Aptamer: A New Class of Oligonucleotide for Therapeutic and Diagnostic Use - Review

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Abstract: *Recently, aptamers get the attention of many scientist, because they have all advantages of antibodies which found in human body. They also have unique merits like thermal stability, low cost, and many more. Aptamers are the short sequence nucleic acid with a high affinity and specificity. It is globally adopted as a one of the advance and promising drug delivery technique. These aptamers are produced by in vitro selection method using SELEX technique. This review contains the discussion on the aspect of design, unique properties, applications of aptamers to aid in cancer diagnosis, prevention and treatment under fine condition. Finally, several medical and analytical applications are presented.*

Keywords*: Aptamer, DNA, RNA, Peptide, Antibody, Generation, Application.*

I. Introduction

The term "Aptamer" was named by Andy Ellington. It was derived from the Latin terms "aptus" meaning to fit and "meros" meaning part. Aptamers are short, single stranded DNA or RNA [ssDNA or ssRNA] molecules that can selectively bind to the specific target. Target includes proteins, peptides, carbohydrate, small molecule, toxins and even living cells. Aptamers acquires variety of shapes due to their tendency to form helices and single stranded loops. They are extremely versatile. It gets bind to the target with high selectivity and specificity. Aptameter binding is determined by its tertiary structure rather than primary.[1] Aptamers also termed as "chemical antibodies" because of their artificial process in vitro based on Systemic Evolution of Ligands by Exponential Enrichment (SELEX).[2]

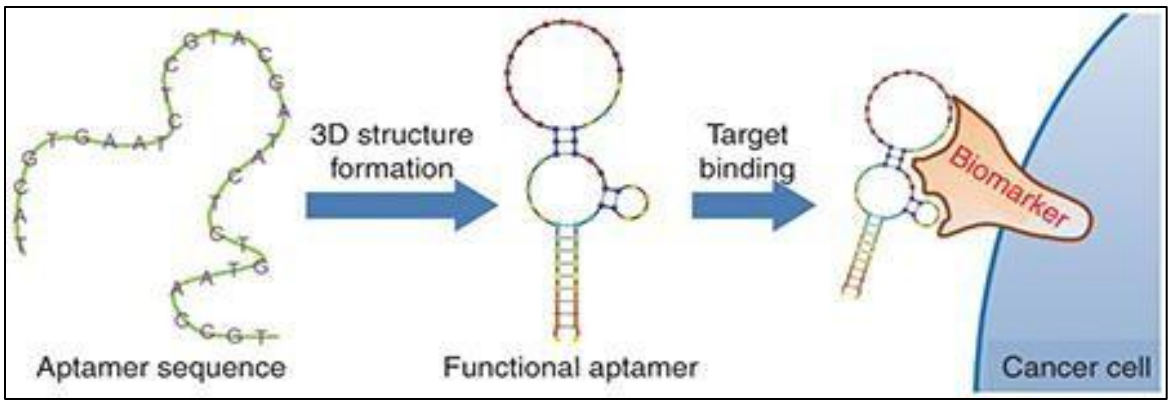


Figure 1: Aptamer binding to its target [3]

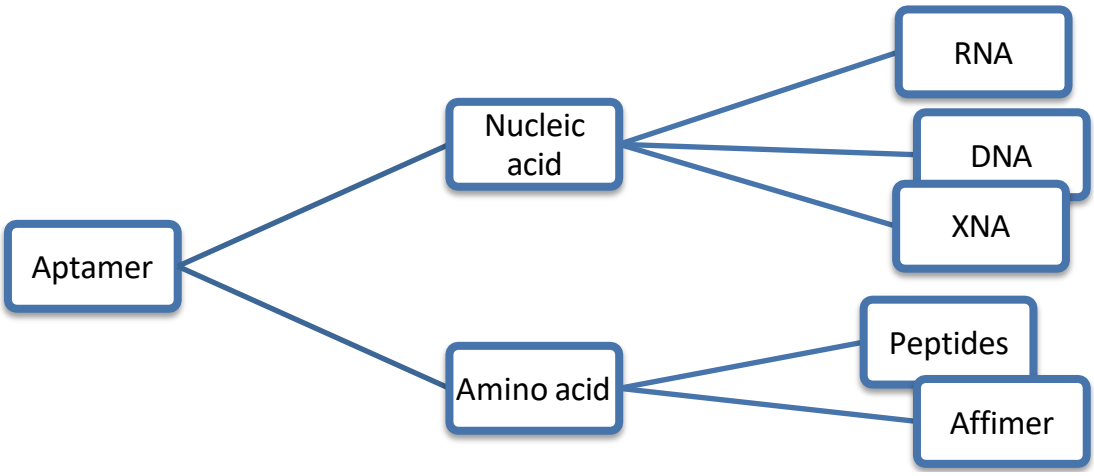
Advantages of Aptamers: [3,4,5,6]
Aptamer has several advantages in order to overcome some difficult scientific challenges.

1. It targets small molecules such as proteins, peptides, dyes and viral particles
2. It peruse toxic and non-immunogenic targets
3. Design stable molecular "sensors" and "switches"
4. They are very efficient to penetrate into tissues and cells.
5. It performs simple chemical modifications
6. Reduce manufacturing time and cost
7. It produces simple product
8. Improve Lot-to-Lot Reproducibility and simplify regulatory procedures

Limitations:[7]

1. Aptamer degradation
2. Aptamer excretion from the bloodstream by renal filtration
3. Control of the duration of action
4. Interaction of aptamer with intracellular targets
5. Generation of aptamer using unpurified target proteins
6. Aptamer cross-reactivity
7. Automation of aptamer generation

Classification of aptamer:[8]
Aptamers are classified based on its structure as follows



Comparison of RNA, DNA, and Peptide aptamer

RNA	DNA	Peptide
Complex secondary and tertiary structure	Complex secondary and tertiary structure	Structure constrains by scaffolds
Diverse 3D structure	Less diverse 3D structure than RNA aptamer	3D structure constrains by scaffolds
Bind target with the entire sequence	Bind target with the entire sequence	Bind targets to the variable region only
Biosensor, Diagnostic, Therapeutics application	Biosensor, Diagnostic, Therapeutics application	Biosensor, Diagnostic, Therapeutics application

Table No. 1: Comparison of RNA, DNA, and Peptide aptamer

Comparison between aptamer and antibody [9]

Properties	Aptamer	Antibodies
Molecular weight	Small (~15-30 kDa)	Relatively big(~150-180)
Secondary structure	Various structures- hairpin, loop, G-quadruplex	B-sheets
Generation time	Few hours to months	Several months (six months)
Batches variation	Low	High
Immunogenicity	Low	High
Minimal target size	Target small sizes~60Da	~600Da
Targets	Wide range of targets	Immunogenic molecules
Shelf-life	Long	Short
Allows chemical modifications	Various modifications	Limited modifications
Nuclease degredation	Sensitive	Resistant
In-vivo half life	Short (~20 min)	Long (~one month)
Stability	Very stable	Sensitive to temp. and pH changes
Cost	Lower	Higher

Table No. 2: Comparison of RNA, DNA, and Peptide aptamer

Generation of aptamers:[10]

In recent years a number of approaches were developed to design aptamer efficiently. This includes the SELEX (Systematic Evolution of Ligands by Exponential Enrichment) approach.
SELEX has different variations such as:

- IP- SELEX
- Capture- SELEX
- CE- SELEX
- AFM-SELEX
- AEGIS-SELEX

Systemic Evolution of Ligands by Exponential Enrichment (SELEX)

In 1990, laboratories independently developed selex [11]. It is developed by Ellington, A.D and Szostak, J.W. It is a standard method of generating aptamers. The conventional selex method mainly consist of three steps: selection, partitioning and amplification (figure.2). Before selection a library of oligonucleotides synthesized. It generally contains up to 10^{15} different unique sequences. Each unique sequence contains random bases (20-50nt) flanked by two conserved primer binding sites, which are used for PCR amplification by annealing primer.

In selection step, library is indicated with target molecule for indicated time. After incubation the unbound sequences are separated from those bound by different method. The target bound sequence is either amplified by PCR (DNA SELEX) or reverse transcription PCR (RNA SELEX). The PCR products, being a new pool utilized for the next round of selection. After several selection rounds, the enriched sequences are sequenced and their binding abilities further evaluated. It takes from weeks to months to obtain specific aptamer candidates, and the hit rates are low. Several modified SELEX methods have been set up for shortening the selection time and enhancing the hit rates.

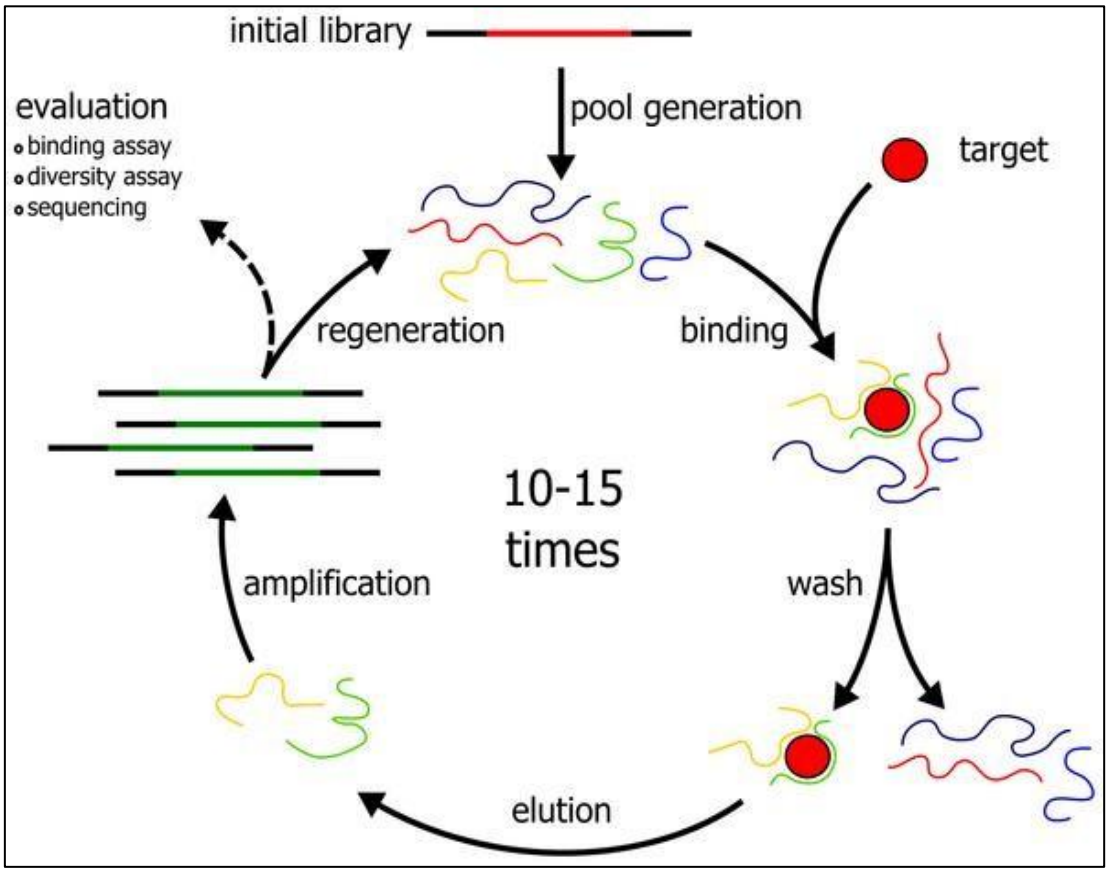
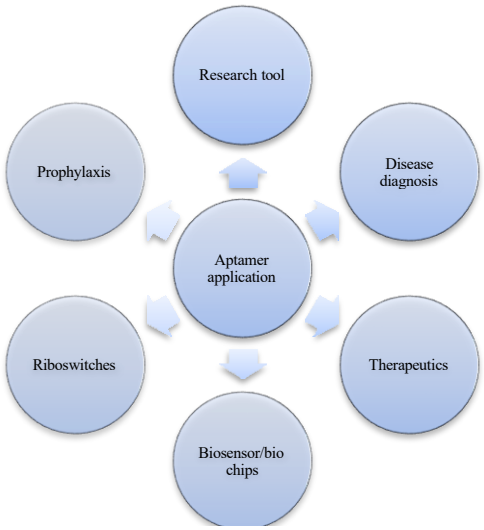


Figure 2: Schematic representation of SELEX method.[12]

Application of aptamer:[13]



The above-mentioned properties of aptamers are used to target ligands and make them ideal tool for diagnostics and therapeutics. Nucleic acid aptamers have wide applications as follows:

1. Aptamers in prophylaxis:

The applicability of aptamer is not only limited for targeted drug delivery to their cognate molecule but also they can function for prophylactic help. These molecules could either be intracellular, extracellular, or cell-surface targets. Nucleic acid aptamers have been projected as convenient molecule to mimics of antibody. It provides basis for developing prophylactic idiotypes in the same ways that of monoclonal antibodies used. Now days Catheter Associated Urinary Tract Infections (CAUTIs) are imposing a big challenge.[14] Proteus mirabilis is a causative agent of CAUTIs. It blocks the flexible tube which connects body cavity. It disallows the fluids to pass into or out it by forming a crystalline biofilm. Therefore, high affinity DNA aptamers were selected using a cell-SELEX in combination with In Silico Maturation (ISM) for the improvement against proteus mirabilissuch as PmA 102, PmA109.

2. Aptamers in Riboswitches:

Riboswitches are the naturally occurring RNA sequences with specific structural recognition of cellular metabolites. They were used to modulate gene expression. The untranslated regions of many mRNA known to act as a sensors of metabolite pool and recognize themselves resulting altered deficiency translation.[15] Such noncoding RNA sequences are naturally occurring aptamers and they are referred to as Riboswitches.[16] Discovery of riboswitches illustrates biological revelance of molecular recognition by natural RNA aptamer.[17] It has been speculated that we should find new aptamers in hitherto unexplored regions of genomes.[18] These structured RNA domains are widespread in bacteria. They help in many fundamental biochemical pathways by sensing the metabolites such as adenine, guanine, FMN, glycine and lysine.[16]

3. *Aptamers in disease diagnosis:*

Aptamers are used in clinical disease due to their small size, stable folding structure, and economy. They get rapid advancement in application of such aptamers in disease diagnosis, imaging and new biomarker discovery. Aptamers are used to detect very low amounts of diseased or tumor cells. For example, immobilized anti-EGFR RNA aptamer on modified glass surface can determine the presence/extent of GBM (glioblastoma) tumor cells.[18] Aptamers can also used in lieu of antibodies in flow cytometry to detect wide variety of cells such as human cancer cells. For example, newly developed CD30 aptamer probe act as antibody free replacement option for the diagnosis of CD30 expressing lymphomas.[19]

The future of the use of aptamer-based probe in noninvasive imaging of many potential clinical applications, such as lesion detection, monitoring of treatment, is becoming increasingly promising and needs for further validation studies to benefit of patient. The principle behind the aptamer-based probe designing is similar to that of developing aptasensor.

Biomarkers plays important and crucial role in diagnosis and treatment of cancer.[20] They can be expressed in different forms such as proteins. It could be soluble or membrane attached. Emerging application of aptamer includes detection of protein biomarker at picomolar concentration. It is also used in biomarker discovery.

4. Aptamers as a biosensors/chips:

Aptamers are used in the biosensors due to the behavior of ligand induced conformational changes. Such aptamers used as a molecular beacon. It is used for the detection of environmental contaminant and monitoring carcinogen or to check level of drugs in blood.[21] Aptamers are used as a fascinating tool for biosensors or biosecurity applications.

Aptamers can be used as chip based biosensor array by immobilizing fluorescent labelled nucleic acid aptamer on a glass slide where they can still rotate that corresponds to their apparent volume and mass.[22] This technique has been proven to be efficient even with complex biological matrices such as human serum or cell extracts.[22]

5. Aptamers as Therapeutics:

Due to their merits as cognate molecules, aptamers are quite attractive for therapeutic application too. As therapeutic agent, RNA aptamer has advantages over classes of RNA tool like short hairpin RNA (shRNA), small interfering RNA (siRNA), ribozyme, or antisense oligonucleotides.[23]

6. Aptamers as a research tool:

Aptamers have wide applicability in pathway elucidation through their effects as a specific inhibitor of intracellular signaling pathway. Example of such inhibitory aptamer is the mitogen activated protein kinase (MAPK) RNA aptamer. [24] MAPK pathway involved in various physiological response such as cell proliferation, apoptosis, and differentiation. Mutations in MAPK lead to inappropriate activation of MAPK signaling cascade and ultimately promote diseased state such as cancer in humans. Inhibitory RNA aptamer has been used as a novel ant cancerous therapeutic tools either alone or in conjugation siRNA.

II. Conclusion and Future Prospects

In this review article, the advantages of aptamer and their application have been presented. An aptamer is a convincing substitute for an antibody because it is very stable under hot and hazard condition. It has been prepared by using SELEX technique with high specificity. The importance of the applicability of aptamers has significantly increased as recent advancement in nanotechnology, microfluidics, microarray, and other technologies for application in clinical field. Aptamers have been proven as multifunctional molecules having tremendous paramedical and medical applications. For detection of small molecules aptamers are utilized pivotally. Recent advancement in aptamer selection and development can potentially deal with targeted drug delivery, targeted inhibition of protein function, and regulation of gene expression. Aptamers also help in cancer detection. Aptamers compete with antibodies to offer promising tools in different applications. Beyond this, there are the different uses of aptamer as drugs, therapeutics, bio-imaging materials, and analytical reagents have been investigated.

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