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Mini review

Recent developments in cell-SELEX technology for aptamer selection

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ABSTRACT

Background: SELEX technique is employed to select aptamers against wide range of targets. The *in vitro* method of aptamer selection using live cells as the target is referred as cell-SELEX.
Scope of the review: The review provides a comprehensive description on the development of aptamers through various cell-SELEX methods and list of aptamers identified through this method. In addition, it pinpoints the advantages and limitations of the cell-SELEX process and its variants.
Major conclusions: The use of aptamers as therapeutic and diagnostic agents is rapidly evolving, selection techniques such as Cell-SELEX could be beneficial in identifying aptamers when the target is in its native conformation and without prior information of the cognate target, thereby bringing the aptamer development one step closer to the clinic.
General significance: The information in this review can serve as a database for the design and development of futuristic oligonucleotide based diagnostics and therapeutics work.

1. Introduction

Aptamers are single stranded oligonucleotides that fold into tertiary structures which allow them to bind with high affinity and high selectivity to the cognate target molecules. Compared with antibodies, aptamers have a unique repertoire of advantages, including, for example, ease of chemical synthesis, small size, low molecular weight, non-immunogenic, ease of modification and conjugation to nanoparticles for therapeutic, diagnostic and imaging purpose [1–5]. Aptamers are selected by a universal process referred as "Systematic Evolution of Ligands by EXponential Enrichment" (SELEX) method developed by Tuerk and Ellington groups separately [6, 7]. SELEX is an iterative process that involves repetitive cycles of binding, partitioning and amplification steps. The SELEX process starts with a combinatorial library of synthetic oligonucleotide consisting of 10^{16} sequences each containing a random region flanked by two known sequence regions to facilitate the primer binding. The library is incubated with a target molecule and if required, can be tagged with a fluorophore, or radiophore for monitoring the process. The partitioning step involves separation of the aptamer sequences that bound to the target from the non-specific sequences. Since partitioning is a critical step in the SELEX process, different strategies have been adopted to simplify the step or enhance the efficiency of the partition. A variety of partitioning techniques such as affinity chromatography, membrane filtration and centrifugation have been adopted to separate the aptamer that binds to the target [8]. Target bound oligonucleotides are eluted and amplified by PCR (DNA SELEX) or reverse transcription (RT)- PCR (RNA SELEX). The resulting double stranded DNA is transformed into new oligonucleotide library, which undergoes multiple cycles of selection and amplification process generating sequences with high binding affinity and specificity.

SELEX is a well-established and widely used technique for selection of high affinity aptamers against the target. Through SELEX, aptamers can be practically selected against any targets including small molecules, proteins, bacteria and viruses, cell lines and even whole cells [3, 9–14]. Over the years, SELEX has evolved and has undergone numerous modifications and improvements that have made this technology faster, more robust and cost-effective. Different SELEX methods have been developed by researchers to suit their needs and make the entire selection process more efficient. Few of these methods are capillary electrophoresis – SELEX (CE-SELEX), rapid-SELEX, microfluidic SELEX and cell-SELEX [13–17]. Except cell-SELEX, all the aforementioned SELEX methods are based on knowledge of the target for aptamer selection and most aptamers identified against the purified protein targets failed to recognize the same protein in its native conformation [14, 17]. It is necessary to maintain a uniform, naturally folded state of the protein to obtain high-affinity aptamers. In order to select aptamers in more physiological conditions, SELEX has been performed directly against purified plasma membranes [18]. For the first time, Morris et al. reported the SELEX could be performed against membranes of human red blood cells (RBC ghosts) instead of purified proteins as long as the target remains unchanged during the *in vitro* selection process [18] (Table 2). After 25 rounds of selection using nitrocellulose filter

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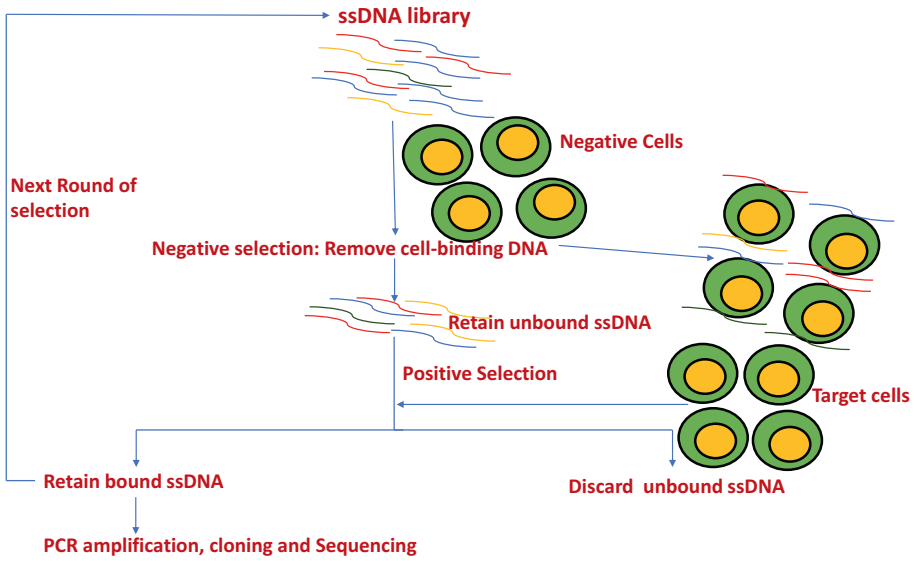

Fig. 1. Schematic Representation of Cell-SELEX technology.

binding, 41 high affinity aptamer sequences for RBCs were obtained. Photoaffinity cross-linking experiments of the obtained sequences demonstrated that pool contained different sequences that recognize different RBC membrane proteins. This work opened the path to the development of whole living cell-SELEX method.

2. Cell-SELEX

Identifying aptamers for biomolecular targets such as proteins, receptors and molecular biomarkers on the cell surface in their native conformation has emerged as a potentially valuable tool for the development of effective probes specific for diseases. The method used to identify aptamers using live cells is commonly known as cell-SELEX [19] (Fig. 1). The first cell-SELEX experiment was performed using a live pathogenic organism, African trypanosomes Trypanosoma brucei [20]. The results from this work demonstrated identification of three classes of high affinity RNA aptamers that are specific for the infective bloodstream life cycle stage of the parasite. The aptamers were selected against the flagellar pocket of the parasite and none of the identified aptamers bind to VSG protein which is one of the most abundant polypeptide on the trypanosoma surface.

2.1. Advantages of Cell-SELEX

1) There are numerous molecules found on the cell surface which could be a potential target in cell-SELEX. Therefore, the generation of panel of aptamers probes through cell-SELEX could be beneficial for accurate disease diagnosis and creating new opportunities for personalized medicine. Daniel et al. successfully isolated the pool of high specificity DNA aptamers against glioblastoma-derived cell line U251 as the target using Cell-SELEX which was followed by identification and characterization of tenascin-C, an extracellular protein found in tumor matrix that aptamer binds [14]. However, the selected aptamer did not demonstrate high affinity at physiological temperature and was prone to nuclease attack. In another study, a nuclease stabilized modified DNA tenascin-C aptamer (K_d = 5 nM at physiological temperature) was selected through tumor cell-SELEX method. The aptamer binds to the fibrinogen-like domain of tenascin-C protein and has potential to be used for exploring the role of tenascin-C protein in tissue remodelling processes [21].

2) Unlike the traditional protein-SELEX where prior exhaustive knowledge of protein target and high purity recombinant protein is necessary prior to selection of aptamers, there is no need to have the prior knowledge of the biomarkers for cell-SELEX method and protein purification is also not necessary. Cell-SELEX technology provides the opportunity to identify aptamers against the known biomarkers and the new biomarkers on the cell membrane surface. In cell-SELEX, a successful selection generates aptamers against the unknown biomarkers. Once the aptamers have been identified, they can be partitioned and purified to identify the target which could be the potential new biomarkers [22].

Using cell-SELEX, high affinity aptamers have been identified against the viable B-cell Burkitt's lymphoma cell line (Ramos cells) and followed by characterization and identification of the target protein for the selected aptamer using mass spectrometry [23]. Berezovski et al. developed a technology referred as “aptamer-facilitated biomarker discovery” (AptaBiD) for cells which was result of the limitation of the conventional methods in biomarker discovery that are prone to false positive and false negative results [24]. The authors used the technique to decipher unknown surface biomarkers on live mature and immature dendritic cells. The AptaBiD method involves three steps: 1) Multiround selection of aptamers to biomarkers of different cells, 2) Isolation of biomarkers from target cells based on their binding to aptamer pool, 3) Identification of biomarkers by means of mass spectrometry method. The application of AptaBiD method is not limited to live cells and can be used for fixed cells, tissue samples and even cell lysates. This cell based aptamer strategy could be useful in discovering novel biomarkers with therapeutic and diagnostic potential.

3) In contrast to other conventional SELEX methods, in cell-SELEX the target molecule is in its own native conformation which makes the development of the identified aptamer for therapeutic and diagnostic applications easier. It also eases the complexity of dealing with target molecule conformation and increases the likelihood of finding an effective aptamer. In addition, since the target molecule is anchored to the cell surface in cell-SELEX, the purification of the target or to fix the target molecule on solid support is not necessary.

Due to wide array of advantages, cell-SELEX has widely been used to obtain highly specific aptamers against targets including cancer cells, tumor associated proteins, parasites, virus infected cells and whole cells [14, 25–28].

2.2. Challenges with cell-SELEX

The advantages in cell-based selection methods allow for the identification of hundreds of aptamers against various important therapeutic and diagnostic targets [29, 30]. However, the cell-SELEX is a