biotin, phosphorothioate/methylphosphonate substitution in the phosphate backbone, 2'-sugar modifications with a fluoro (F), amino (NH2) or O-methyl (OCH3) groups and locked nucleic acid (LNA) modification.
   The LNA modification is an intramolecular 2'-O to 4'-C methylene bridge, and LNAs show high resistance to ribozymes. The most common modifications are a substitution of a sulfur atom for a non-bridging oxygen in the DNA or RNA phosphate backbone as well as the modifications of RNA aptamers at the 2'-position on the ribose ring. Modified RNA

aptamers have half-life ranging from several to hundred hours but unmodified RNA usually has a half-life of seconds in human serum.

2. **Renal filtration**

Because of small mass (6–30 kDa) and short diameter (< 5 nm) of aptamers, they are easily filtered through the glomerular capillaries.

To extend the circulation time of aptamers, they are conjugated to compounds with high molecular mass, e.g., polyethylene glycol (PEG), cholesterol, an antibody, liposome or other nanomaterials.

PEG has been widely been used to prolong circulation half-life of biological agents. Besides half-life extension in the blood circulation, conjugation of PEG can improve drug solubility and stability.

The first aptamer-based drug (Macugen) is based on the PEGylation. This PEGylated aptamer has a half-life of 9.3 h according to the pharmacokinetic experiments performed on rhesus monkeys.

Pegaptanib

The first aptamer therapeutic approved for use in humans (Macugen)

Aptamers are oligonucleotide ligands that are selected for high-affinity binding to molecular targets. **Pegaptanib sodium** (Macugen; Eyetech Pharmaceuticals/Pfizer) is an RNA aptamer directed against vascular endothelial growth factor (VEGF)-165, the VEGF isoform primarily responsible for pathological ocular neovascularization and vascular permeability. Pegaptanib was shown in clinical trials to be effective in treating **choroidal neovascularization associated with age-related macular degeneration**.

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