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Cell-SELEX-based selection and characterization of a G-quadruplex DNA aptamer against mouse dendritic cells

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ABSTRACT

Targeting of dendritic cells (DCs) by aptamers increases antigen capture and presentation to the immune system. Our aim was to produce aptamers against DC molecules using the cell-SELEX procedure. For this purpose, 18 rounds of cell-SELEX were performed on mouse macrophage J774A.1 and CT26 as target and control cells, respectively. The selected aptamers were truncated and their binding to mouse macrophages, and immature and mature DCs analyzed. Two macrophage-specific aptamers, Seq6 and Seq7, were identified. A truncated form of Seq7, Seq7-4, 33 nucleotides in length and containing the G-quadruplex, bound macrophages and immature DCs with KD values in the nanomolar range. We anticipate that Seq7-4 has potential as a therapeutic tool in targeting of mouse macrophages and immature DCs to efficiently improve different immunotherapy approaches.

1. Introduction

Antigen-presenting cells (APCs), which include dendritic cells (DCs) and macrophages, play essential roles in the immune system through the uptake, processing, and presentation of antigens to the T lymphocytes [1]. Studies have demonstrated that antigen targeting produces much stronger immunity than soluble antigen [1]. DC targeting can direct appropriate immune responses in immunotherapy and vaccine technology [2].

Recent advances in the field of targeted therapy using antibodies, peptides, and aptamers are improving treatments against infectious diseases, allergies, and cancers [3]. Recently, antibodies were major targeting agents; however, immunogenicity and the high cost of production limited their clinical applications [4]. Oligonucleotide aptamers offer several advantages over antibodies; these include lack of immunogenicity, lower molecular weights, higher stabilities, and greater tissue penetration. These characteristics make aptamers ideal candidates as probes for diagnosis, imaging, targeting therapy, and drug delivery [4,5].

Oligonucleotide aptamers are generated from single-stranded (ss) DNA or RNA libraries using the SELEX (systematic evolution of ligands by exponential enrichment) process [6]. Cell-SELEX is a modified form of the SELEX procedure by which aptamers are generated against molecules with natural folding structure of target cells [7] (Fig. 1A). The aptamers isolated in the cell-SELEX cycle are often used in targeting therapies, biomarker discovery, and cancer diagnosis and therapy [8,9].

Aptamers can fold into three-dimensional (3D) structures that bind to a wide variety of targets with high affinities and specificities. One aptamer structure, known as the G-quadruplex, is regarded as a four-stranded DNA in which guanine (G) nucleotides are linked by Hoogsteen hydrogen bonding in a square-planar array [10] (Fig. 1B). One example of a DNA aptamer with a G-quadruplex structure is thrombin-binding aptamer [11].

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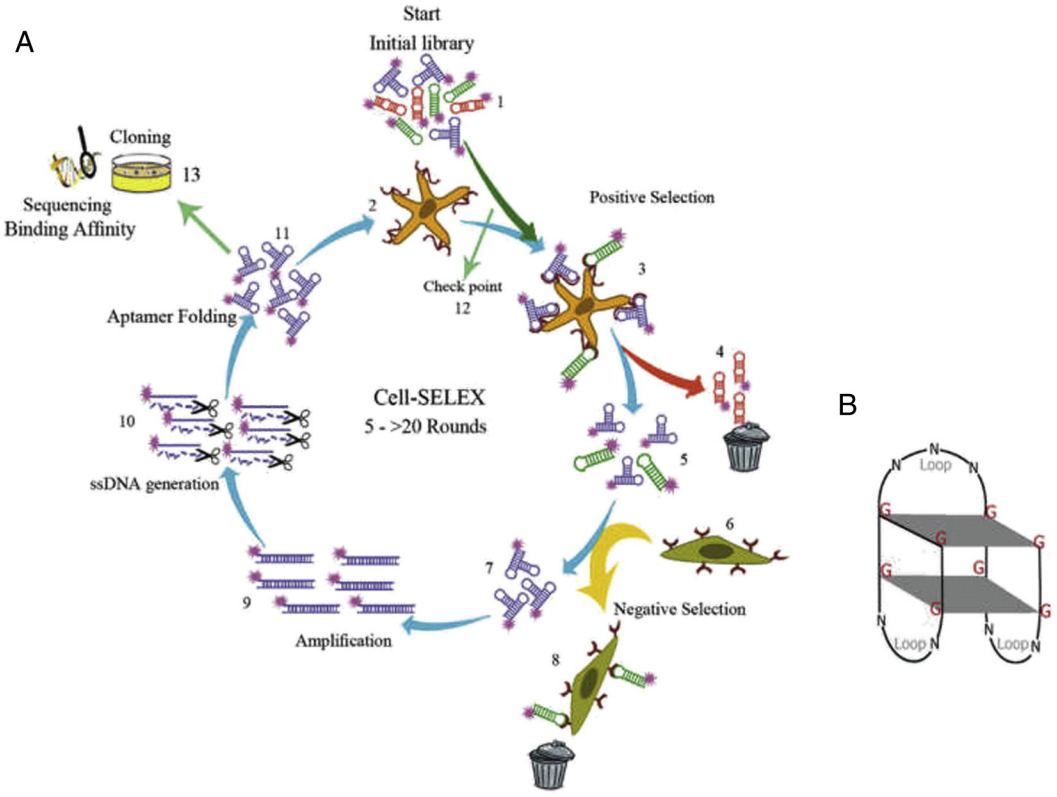

Fig. 1. (A) Schematic diagram of DNA aptamer selection using cell-SELEX. Briefly, the single-stranded (ss) DNA library, *1*, was incubated with J774A.1 cells as targets, *2*, in the positive selection step. After washing, the unbound aptamers, *4*, were removed and bound aptamers, *5*, were eluted by heating and then incubated with CT26 cells as controls, *6*, for negative selection. The unbound aptamers, *7*, were collected, and the sequences that bound to the control cells, *8*, discarded. After amplification of the collected sequences, 9, the double-stranded. DNAs were converted into ssDNAs, *10*, and refolded, *11*, by heating and snap cooling. Cell-SELEX progression was ended based on the highest mean fluorescence intensity (MFI) of each selected pool in the checkpoint step, *12*, by flow cytometry. When the selected aptamer pool was sufficiently enriched, the PCR products were cloned and sequenced, *13*, for aptamer identification [13]. (B) A G-quadruplex structure of the DNA aptamer with three loops that play important role in binding to target molecules.

The J774A.1 mouse macrophage cell line and mouse DCs express many of the same molecules [12]. Herein, we used J774A.1 cells as targets and fibroblastoid colon carcinoma CT26 cells as controls to generate DNA aptamers against mouse macrophages by the cell-SELEX approach. The aptamers were then truncated based on their secondary structures. Finally, binding affinities of the truncated aptamers to mouse DCs were measured.

2. Materials and methods

2.1. Cell lines and buffers

Mouse macrophage J774A.1 (ATCC Number: TIB-67) and fibroblastoid colon carcinoma CT26 cell lines (ATCC Number: CRL-2638) were obtained from the Pasteur Institute, Tehran, Iran. Cells were cultured in RPMI-1640 medium (Gibco, NY, USA) to form monolayers at 37 °C under a humidified 5% CO_2 atmosphere in 60-mm tissue culture dishes (SPL Life Sciences, Pocheon, Korea). Complete RPMI-1640 medium was prepared with heat-inactivated 10% fetal bovine serum (FBS) (Gibco, NY, USA), 100 units/ml penicillin (Sigma, MO, USA), 100 μg/ml streptomycin (Sigma, MO, USA) and 2 mM L-glutamine (Gibco, NY, USA). On the day of the experiment, the medium was removed and the monolayer cells with >95% viability and confluency at a density of 2×10^6 cells/dish were washed three times with cold washing buffer. Washing buffer, pH 7.6, was prepared by dissolving 4.5 g/L glucose and 5 mM $MgCl_2$ in Dulbecco's phosphate buffered saline (DPBS) buffer. DPBS, pH 7.6, was prepared with 137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4, 0.9 mM $CaCl_2$, and 0.5 mM $MgCl_2$. Binding buffer, pH 7.6, was prepared by adding 1 mg/ml bovine serum albumin (BSA) (BioSera, Sussex, UK) and 0.1 mg/ml yeast tRNA (Sigma, MO, USA) to the washing buffer.

2.2. ssDNA library and primers

The ssDNA library, 5′-GCTGTGTGACTCCTGCAA-N42-GCAGCTGTATCTTGTCTCC-3′, was composed of 42 random nucleotides flanked by two fixed-primer hybridization sites. The sense primer was labeled at the 5′-end with FAM (5′-FAM-GCTGTGTGACTCCTGCAA-3′) to monitor the enrichment of the selected pools by flow cytometry, and the antisense primer was phosphorylated at the 5′-end (5′-phosphate-GGAGACAAGATACAGCTGC-3′) to allow digestion of the antisense strands by lambda exonuclease (Thermo Scientific, Schwerte, Germany). The primers and ssDNA library were synthesized and purified by HPLC (Bioneer, Shanghai, China).

2.3. Cell-SELEX procedure

For the first cell-SELEX round, 1 nmol of the ssDNA library was dissolved in 700 μl of binding buffer. The library was refolded by heating at 95 °C for 5 min followed by snap cooling on ice for 10 min. The refolded ssDNA library was incubated with 2×10^6 J774A.1 cells at 37 °C for 45 min on a rotary shaker. After incubation, the cells were washed two times with 1 ml of washing buffer to remove unbound sequences. Cells were harvested and bound sequences were eluted in binding buffer by heating at 95 °C for 10 min. The supernatant was collected and the sequences were recovered by phenol extraction and ethanol precipitation. The recovered sequences were amplified by PCR using 2× master mix (Parstous, Mashhad, Iran) with FAM- and phosphate-labeled primers. PCRs of 6 to 30 cycles were performed at 94 °C for 30 s, 64 °C for 20 s,