Published in final edited form as:

Curr Med Chem. 2011 ; 18(27): 4117–4125.

Recent Developments in Protein and Cell-Targeted Aptamer Selection and Applications

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Abstract

Because of their easily modified chemical structures and wide range of targets, aptamers are ideal candidates for various applications, such as biomarker discovery, target diagnosis, molecular imaging, and drug delivery. Aptamers are oligonucleotide sequences that can bind to their targets specifically *via* unique three dimensional (3-D) structures. Usually, aptamers are obtained from repeated rounds of *in vitro* or *in vivo* selection termed SELEX (Systematic Evolution of Ligands by EXponential enrichment), which can generate aptamers with high affinity and specificity for many kinds of targets, such as biomedically important proteins and even cancer cells. In this review, some basic principles and recent developments in the design of SELEX process are discussed, hopefully to provide some guidelines towards performing more efficient aptamer isolation procedures. Moreover, the biomedical and bioanalytical applications of aptamers are further reviewed, based on some smart biochemical modifications of these oligonucleotide structures.

Keywords

Aptamer; biomarker discovery; detection; drug delivery; imaging; molecular probe; SELEX; visualization

INTRODUCTION

In the development of medical science, knowledge of the molecular features of an organism becomes increasingly important. For that reason, a great deal of effort in current research focuses on discovering bioactive molecules, thereby leading to a high demand for target-

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specific molecular probes. Aptamers (RNA or ssDNA oligomers), which bind to their targets with high selectivity and sensitivity, are important in meeting this demand. The targets of aptamers vary from cells, proteins and other complex molecules, to drugs and even metal ions [1–4]. Aptamers are generated *via* SELEX, which is short for **S**ystematic **E**volution of **L**igands by **EX**ponential enrichment. Since first described in 1990 [5, 6], this method has become widely developed in many research areas [7, 8]. This review examines the use of SELEX for generating aptamers for biomedically important targets, such as proteins, cells, and others.

Since aptamers and antibodies bind to their targets with high affinity and specificity, as the new-comers, aptamers have sometimes been called “chemical antibodies”. A comparison of these two types of molecules has been summarized in Table 1. Both molecules have their own strengths, while, as the SELEX process is rapidly evolving, the advantages of aptamers over antibodies are becoming increasingly evident [1–4, 9]. For instance, aptamers can be synthesized and modified easily by inexpensive and conventional chemical methods [10–13]. In addition, the long-term stability, the ability to sustain reversible denaturing and the lack of toxic or immunogenic properties [14] are also valuable merits of aptamer molecules. From an engineering point of view, the *in vitro* chemical synthesis of aptamers results in less batch-to-batch variation compared to the *in vivo* process used for production of antibodies [15]. All these considerations are leading to a wider application of aptamers [16, 17].

GENERAL PRINCIPLE OF SELEX

As shown in Fig. (1), SELEX involves iterative cycles of binding, extraction and probe amplification. First, a random library of oligonucleotides is designed and synthesized as a pool of candidates. After incubation with positive targets, the bound oligonucleotides are extracted. (As described further below, if the target is a cell or other complicated target, the extracted sequences will be further incubated with negative target and only the unbound fraction is retained.). Next, the extracted sequences are replicated by enzymatic amplification processes, in preparation for the next round of selection. This sequenced selection process is repeated several rounds, until the desired sequences that bind specifically to the target with high affinity are isolated.

The success of the aptamer isolation process requires some careful considerations in the design of the SELEX procedure. First of all, the initial polynucleotide pools should contain a large number of candidates [18]. Nucleic acid libraries for SELEX procedure always contain 10^{13} to 10^{16} single stranded DNA or RNA (ssDNA or ssRNA) molecules [19], thereby assuring the presence of a range of nucleic acid sequences able to fold into various secondary and tertiary structures, forming the basis for target recognition. Generally, aptamers are composed of a random sequence region (normally 20–80 nucleotides (nt) long), flanked by 18–21nt primer binding sites for polymerase chain reaction (PCR) amplification. Excessively long random regions may cause problems during the amplification step, while in contrast, if the random region is too short, the possible geometry of secondary and tertiary structures will be limited. In some cases, chemically modified oligonucleotide libraries, instead of natural nucleic acids, can be equally, if not more, useful in the SELEX procedure. More potential geometries and new features can be introduced into

the library as a result, enhancing the possibility of isolating the desired oligonucleotide sequences [19, 23–26].

Secondly, after the selection process, aptamers are often truncated to obtain some smaller-sized functional sequences. This step requires some careful thinking and some computer software can be helpful. This post-SELEX procedure improves the performance of aptamers, since the binding affinity as well as target selectivity can be enhanced as a result [20]. Meanwhile, the selected aptamers can be further analyzed to determine their binding motifs and can be post-engineered to enhance their affinity or functionality [21, 22].

Thirdly, some physical parameters during SELEX procedure also affect the aptamer isolation, such as the buffer systems and temperature conditions. For example, during the cell-targeted aptamer selection process, the nucleic acid pools are most-likely incubated with the target cells at the temperatures of 4°C or 37°C. In most cases, 4°C conditions help to maintain the target-binding activity, while 37°C is used to mimic the environment of the human body.

If all parameters were adequately considered, to complete the selection process, at least 12 cycles are generally required [27], but the actual number depends on several other considerations, such as the library used, the target or negative target (if used as well) features and concentration, the efficiency of the amplification method, etc. These features will also influence the affinity and specificity of the selected aptamers. We will discuss below how to optimize these conditions to achieve better selection procedures and improve the performance of generated aptamers; biomedically important targets-based SELEX (proteins or cells), will be discussed in detail for this purpose.

PROTEIN-TARGETED APTAMER SELEX

Although the SELEX process for the selection of specific target-binding aptamers is the repetition of four main steps: binding, partition, elution and PCR amplification, there is no standardized aptamer selection protocol for protein targets. In SELEX, the separation of target-binding species from unbound nucleic acid sequences is the most vital step, and it determines SELEX efficiency and some of the characteristics of the resultant aptamers [28]. This section summarizes the separation methods used with protein targets.

Separation Strategies for Free Protein Targets

When free protein targets are incubated with a nucleic acid library in solution, the sequences are able to access all potential binding sites without interferences from external molecules. After incubation, nitrocellulose membranes and electrophoresis are usually used to separate the bound and unbound sequences.

Due to its facile operation and rapid separation, nitrocellulose membrane filtration is extensively used as the separation method of protein-binding aptamers [6]. The nitrocellulose membrane retains protein-nucleic acid complexes, but not free nucleic acids. The bound nucleic acids are recovered and amplified for the next round of selection. To date, RNA nucleic acid aptamers of more than 42 proteins have been selected with nitrocellulose

membrane filtration [27], but only a few DNA aptamers have been selected by this method, indicating that nitrocellulose membranes may be more suitable to separate RNA than DNA [29]. This method often requires large amounts of target and library in the initial selection, and usually requires up to 12 cycles of selection [27].

Gel electrophoresis is an effective alternative to separate sequences bound to free proteins. In gel electrophoresis, the sample components are separated based on their different migration velocities in an electric field, primarily on the basis of their individual sizes and charges. For example, using this separation method, Smith *et al.* have selected RNA-based irreversible inhibitor of human neutrophil elastase [30]. DNA-aptamers against integration host factor [31] were also selected by gel electrophoresis. In practice, DNA, which is more stable and feasible for large-scale syntheses, is more easily separated by gel electrophoresis. After separation, the DNA from the band of protein-DNA complex is recovered by a “crush-and-soak” method, which accelerates the diffusion of DNA from gel matrix block, for amplification and the next cycle of screening. Selection of aptamers based on such gel separation is time-consuming, laborious and sometimes requires labeling with radioisotopes. Moreover, the loss of DNA during electrophoresis and recovery may result in low enrichment efficiency.

Separation Methods for Immobilized Protein Targets

In comparison with free protein targets, the separation process can be facilitated by immobilizing the protein on a solid matrix. When the nucleic acid library is applied to the protein-modified matrix, the target-binding sequences on the solid support can be efficiently separated from inactive sequences left in solution by washing, attraction to a magnetic field, or centrifugation. Using this strategy, many aptamers have been efficiently selected against various proteins, noncovalently or covalently fixed on titer plates [32, 33], beads [34, 35] or SPR chips [36].

Although separation based on an affinity matrix is an efficient tool for selecting aptamers with high affinity and specificity, there are some drawbacks to this method. First, the immobilization of protein targets on a solid support prevents the nucleic acid sequences from accessing the conjugation side of the targets and may lead to steric hindrances to binding, thereby causing some loss of potential aptamers and decreasing the efficiency of SELEX. Second, the presence of a linker between the target and matrix may act as an artificial positive target and enhance interferences. And finally, nonspecific binding of the library to the matrix may also occur. These drawbacks of the current protein-targeted aptamer SELEX procedures have induced the development of various novel SELEX processes with higher selection efficiency.

Modifications of Protein-SELEX

Usually, generation of aptamers with high binding affinity and specificity requires 8 – 15 cycles of selection. To achieve more efficient enrichment of aptamers, various modified SELEX procedures have been developed, including photo-SELEX, capillary electrophoresis (CE) -SELEX, microfluidic-SELEX and automated-SELEX.

Photo-SELEX is a modified screening method, in which nucleic acid sequences labeled with a photo-reactive chromophore (e.g. 5'-IdU or 5'-BrdU) are incubated with protein targets to form covalent-linked complexes upon irradiation with monochromatic UV light. The resultant protein-nucleic acid complexes are then separated from the free nucleic acid sequences by gel electrophoresis. The formation of a covalent bond between the nucleic acid and protein target allows vigorous washing with denaturants to improve the screening and selectivity. Using this strategy, nucleic acid ligands have been selected against human immunodeficiency virus type 1 [37] and basic fibroblast growth factor [38]. Photo-SELEX could be further used to study the interaction between the aptamer and the protein.

An important development in this area is the incorporation of capillary electrophoresis (CE) in SELEX, and this has led to significant improvements in the partition efficiency [39]. CE separates the bound and unbound sequences based on their different migration velocities (different charge-to-size ratios). With this strategy, high affinity aptamers against immunoglobulin E have been selected with only 2 – 4 cycles, and the affinities of these aptamers are almost the same as those of the aptamers obtained using nitrocellulose membrane filtration, which requires 18 selection cycles. This result indicates that a SELEX procedure could be achieved in a very efficient manner, even with only 2- 4 days, by CE separation. Krylov *et al.* [40,41] further expanded CE-SELEX by developing two models of CE-SELEX (i.e., NECEEM and ECEEM), allowing aptamers with predefined parameters (e.g. K_d) to be selected. As a result of its high partition efficiency, CE-SELEX has been used to select nucleic acid ligands against various protein targets, such as neuropeptide Y [42], HIV-1 reverse transcriptase [43], protein kinase C-delta [44], H4-K16Ac histone protein [45], egg white lysozyme [46] and signal transduction proteins [47]. However, there are still two drawbacks of CE-SELEX: the velocities of the target-sequence complexes and the free sequences must be different, and there can be only *ca.* 10^{13} sequences as aptamer candidates (which could be high as 10^{16} for other methods), due to the limitations of the separation.

Recently, Soh *et al.* [48] have developed a novel, rapid and universal automated aptamer selection method, named microfluidic SELEX (M-SELEX), which integrates magnetic bead and microfluidic chip technology. With this method, a high affinity DNA aptamer (K_d = 33 ± 8 nM) has been selected from *ca.* 10^{14} sequences against the light chain of recombinant Botulinum neurotoxin type A within only one selection round. The high partition efficiency is attributed to two features. First, the embedded ferromagnetic structure within the microchannel precisely controls the local magnetic field gradients, allowing the manipulation of a small number of magnetic beads. Second, the implementation of a multistream, laminar-flow fluidic architecture prevents the unbound DNA sequences from diffusing into the collection port, thereby increasing the partition efficiency. However, similar to most existing methods, M-SELEX has a number of practical drawbacks, such as microbubbles in the flow streams and blockage in the micro-channel caused by magnetic bead aggregates, which impair partition efficiency and aptamer recovery. To address these problems, Soh *et al.* [49] further developed a more robust and facile microfluidic separation device, the micromagnetic separation (MMS) chip, which avoids the bubbles and the blockage in microchannels, and enables high partition efficiencies without any tuning or optimization. Using the improved MMS device, high affinity aptamers against strepavidin

have been selected by three cycles of screening. Moreover, a negative selection was for first time introduced in the microfluidic device to enhance the specificity of the selection.

Conventional SELEX methods are repetitive, time-consuming and unsuitable for high-throughput selection. To overcome these limitations, there have been several efforts to develop automated SELEX systems. Cox and Ellington built the first automated system based on a Beckman Biomek 2000 pipetting system, and they successfully demonstrated the autoselection of anti-lysosome aptamer [50]. But for this system, individually purified protein was needed. To improve automated selection further, they developed a procedure with *in vitro* transcription and translation of individual genes [51]. In another research, an automated selection system was developed based on a RoboAmp 4200E workstation, which was integrated with a fluorescence reader, vacuum pump and thermo cycler [52]. The development of automated selection provides high flexibility and versatility of conditions during the selection procedure, and it paves the way to realize high-throughput screening of aptamers.

CELL-SELEX

The cell-targeted screening method called Cell-SELEX is quite different from Protein-SELEX, in that aptamer selection is against much more complicated targets [19, 53–59]. Compared with Protein-SELEX as described above, use of whole cells for targets ensures that the target molecules (e.g., proteins) will be in their native conformations, which are critical for their biological functions. Furthermore, prior knowledge of the target signature is not needed, and the selected aptamers can be used to identify the cell markers for a particular disease.

The most popular method involves cells raised *in vitro* for selection. Usually cancer cell lines are used as target cells, because of their biomedical importance and rapid and easy growth [60–62]. As shown in Fig. (1), Cell-SELEX usually involves two cell lines: the target cells (positive cells) and a negative cell line. The target cell surface contains a variety of molecules, many of which are also present on non-target cell surface. To exclude the aptamer candidates which bind to these nonspecific surface markers, sequences which bind to target cells are subsequently incubated with non-target (negative) cells, and only the unbound candidates are retained. This phase of the process is called “counter-selection”.

There are no set rules for negative cell line choice, but such choice is important; depending on the choice, different aptamers may be selected. For example, using the cancer cell line as positive cells, many investigators choose a normal cell line from the same source as the negative cells. Thus, the aptamers recognize only the cancer cells, not normal cells. Sometimes, another similar subtype cancer cell line is chosen for counter-selection, in order to obtain an aptamer with high specificity for the positive cell line, but not for similar cell types. Different combinations of positive and negative selection may be carried out to meet the experimental needs. The target molecule may be expressed on the surfaces of both A and B cells, but not on C cell. The best choice of selection combination would use both A cells and B cells for positive selection, with C cells for counter-selection. In that way, aptamer probes can be selected to profile the molecular characteristics of the target cells, which is a

main objective in the development of cell-based aptamers for use in cancer diagnosis and treatment [63].

As the selection proceeds, the affinity and specificity between pools and target cells should be enriched step-by-step, until a plateau is reached. Therefore, binding between pools and cells must be tested every few rounds, commonly by flow cytometry. For example, a fluorescent dye can be included on one side of the primer [64], so that bound sequences will show fluorescence signal enhancement, and the binding action between the target and aptamer may be converted to a measurable fluorescent signal output.

For this review, Cell-SELEX procedures are discussed according to target cell growth pattern: suspension cells and adherent cells. In addition, for some specialized cases, cells must be pretreated before selection, by infection [65, 66] with a virus or bacteria. This type of SELEX may help identify markers for infectious diseases.

Cell-Based Selection of Aptamers Specific to Suspension Cells

For SELEX against suspension cells, which grow singly in the supernatant, it is only necessary to wash the cells with washing buffer, followed by direct incubation with the DNA pool using mild shaking. One of the benefits is that suspension cells do not have to be detached before running flow cytometry [60, 67], which is the test method of choice due to its quantitative nature, high statistical precision, speed and reproducibility. Without treatment with detaching reagents, there is no loss of molecules on the cell surface. Compared to SELEX for adherent cells, there is an additional advantage that suspension cells grow singly in blood, so that the growth state under *in vitro* conditions is very similar to that *in vivo*. Overall, the selection of aptamers is simpler for suspension cells than for adherent cells.

Cell-Based Selection of Aptamers Specific to Adherent Cells

For adherent grown cells, there is an additional step in the selection process. It is generally believed that detaching the cells into single units makes their exposure to the nucleic acid library more complete. The choice of different detaching reagents or methods can also affect the SELEX process, since excess molecules on the cell surface could be removed. For example, Van Simaeys *et al*. [68] were able to obtain an aptamer with stable binding to target ovarian cancer cells with trypsin pretreatment. Because trypsin may digest the proteins on the cell surface, the selected aptamer may more preferably bind to nonprotein molecules on the cell. It is also possible to detach target cells using non-enzymatic buffer to maximize retention of proteins on the cell surface, with loss of only some skeleton connection between cells. In most cases, SELEX is performed in a dish, and the cells are washed before incubation and incubated directly with the DNA pool to keep the membrane intact. Alternatively, adherent cells can be detached in trypsin for only 30s to 1min, followed immediately by incubation in cell culture media [69]. Adherent negative cell lines are normally never detached before binding, to assure that the aptamer candidates are exposed to all markers on the negative cells.

Cell-Based Selection of Aptamers Specific to Infective Cells

Because traditional methods for the detection and identification of pathogenic viruses or bacteria tend to be slow and cumbersome, aptamer probes are being sought for rapid detection of the presence of viral [65, 69] and bacterial infection [70, 71]. Some previous work has involved SELEX of the pathogen itself. For example, Nitsche *et al.* [72] reported aptamers generated for detection of vaccina virus, but they cannot detect infected cells. Infected Cell-SELEX allows selection of aptamers which target proteins specifically expressed on the surface of infected cells, and hence bind to target proteins which may be highly antigenic and structurally unique [73]. The key feature in the SELEX procedure for infective cells is the pre-treatment of target cells before selection [65]. In this variation of cell-SELEX, the positive cells are always the infected cells, while the negative cells are the non-infected cells of the same type.

Cell-Based Aptamer Selection Applied In Vivo

Recently, some researchers have been investigating the SELEX process directly *in vivo*. For example, *in vivo* selection was applied to an animal model of tumor cell metastases [74]. The previously implanted tumor was intravenously injected with the random RNA library. After incubation, live tumors were harvested, the RNA molecules were extracted and amplified, and the process was repeated. The greatest advantage of *in vivo* selection is that the process recognizes the *in situ* context of potential targets. This strategy could have potentially very broad applications in creating reagents that allow for the discovery of aptamers that distinguish tissues of interest and in the creation of reagents that may be useful for target inhibition.

Other Types of SELEX

Aside from proteins and cells, numerous aptamers have been also selected for ions and small molecules (e.g., K^+ [75] and toxins [76]), and other biologically important targets (e.g., viruses [77], bacteria [71]). Some of these targets may not have the corresponding antibodies, but aptamers can be still generated for them. As limited by the space in this review, SELEX targeting other molecules will not be discussed in detail here. We will discuss next the biomedical and bioanalytical applications of these aptamers, which are generated through the above-reviewed SELEX procedure.

VARIOUS APPLICATIONS OF APTAMERS

Currently, investigations at the molecular level are limited by the lack of appropriate target-specific tools, while aptamers have the potential to fill this gap. Aptamers can serve as agents for molecular profiling, be used to modify therapeutic reagents to develop targeted cancer therapies, and aid in the discovery of biomarkers through the recognition of targets. As shown in Fig. (2), many molecular assemblies have been designed to integrate the properties of aptamers with nanomaterials or other devices [63]. The various applications of aptamers are based mainly on their two properties: the wide range of targets and easy and controllable modification.

Aptamers for Biomarker Discovery

Biomarker discovery is a pressing task in molecular investigations. Although 30% of all proteins are associated with cell membranes, less than 5% of them could be recognized by 2D-gel electrophoresis-mass spectrometry (2D-GE-MS) [78], which is a commonly-used analytical tool in biomarker identification. Aptamers generated by SELEX, especially Cell-SELEX, can greatly facilitate the discovery of biomarkers, because the selection procedure does not require pre-understanding of the target signature. Surface markers distinguishing mature from immature cells [57], cancer from normal cells [79], infected from uninfected cells [65, 69] can be identified by aptamer selection. However, most aptamer binding targets are still unidentified membrane structures (membrane proteins and other biomarkers), and further investigations are needed to learn the details of binding targets. For example, the target of aptamer sgc8 was found to be protein tyrosine kinase 7 (PTK7) [79], which has extremely high expression on CEM cells (human T cell lymphoblast-like cell line). With further developments in binding elucidation, biomarker discovery based on aptamer SELEX will be improved and will lead to more biomarker discovery in the future.

Aptamers for Molecular Imaging

Molecular imaging is essential for disease detection, monitoring and pathogenesis characterization, all of which increase the range of treatment options [80]. By including fluorophores [81, 82], radiolabels [83] or other devices [84], novel methods for molecular imaging have been developed based on selected aptamers. Depending on the specific binding between aptamers and their targets, whole cells can be imaged conveniently *in vitro*. For example, aptamers generated by Cell-SELEX bind to the cell surface [85], making it possible to image the outlines of the target cells. Aptamers, such as sgc8 [86], which can be internalized through the cell surface, will contribute to the molecular image of the nucleus (Fig. 3). Instead of using fluorescence signals, imaging can be taken a step further by conjugating aptamers to magnetic beads. The aptamer serves as the cross-linker between the cells and the magnetic beads, which act as contrast agents for enhanced magnetic resonance imaging (MRI) [84].

Aptamers can also be applied for *in vivo* imaging. Shi *et al.* [82] used the Cy5 fluorescent dye conjugated to TD05 aptamers, which were generated against Ramos cells (B cell lymphoma), to obtain an *in vivo* tumor image in live mice. After intravenous injection of Cy5-TD05 into the Ramos tumor-bearing mice, whole-body fluorescence imaging then allowed the spatial and temporal distribution to be directly monitored noninvasively. In these *in vivo* studies, the aptamers have showed exceptional biostability and excellent specificity and sensitivity, while some modification of nucleic acid structures will still be necessary to ensure sufficient *in vivo* biostability.

Aptamers for Diagnosis

Since SELEX can be performed with whole cells and many receptor proteins exist on the cell membrane surfaces, aptamers are also gaining attention as molecular probes, and have demonstrated their potential in many diagnostic and therapeutic applications, especially for cancer [19, 53–59] and infective diseases [65, 69]. Aptamers aid in distinguishing molecular-level differentiation of diseases cells for accurate and early diagnosis [87].

Cancer is a group of diseases which originate from mutations and alterations at the genetic level. The early detection of cancer is a key factor in treatment and long-term survival. It is unfortunate that almost all of the traditional detection methods, such as computed tomography (CT), MRI and positron emission tomography with radio-labeled 2-fluoro-deoxy-glucose (FDG PET), cannot meet that goal. But aptamers, which can discriminate the molecular characteristics of cancer, can be of great benefit in early diagnosis [88]. Based on their high recognition specificity and high affinity, aptamers can help distinguish different types, and even different subtypes, of cancer cells [89] and can detect the cancer cells at relatively low levels, leading to early and sensitive diagnosis. Because aptamers have the advantage of predictable structures and easy site-specific chemical modifications, they can be further modified for binding and signaling enhancement. Medley *et al.* [90] developed a colorimetric assay for the direct detection of diseased cells using aptamer-conjugated gold nanoparticles (ACGNP). In another study, the addition of fluorophores at either 5' or 3' ends improved the cancer visual detection [91]. Pu *et al.* are also trying to use this method to capture circulating tumor cells (CTCs), aiming to achieve both early cancer diagnosis and treatment.

SELEX for the cell model of a particular pathogenic infection, or the pathogen itself, or the toxin released by the pathogen after infection will provide valuable molecular tools for infective disease investigation. Based on the changes occurring on the cell membrane after infection, it is possible to select different kinds of aptamers for particular infective live cells [69]. Parekh *et al.* [65] built a cell model for vaccina virus-infection, and by using Cell-SELEX, they were able to extract a map of the differences between the infected and uninfected living cells. Isolation of aptamers directed against virus-encoded surface proteins allowed the design of a molecular probe for vaccina virus infection diagnosis.

Aptamers for Drug Delivery

The conjugation of antitumor chemicals to targeting reagents, such as aptamers, can increase the efficacy of chemotherapy and reduce its overall toxicity. However, for aptamer-based intracellular delivery, there is the problem that aptamers may bind to the cell surface and not be endocytosed by the target cells. To overcome this difficulty, researchers have incorporated aptamers into different delivery vector systems, such as the liposome vesicles [92]. There is also a small number of aptamers which can be directly taken up by cells, such as sgc8 by leukemia cells [86] and anti-PSMA (prostate-specific membrane antigen) aptamers [93–95]. Colocalization of internalized sgc8 and transferrin indicated that the aptamer was taken up by the endosome without cytotoxicity (Fig. 3). In one application, the antitumor drug doxorubicin (Dox) was linked covalently to sgc8 aptamers, which maintained their high binding affinity and capability for internalization into the endosomes of target cells [96]. Although cell viability testing showed no obvious difference between the conjugated group and unconjugated group, nonspecific uptake of membrane-permeable Dox to non-target cell lines could also be inhibited by linking the drug with the aptamer. In short, the drug-aptamer conjugation method will have broad implications for targeted drug delivery. Furthermore, there is a group of aptamers which bind with their target cells with cytotoxicity, such as AS1411 [97]. In that case, without any extra modification, the aptamer itself can act as an anti-cancer drug.

CONCLUSIONS

This review has provided an overview of the development of aptamers by SELEX; the technologies, advantages, and applications of aptamers were also discussed. In short, SELEX provides an effective approach for generation of aptamer probes that specifically target a variety of biomarkers. These aptamers have wide applications in many areas of biomarker discovery, molecular imaging, diagnosis of various kinds of diseases, and drug delivery.

The aptamer panels generated by SELEX show the high selectivity required for recognition of different kinds of targets, and the ease of site-specific chemical modification makes it possible to conjugate aptamers to many reagents, which improve the capability of the aptamer itself. However, even though the past 20 years have witnessed some rapid development of aptamer techniques, mature technology based on aptamers for real application and commercial use is still far away. Only a limited number of aptamers has been isolated. Furthermore the SELEX procedure is not routine and is both tedious and inefficient. Developing a low-cost and easy-to-manage aptamer system is still difficult. Further research work on the combination of aptamers and microchips or microfluidic devices will allow multiple detection capability; meanwhile, engineering some regulable or multifunctional aptamer machines may also be a useful research direction [98].

As therapeutic agents as well, several aptamers are currently being evaluated in clinical trials. In particular, the drug “Macugen” has already been approved by the FDA [99] in a 3-year study. However, so far only a few aptamers have been adapted for practical applications (Table 2). Most aptamers were selected and studied in buffer system, which leads to shortcomings in issues such as biostability and cell internalization efficiency. The bioavailability of aptamers could be improved through some chemical modifications, commonly employed methods including the use of non-natural nucleotides, macromolecules or delivery vehicles [98]. These modifications are important to enhance aptamer biostability and target binding affinity for some *in vivo* applications.

In the future, there will be a continuing need for new aptamers to meet a variety of demands. For example, the clinical possibility of using aptamers as drugs and diagnostic tools will be demonstrated. The potential applications of aptamers in some forefront areas, such as personalized medicine, disease diagnosis or drug screening, will be smartly designed by aptamer researchers as well, aiming to develop a major technology for future biomedical applications.

Acknowledgments

The authors would like to thank many of our co-workers whose work is reported here. We also acknowledge Dr. Kathryn R. Williams for manuscript review and Interdisciplinary Center for Biotechnology Research (ICBR) at the University of Florida for technical support. This work has been supported by the National Key Scientific Program of China (2011CB911000) and China National Grand Program (2009ZX10004-312), and by the Healthy Department of Hunan Province Grants (D2010-007), Science and Technology Committee of Hunan Province Program (2010SK3098) as well as by the grants from the National Institutes of Health (GM066137, GM079359 and CA133086).

Biographies

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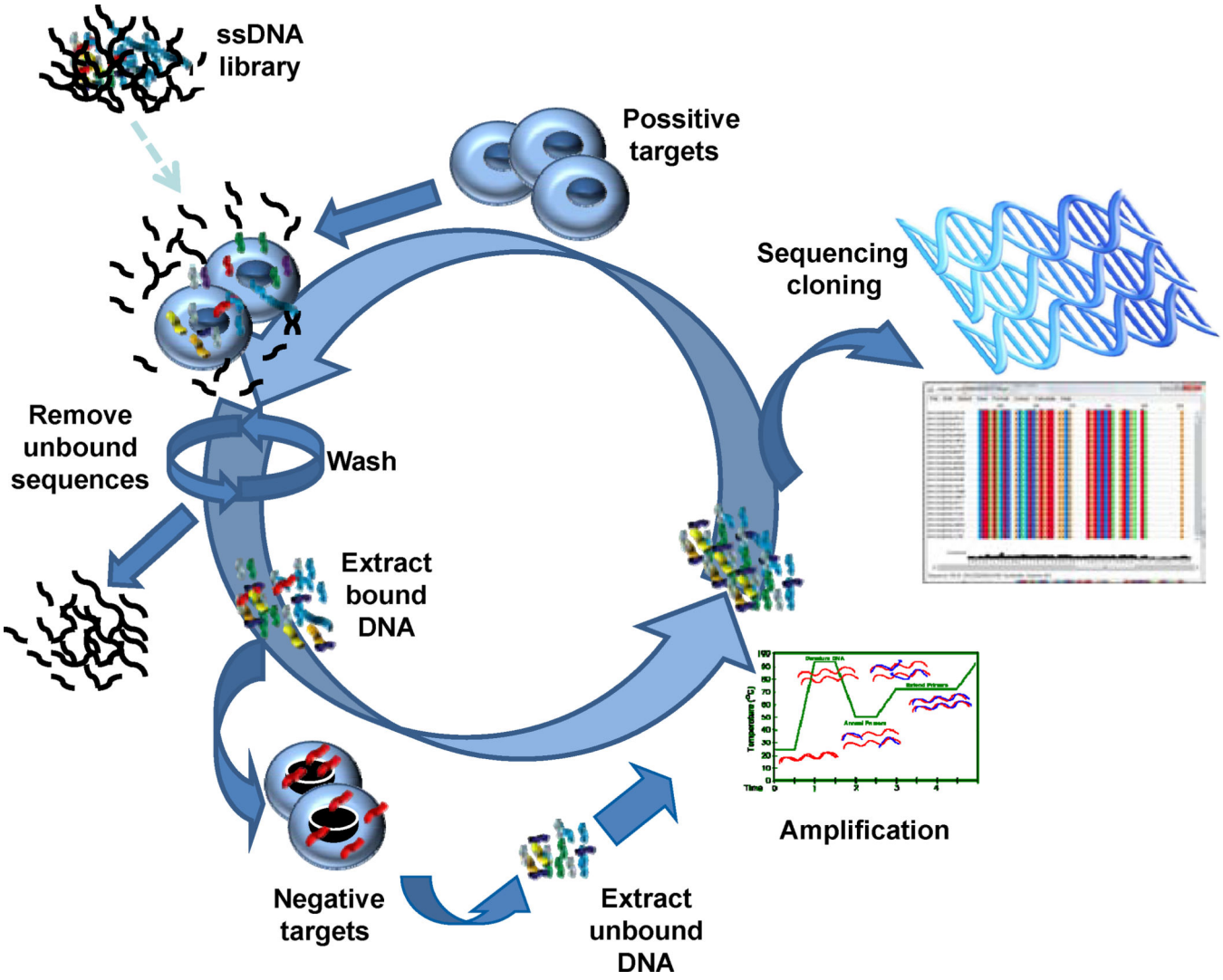

Fig 1.
Schematic Representation of the SELEX Process.

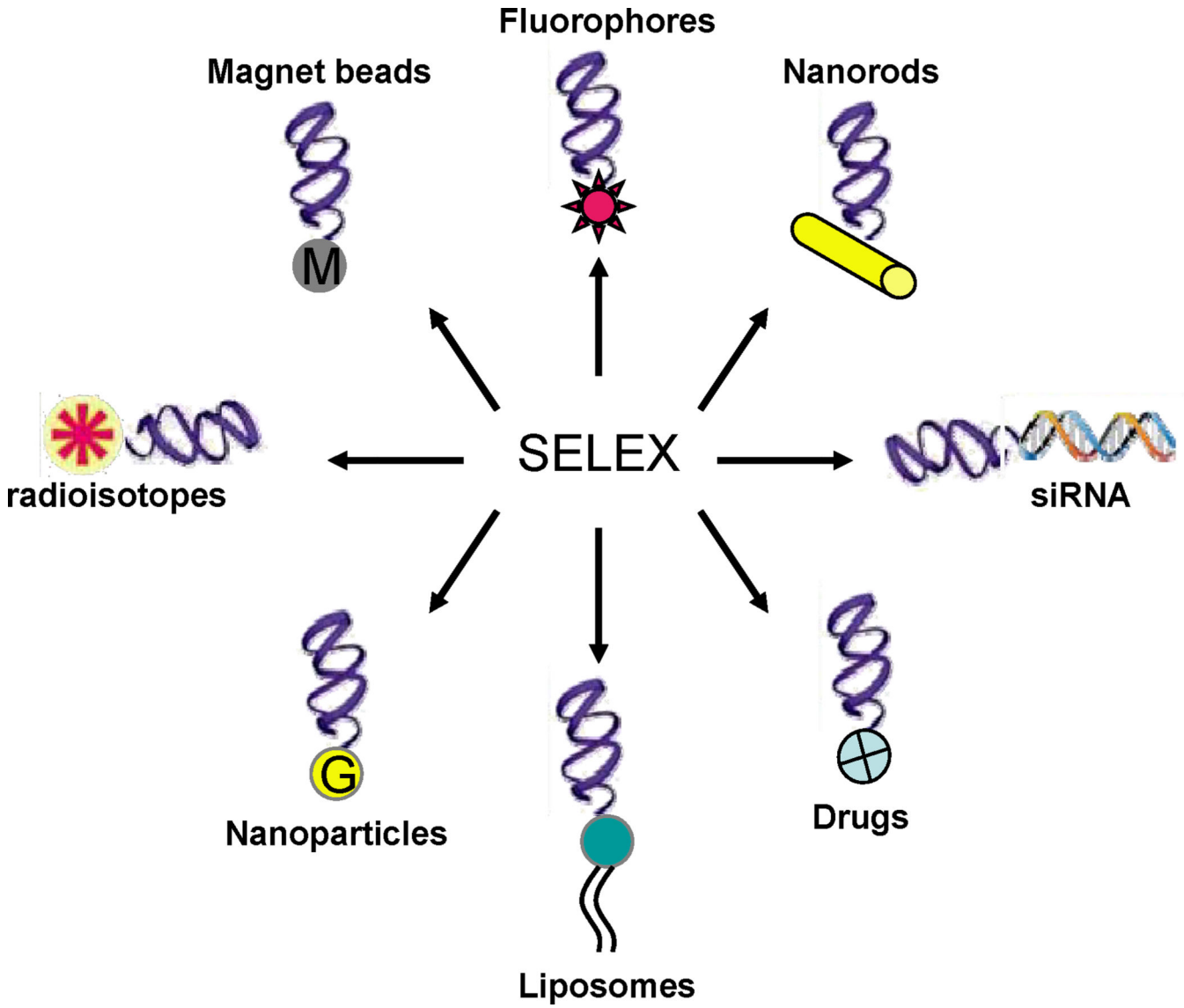

Fig 2.
Conjugation of aptamers for different applications.

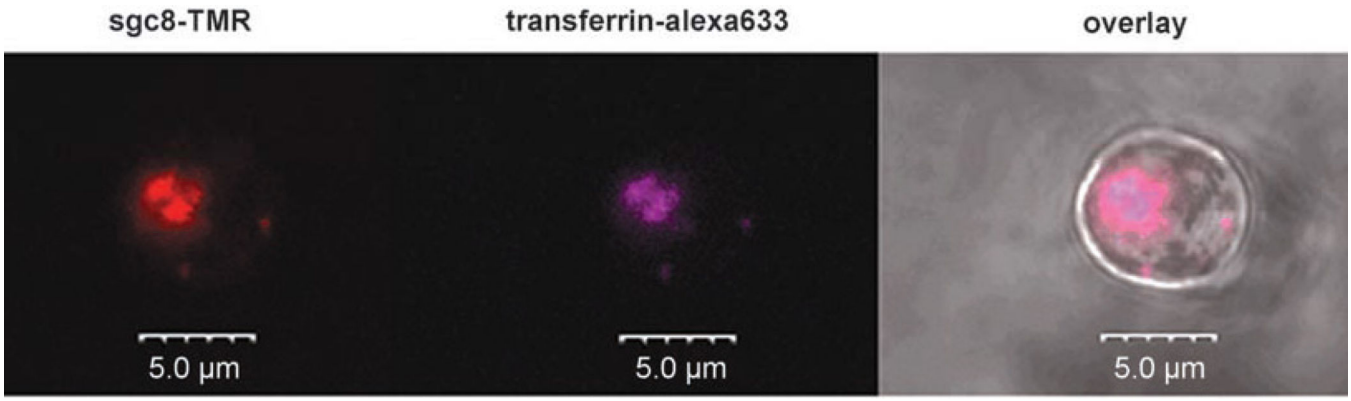

Fig 3. Sgc8 located inside the endosome (Confocal Picture) [86]

Sgc8 aptamer was labeled with TAMRA dye. Overlay of the fluorescence channel (left two) and bright field channel (right one) confirm that sgc8 was colocalized with alexa633-labeled transferrin, a commonly used endosome location indicator.

Table 1

Comparison of the Features of Aptamers and Antibodies

Features	Aptamers	Antibodies
Production	<8 weeks (*in vitro, low cost*)	> 10 weeks (*in vivo, high cost*)
Selection conditions	Versatile	Physiological conditions
Target range	Various	Proteins
*Immunogenic*ity *and toxicity*	None observed	Immune reactions observed
Molecular weight	Small (*many can pass through cell membranes smartly*)	Huge (*can not pass through cell membranes or the blood-brain barrier*)
Batch to batch variation	No	Yes
Convenient chemical modification	Yes	No
Inhibitory potential	High	Low, 1 out of 200
Stability	Stable (*powder stored for several years*)	Labile (*cold storage, only a few months to a year*)
Binding affinity	High	High

Table 2

Aptamers in the Clinical Pipeline

Aptamer Candidate	Target/Indication	Developer	Clinical phase	Type
Macugen (Pegaptanib)	VEGF/AMD	Eyetech(Cedar Knolls, JN, USA)	FDA approved	RNA
Edifoligide (E2F decoy)	E2F/CABG surgery	Anesiva (formerly Corgentech, San Francisco, CA, USA)	No better than placebo in Phase III	RNA
Avrina (NF-kB decoy)	NF-kB/eczema	Anesvia (formerly Corgentech, San Francisco, CA, USA)	Phase III	ds DNA
AS 1411	Nucleolin/anticancer (AML, renal cell carcinoma)	Antisoma (London, UK)	Phase II	DNA
REG1	FIX/arterial thrombosis	Regado Biosciences (Durham, NC, USA)	Phase II	RNA
REG2	FIX/venous thrombosis	Regado Biosciences (Durham, NC, USA)	Phase I	RNA
ARC 1779 (TMA/TTP)	Vwf/TMA, TTP, CEA	Archemix (Cambridge, MA, USA)	Phase II	DNA/RNA
ARC 183	Thrombin/anticoagulation	Archemix (Cambridge, MA, USA)	Phase I completed, not in development	DNA
NU172 (ARC2172)	Thrombin/anticoagulation (PCI, CABG)	Nuvelo/Archemix (San Carlos, CA/Cambridge, MA, USA)	Commence a Phase II study	DNA
E10030	PDGF-B/wet AMD	Ophthotech (Princeton, NJ, USA)	Phase I	DNA
ARC 1905	C5/wet and dry AMD	Ophthotech (Princeton, NJ, USA)	Phase I	RNA

All the above information is collected from the references [99–105].