WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 7, Issue 9, 34-42 Conference Article ISSN 2277– 7105

APTAMER: A MIRACLE TOOL FOR TARGETED DRUG DELIVERY

Asutosh Parhi* and Manoj Panda

University Department of Pharmaceutical Sciences, Utkal University, Vani vihar, Bhubaneswar, Odisha.

Article Received on
18 March 2018,
Revised on 09 April 2018,
Accepted on 29 April 2018,
DOI: 10.20959/wjpr20189-12079

***Corresponding Author**
Asutosh Parhi
University Department of Pharmaceutical Sciences, Utkal University, Vani vihar, Bhubaneswar, Odisha.

INTRODUCTION

Aptamers are oligonucleotides (ss-RNA or DNA) having high affinity and specificity in identifying the target molecules, by folding into 3-D structures. They are identified from an initial library containing 10^{13} - 10^{16} random ssDNA or ssRNA sequences through an *in vitro* selection process termed SELEX (systematic evolution of ligands by exponential enrichment). Aptamers are different from antibodies, Aptamers are having novel properties, such as highly selective and specific target recognition and binding. Aptamers have advantages on antibodies like such as easier and more economical to produce; compared to antibodies, toxicity and low immunogenicity of particular antigens do not interfere with the aptamer selection; having greater specificity and affinity; can easiy be modified chemically to yield improved, custom tailored properties, much more stable at ambient temperature. These properties make aptamers ideal candidates for use in molecular medicine to cure of diseases, particularly cancer and infectious diseases.

Aptamers as therapeutic agents

In current research, aptamers have been effectively used for therapeutic applications, such as cancer cell detection and diagnostics and targeted therapy, as well as sorting and enrichment. Aptamers are theraupeutically used as DNA aptamers for mesenchymal stem cells, porcine endothelial precursor cells and live bacterial cells have also been developed by other research groups during the past years. With their superior targeting performance, incorporation of aptamers with a defined therapeutic function and recognition capability for cancer therapy.

SELEX: aptamer selection process

The aptamers are selected by incubating the target molecule in a large pool of oligonucleotide. Aptamers can distinguish between closely related but non identical members

of a protein family, or between different fuctional or conformational states of the same protein. Aptamers have high specificity. example- small molecule theophylling binds with 10000 fold lower affinity to caffeine, that differs from theophylline by a single methyl group.

IN VITRO SELECTION

- Toxic or poorly immunogenic target.
- Not limited to biological condition.
- Tuneable target specificity.

Range of targets

Small molecules

- Peptides, proteins, and complexes.
- Viruses, cells and tissues.
- Whole organisms.

Different Methods

- Nitrocellulose membrane filtration – Based SELEX.
- Affinity chromatography and Magnetic Bed – based SELEX.
- Capillary electrophoresis – Based SELEX.
- Micro fluidic –Based SELEX.
- Cell – SELEX.

Cell-SELEX: a promising tool to generate clinically useful aptamers

Cell-SELEX is the process whereby live cells are used to select aptamers for target recognition. It is a promising tool to generate clinically useful aptamers. The aptamers that bind preferentially to diseased cells, compared with normal cells for those, cell-SELEX is a promising selection strategy for the development of aptamers able to transports nanomaterials to diseased cells. Live cells of different cancers have been used in this process, and as a result, cell-SELEX was successfully used to develop an aptamer against hepatocellular carcinoma (HCC), one of the most common and highly malignant cancers in the world, found in a human T cell acute lymphoblastic leukemia cell line CEM (used cell-SELEX target). More recently, cell-SELEX was applied to isolate aptamers that recognized acute myeloid leukemia (AML) cells with dissociation constants (K_d) in the nanomolar range.

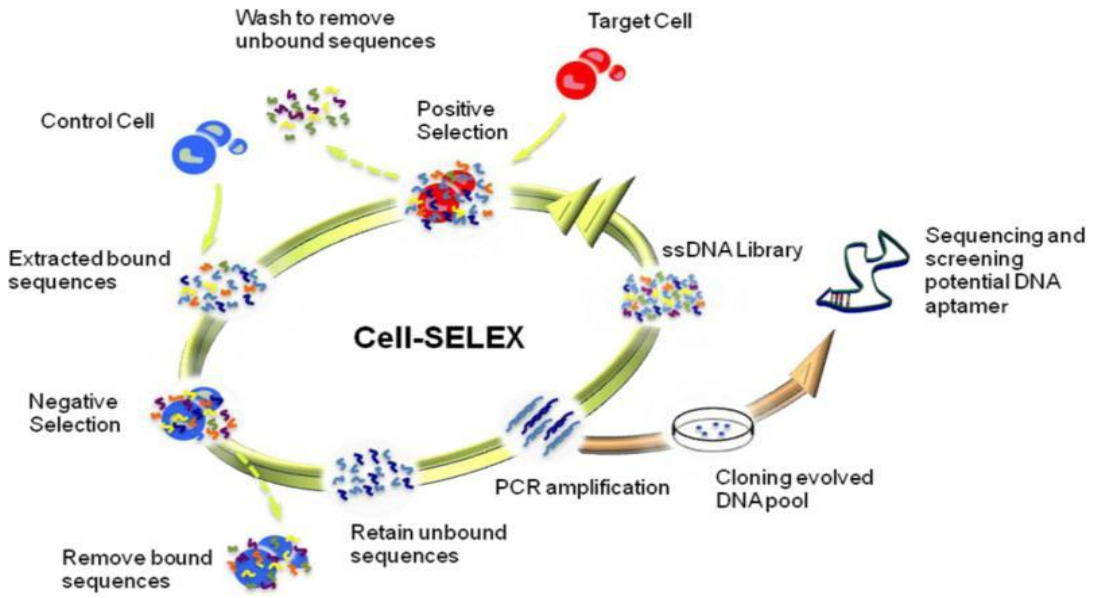

Figure 1: Schematic representation of DNA aptamer selection using the cell-SELEX strategy. DNA sequences that have specific recognition to target cells are evolved to enrich the selection pools.

Aptamers as delivery agents

Aptamers can be designed as targeting ligands, particularly when generated by cell-based SELEX, and can differentiate diseased cells from healthy cells, thus enabling the selective delivery of therapeutic compounds to target cells (Table 1).

Table-1.

Target Name	Aptamer	Selection Technique	Delivery application
Epidermal growth factor receptor(EFGR)	RNA	Purified extracellular domain of EFGR	Nanoparticle delivery
Immunoglobin heavy mu chain	DNA	Cell-SELEX	Micelle nanoparticle for drug delivery
Mucin-1(MUC 1)	DNA	Recombinant peptides	Photodynamic Therapy(PDT) Radionuclide delivery
Prostate-specific membrane antigen(PSMA)	RNA	Purified extracellular domain of PSMA	siRNA delivery, cytotoxin delivery, chemotherapeutic drug delivery
Protein tyrosine kinase-7(PTK-7)	DNA	Cell-SELEX	Chemotherapeutic drug delivery

Aptamers in nanotechnology

The ability of DNA or RNA aptamers to act as targeting agents enables these molecules to be conjugated with therapeutic agents for use in targeted drug delivery. Some cancer cells, especially those in the early stages of disease development, may have a very low density of

target on the cell surface available for detection. With their relatively small size, aptamers have shown promise in specifically targeting tumor cells and transporting small molecules, such as proteins, drugs or siRNA, through the microvasculature or the tumor interstitium. Each type of nanomaterial has different optical, electrochemical, and mechanical properties, medical diagnostic and drug delivery agents with diverse characteristics can be used for different applications, moving aptamer-based nanomedicine closer to reality.

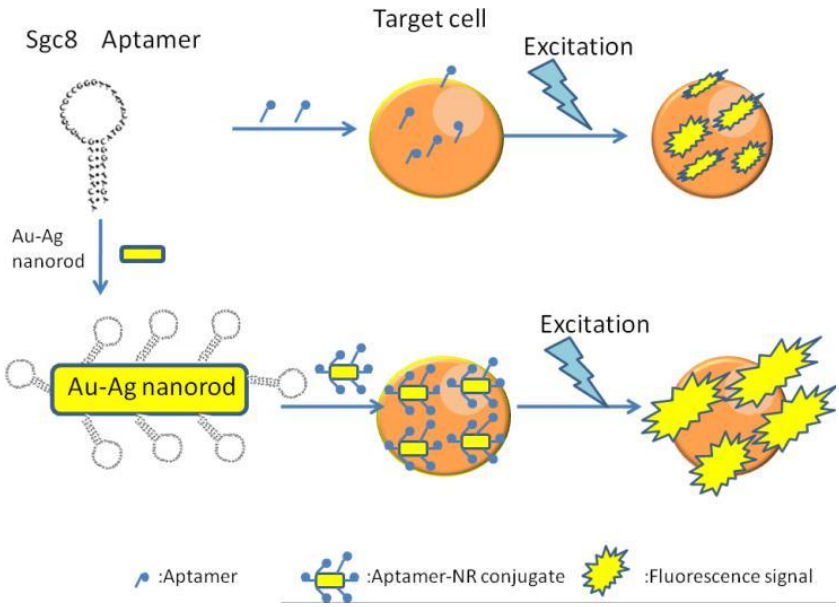

Figure 2: Schematic representation of aptamer-nanorod signal enhancement.

Advanced aptamer–nanomaterial conjugates

Hydrogels are networks of polymer chains that are water-insoluble and superabsorbent, and they possess a degree of flexibility very similar to natural tissue. Target-responsive hydrogels that cross-link DNA aptamers with linear polyacrylamide chains have been fabricated (Figure 3). Competitive binding of the target to the aptamer causes a decrease in cross-linking density and, hence, dissolution of the hydrogel. Therapeutic applications can therefore be devised using small molecules and proteins as the targets. To this end, an *in situ* injectable hydrogel has been functionalized with nucleic acid aptamers to control the release of proteins for human disease treatment. The results showed that the protein release rate can be controlled by adjusting the affinity of the aptamers. Both of these studies, demonstrate that aptamer-based hydrogels provide a highly selective and controllable system, whereby efficient release of therapeutic agents can occur in the specific environment where the target biomarker is found.

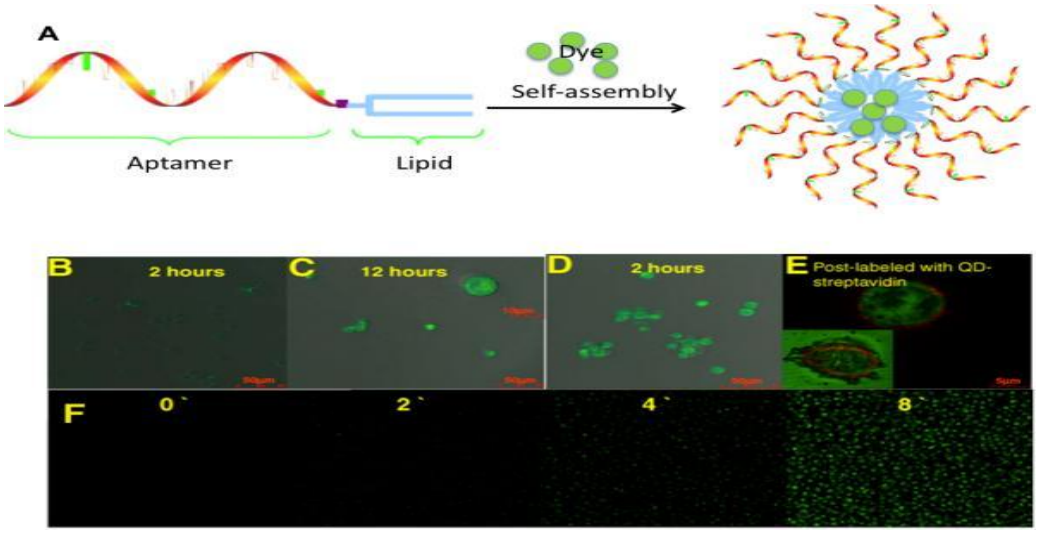

Figure 3: (a) Design scheme of aptamer micelles containing dye. Fluorescent images of Ramos cells for (b) 2 h and (c) 12 h, or (d) incubation with biotin-TDO5-micelle for 2 h. (e) Enlarged fluorescent image after post-labeling the biotinylated TDO5 aptamer with.

In another report, a diacyl lipid tail was incorporated at the 5' end of oligonucleotides by solid-phase DNA synthesis. When dispersed in an aqueous solution, these amphiphilic DNA molecules spontaneously self-assembled into monodispersed micelle structures. In one application of this technology, a self-assembling aptamer–micelle nanostructure was formed of hydrophilic aptamers linked to hydrophobic lipids by poly (ethylene glycol) (PEG). In aqueous solution, these conjugates self-assembled into3D spherical micelle structure with a hydrophilic aptamer targeting Ramos cells (from human Burkitt's lymphoma cell line) on the outside, and the lipid core. The presence of more than one aptamer on the micelle surface provides an approximately 750-fold increase in target binding affinity. The aptamer-micelle assembly is also able to be internalized, indicating it is a promising strategy for clinical applications by increasing therapeutic effectiveness. Furthermore, after two days of incubation with the aptamer-micelle assembly, normal cells maintained over 80% viability.

Another application of aptamers is in photodynamic therapy (PDT). Singlet oxygen ($^1O_2$), one of the most important cytotoxic agents generated during PDT, is gaining wide acceptance as an alternative noninvasive treatment of cancers. The photosensitizer used in PDT, generally a chemical, transfers the light energy to tissue oxygen to generate highly reactive $^1O_2$, which can react aggressively with molecules in the cell, leading to cell damage and ultimately cell death. A novel molecular complex consisting of photosensitizer, a ssDNA aptamer, and single-walled carbon nanotubes (SWNTs) has been engineered for

controllable $^1O_2$ generation (Figure 4). In the absence of a target, the close proximity of the photosensitizer to the SWNT surface causes efficient quenching of $^1O_2$. In the presence of its target, the binding between the aptamer and target molecule disturbs the DNA-SWNT interaction and causes the DNA to detach from the SWNT surface, resulting in a restoration of $^1O_2$ for PDT applications.

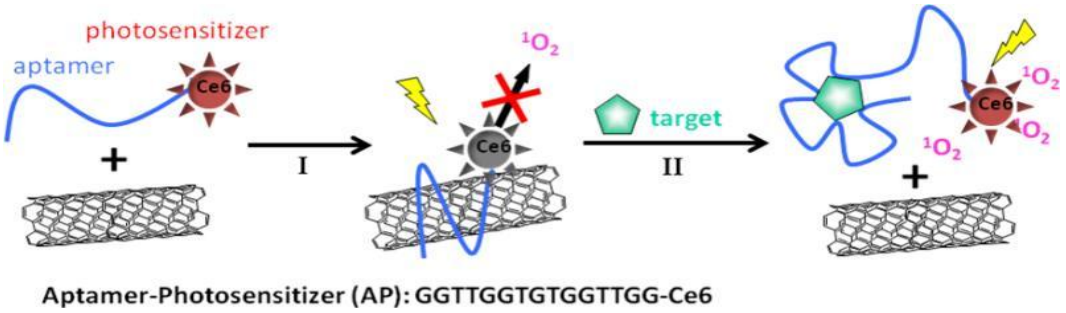

Figure 4: Schematic representation of aptamer-photosensitizer-SWNT complex and the regulation of SOG upon target binding: (I) AP and SWNTs were mixed together to form an AP-SWNT complex. The ssDNA aptamer is wrapped onto the surface of SWNTs, which brings the photosensitizer.

Because most aptamers cannot directly pass through the cell membrane, finding ways to increase membrane permeation has been an active area of research. Aptamer-conjugated, multifunctional liposomes have been fabricated to encapsulate and deliver cisplatin to breast cancer cells (MCF-7). The cell membrane is basically a dynamic lipid bilayer; therefore, this liposome nanostructure can efficiently increase cell permeability and enhance drug delivery. A cell-permeable sgc8-PEG-liposome nanostructure has also been reported for drug delivery. With approximately 250 aptamers on each liposome, this design leads to multiple aptamer-receptor interactions, provides better binding to the target cells, and facilitates translocation through the plasmic membrane. Although liposomes are immobilized on the surface membrane of the CEM cells via aptamer recognition, no binding to the non-target NB4 cells is observed, again demonstrating aptamer specificity. Another advantage of liposomes is their ability to increase the plasma residence time of aptamers from several minutes to 23 h. Here, polymeric nanocarriers have shown the benefit of being able to carry multiple drugs in the same vehicle. This, combined with aptamer-mediated targeting, can be used to selectively deliver dual-drug payloads to cancerous cells with high efficiency and specificity.

The lack of tumor-cell specificity often results in severe toxic effects for cancer patients undergoing traditional chemotherpay. To overcome this problem and to improve the

selectivity of cancer therapy, cytotoxic drugs conjugated to aptamers have been designed for targeted delivery to tumor-specific sites. An antitumor agent doxorubicin (Dox) was covalently linked to the DNA aptamer sgc8c to specifically kill CEM cells, with minimal toxicity towards non-target cells. The results demonstrated that the sgc8c-Dox conjugate possesses many of the properties of the sgc8c aptamer, including high binding affinity (K_d = 2.0 ± 0.2 nM) and efficient internalization by CEM cells. Moreover, the acid-labile linkage between sgc8c and Dox could be cleaved inside acidic endosomal environment. The delivered drug displayed potency similar to that of independent Dox, but with the target specificity lacking in most current drug delivery strategies. Furthermore, the nonspecific uptake of membrane-permeable Dox to non-target cell lines could also be inhibited by linking the drug with the aptamer; thus, it makes the conjugates selective to the cells which express higher amounts of target proteins. Compared to other reported Dox-antibody conjugates, these sgc8c-Dox conjugates make targeted chemotherapy more feasible with drugs having various potencies. When combined with the large number of recently created DNA aptamers that specifically target a wide variety of cancer cells, this drug-aptamer conjugation method has broad implications for targeted drug delivery.

Aptamer-mediated cell-type-specific siRNA delivery

Also known as chemical antibodies, aptamers are poised to surpass natural antibodies in therapeutics, diagnostics, and drug development. The pharmacologic properties of aptamers include wide therapeutic margins, stability, adjustable pharmacokinetics, and very low immunogenicity and toxicity – advantages that are drawing attention from major pharmaceutical companies. It has also been demonstrated that exogenous siRNAs can silence gene expression via the RNAi pathway in mammalian cells. The challenge is learning how to direct those RNA molecules to the target cells and then deliver them through the membrane. With their specific recognition ability, aptamers are ideal candidates for this purpose. For the first time, cell-type–specific delivery of anti-human immunodeficiency virus (anti-HIV) siRNAs through fusion to an anti-gp120 aptamer has been demonstrated. The envelope glycoprotein is expressed on the surface of HIV-1-infected cells, allowing binding and internalization of the aptamer–siRNA chimeric molecules. The chimera is specifically taken up by cells expressing HIV-1 gp120, and the appended siRNA is processed by Dicer; this releases an anti-*tat/rev* siRNA, which, in turn, inhibits HIV replication.

In a further study of this method, an anti-PSMA RNA aptamer (A10) was appended to a 21-mer siRNA portion, resulting in a chimera that targets polo-like kinase 1 (PLK1) and BCL2,

two survival genes overexpressed in most human tumors The aptamer portion of the chimeras selectively binds to PSMA, while the therapeutic siRNA portion interferes and knocks down gene expression and inhibits the xenograft growth activity of the cancer cells both in cell culture and *in vivo*. Because this delivery system consists only of RNA components, it offers several potential advantages as a therapeutic agent, including lack of immunogenicity, the possibility for chemical synthesis, and stabilizing modifications for *in vivo* application.

CONCLUSION AND FUTURE PERSPECTIVES

Aptamers are rapidly maturing into therapeutic tools with commercial potential. Given that aptamers mimic and extend many features of monoclonal antibodies (mAbs), they have the potential to make an impact in molecular medicine. For instance, aptamers that are highly specific to cancer cells can be used as drug targeting agents, thereby reducing toxicity (mice body weigh loss only around 7%) while improving upon the efficacy of current therapeutic drugs (tumor size reduction from 5^{th} day of injection and all the mice suvived in 109 days treatment. Compared to antibodies in the 1990s, aptamers are on an even more accelerated trajectory for commercialization. Using cell-SELEX, aptamer probes capable of recognizing vaccinia virus-infected lung cancer A549 cells have been created. It is therefore possible that a range of antiviral aptamers can be generated easily and that these might show synergistic activity, opening new prospects for antiviral prophylaxis or therapy.

With the improvements in SELEX technology and aptamer functionalities, as illustrated above, we believe that aptamers are poised to successfully compete with mAbs in therapeutics and drug development within the next few decades.

REFERENCES

1. Cerchia L, de Franciscis V. Targeting cancer cells with nucleic acid aptamers. Trends in Biotechnology, 2010; 28(10): 517–525. [PubMed].
2. Keefe AD, Pai S, Ellington A. Aptamers as therapeutics. Nature Reviews Drug Discovery, 2010; 9(7): 537–550. [PubMed].
3. Osborne SE, Matsumura I, Ellington AD. Aptamers as therapeutic and diagnostic reagents: problems and prospects. Current Opinion in Chemical Biology, 1997; 1(1): 5–9. [PubMed].
4. Famulok M, Hartig JS, Mayer G. Functional aptamers and aptazymes in biotechnology, diagnostics, and therapy. Chemical Reviews, 2007; 107(9): 3715–3743. [PubMed].

5. Navani NK, Li YF. Nucleic acid aptamers and enzymes as sensors. Current Opinion in Chemical Biology, 2006; 10(3): 272–281. [PubMed].
6. Jiang YX, et al. Specific aptamer-protein interaction studied by atomic force microscopy. Analytical Chemistry, 2003; 75(9): 2112–2116. [PubMed].
7. Griffin LC, Toole JJ, Leung LL. The discovery and characterization of a novel nucleotide-based thrombin inhibitor. Gene, 1993; 137(1): 25–31. [PubMed].
8. Ferreira CS, et al. DNA aptamers against the MUC1 tumour marker: design of aptamer-antibody sandwich ELISA for the early diagnosis of epithelial tumours. Anal Bioanal Chem, 2008; 390(4): 1039–50. [PubMed].
9. Balogh Z, et al. Selection and versatile application of virus-specific aptamers. FASEB J., 24(11): 4187–95. [PubMed].
10. Shangguan D, et al. Aptamers evolved from live cells as effective molecular probes for cancer study. Proceedings of the National Academy of Sciences of the United States of America, 2006; 103(32): 11838–11843. [PMC free article] [PubMed].