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Ligand-guided selection of aptamers against T-cell Receptor-cluster of differentiation 3 (TCR-CD3) expressed on Jurkat.E6 cells

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ABSTRACT

We recently introduced a screening technology termed ligand-guided selection, (LIGS), to selectively identify target-specific aptamers from an evolved cell-SELEX library. Cell-SELEX utilizes a large combinatorial single-stranded oligonucleotide library and progressively selects DNA ligands against whole cells with variable DNA-binding affinities and specificities by repeated rounds of partition and amplification. LIGS exploits the partition step and introduces a secondary, pre-existing high-affinity monoclonal antibody (mAb) ligand to outcompete and elute specific aptamers towards the binding target of the antibody, not the cell. Here, using anti-CD3ε mAb against the cluster of differentiation 3 (CD3ε), as the guiding ligand against one of the domains of the T-cell Receptor (TCR) complex expressed on Jurkat.E6 cells, we discovered three specific aptamers against TCR complex expressed on an immortalized line of human T lymphocyte cells. In sum, we demonstrate that specific aptamers can be identified utilizing an antibody against a single domain of a multidomain protein complex in their endogenous state with neither post- nor pre-SELEX protein manipulation.

Introduction

DNA aptamers are small synthetic nucleic acid strands that specifically bind to a target molecule with high affinity [1,2]. The method of aptamer selection known as SELEX (Systematic Evolution of Ligands by Exponential enrichment) was originally introduced by two independent groups [1,2]. SELEX screens short single-stranded oligonucleotides against a variety of target ligands via an iterative and evolutionary process of continuous enrichment to identify target-specific binders. A typical SELEX library is vastly heterogeneous with a large number of distinct nucleic acid molecules (~approximately 10^{13} molecules). Each molecule folds into a unique secondary structure, which leads to a distinct geometrical shape. Depending on shape complementarity and noncovalent electrostatic or hydrophobic interactions, a few nucleic acid sequences can specifically bind to the desired target. Subsequently, bound sequences are separated and amplified using Polymerase Chain Reaction (PCR) to generate an evolved library. The process is repeated until high-affinity binders are enriched, resulting in a homogeneous library with high-affinity nucleic acid aptamers against the target of interest.

SELEX has resulted in generating a significant number of aptamers against targets ranging from small molecules to whole cells; however, translational applications of aptamers have been limited [3]. Therefore, steps to improve SELEX have been introduced, for example, cell-SELEX, which was introduced as a method to select aptamers against membrane receptors in their endogenous state [4]. In addition, a bead-based selection method has been introduced to increase selection diversity aimed at generating therapeutic aptamers [5]. To expand target specificity, "internalizing cell-SELEX technology" and hybrid-SELEX have also been introduced. Hybrid-SELEX incorporates the enrichment of the SELEX library against the purified protein target first, followed by cell-SELEX, utilizing cells that express the same protein, while cell-internalization-SELEX is designed to select aptamers towards RNA molecules capable of internalizing into cells [6,7,18]. To increase the clinical practicality of aptamer selection, development of methods to identify aptamers

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able to specifically recognize predetermined epitopes in their endogenous state with no prior- or post SELEX sample manipulations on receptor proteins would be most desirable.

To address this, we recently developed a method called Ligand-guided Selection, (LIGS), which selects aptamers that specifically bind a predetermined epitope expressed on the target cell surface [8]. LIGS takes advantage of the partition step in cell-based SELEX and introduces a secondary, pre-existing high-affinity ligand, in effect a monoclonal antibody (mAb), to outcompete and elute specific aptamers binding to the receptor target of the antibody, not the cell. Conventional SELEX is designed to winnow out low-affinity binders through a competitive process whereby high-affinity binders move on by repeated rounds of partition and amplification through the selection process. We hypothesized that the addition of a secondary stronger specific ligand in excess against a specific epitope of interest will selectively outcompete specific aptamers competing to bind to the same epitope or a related epitope from an evolved pool pre-incubated with the whole cell. Therefore, at a partial enrichment stage in the cell-SELEX iterative process, LIGS interrupts the process and exploits this competitive selection by introducing a stronger, known high-affinity ligand against a specific protein receptor (epitope) target of interest in order to 1) directly outcompete and replace aptamers specific towards the target of interest and, more importantly in terms of LIGS, 2) elute aptamers resulting from conformational changes induced through the interaction of the secondary ligand with its target epitope at its endogenous state. Therefore, based on the specificity of a natural pre-existing ligand towards its target and the conformational changes induced through antibody-protein receptor binding, the aptamers identified by LIGS are expected to show higher specificity towards the target protein or a protein co-expressed with the target protein compared to those aptamers evolved as binders through cell-SELEX (Scheme 1).

Here, we utilized LIGS to identify aptamers against CD3ε expressed on Jurkat.E6 cells from a partially enriched SELEX library. Using high-affinity anti-CD3 antibody against a specific epitope on the CD3ε chain as the secondary ligand, we successfully identified three specific aptamers against CD3ε, one of the domains of the T-cell Receptor (TCR) complex expressed on T lymphocytes. CD3ε is one of the ectodomains of the TCR complex expressed on T-cells. The TCR complex is a multidomain, transmembrane protein consisting of a αβ heterodimer and both CD3εγ and CD3εδ ectodomains. The main αβ heterodimer consists of a variable and constant domain, while the CD3ε domain is conserved and non-glycosylated, making CD3ε an attractive target for aptamer development [9].

Methods and materials

Cell lines, Jurkat.E6 (T lymphocyte) and Ramos (Burkitt's lymphoma), HL60 (Myeloid Leukemia) were generously provided by David Scheinberg and Morgan Huse labs, Memorial Sloan Kettering Cancer Center. All cells were cultured in RPMI 1640 medium supplemented with 100 units/mL penicillin–streptomycin and 10% fetal bovine serum (heat-inactivated; Invitrogen). Cell lines were validated by flow cytometric assays utilizing antibodies against surface markers uniquely expressed on each cell line.

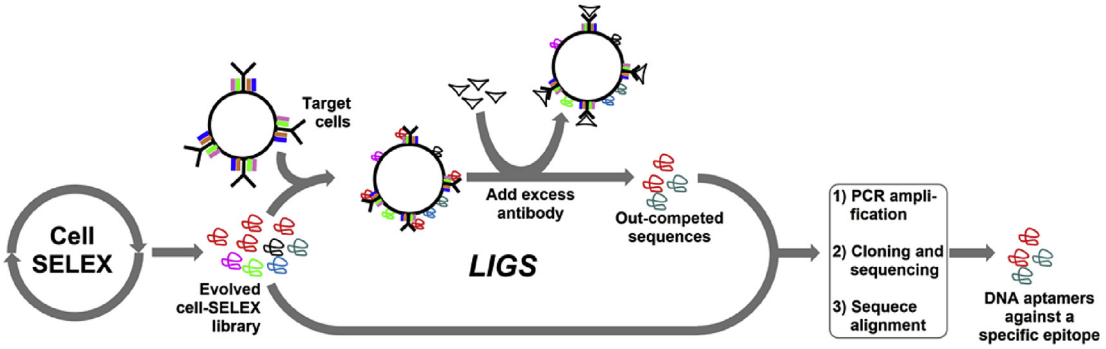
Buffer compositions

Washing buffer was composed of 1× DPBS containing 4.5 g Glucose/1 L and 5 mL of 1 M $MgCl_2$/1 L. DNA Binding Buffer (DB) was composed of 1× DPBS containing 4.5 g Glucose/1 L, 5 mL of 1 M $MgCl_2$/1 L, and 100 mg/1 L tRNA. Cell Suspension Buffer (CSB) was composed of 1× DPBS containing 4.5 g Glucose/1 L, 5 mL of 1 M $MgCl_2$/1 L, 100 mg/1 L tRNA, and 2 g/1 L BSA.

Phosphoramidites: All of the DNA reagents needed for DNA synthesis were purchased from Glen Research or ChemGenes. The DNA oligo sequences were chemically synthesized with a FAM-dT at the 3′-end using standard solid phase phosphoramidite chemistry on an ABI394 DNA (Biolytics) synthesizer using a 0.2 μmol scale. Aptamer candidates were synthesized in house using a solid phase DNA synthesizer according to the manufacturer's protocol (Applied Biosystems, Inc. Model 394). The completed DNA sequences were deprotected using conditions required for modifications and purified using HPLC (Waters) equipped with a C-18 reversed phase column (Phenomenex). DNA concentration was determined by a UV-VIS spectrophotometer (Thermo Scientific; Evolution 300) and stored in DNA Binding Buffer (DB) at −20 °C.

Cell-SELEX procedure: We routinely conducted PI staining of the cells and flow cytometric analysis of CD3ε expression utilizing PE-labeled anti-CD3ε antibody (BD Pharmingen mouse anti-human) along with an isotype control (mouse IgG1 BioLegend) to ensure high-quality cells expressing CD3ε prior to performing each round of SELEX.

The ss-SELEX DNA library in DB buffer was heated at 95 °C for 5 min and “snap-cooled” in ice for 30 min prior to selection. Cells were washed three times with the wash buffer to remove cell debris and apoptotic cells and subsequently re-suspended in 100 μL of a cell suspension buffer prior to incubation with 100 μL of an ss-DNA library for 40 min on ice. The first round of selection was done



Scheme 1. LIGS. Conventional cell-SELEX is first employed against target cells until a partial enrichment of DNA aptamer library is achieved. Next, the partially enriched library is divided into fractions. The first fraction is PCR-amplified, cloned and sequenced. These sequences are enriched towards target cells. An excess of mAb is then introduced on the second fraction, which is preincubated with target cells to selectively outcompete and elute potential aptamers that would tend to bind to the cognate epitope less strongly compared to mAb. The sequences outcompeted by antibody are next PCR-amplified, cloned, and sequenced. By virtue of antibody-cognate epitope binding, these LIGS-generated sequences are specific towards the target surface protein of the antibody. Finally, sequences obtained from DNA sequencing of both fractions are aligned using the ClustalX.2 program, and based on set criteria, specific aptamer candidates against respective epitopes on the target cells are identified.