Chapter 3

Developing Aptamers by Cell-Based SELEX

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Abstract

The reliable targeting of cell surface disease-associated proteins is a major challenge in chemical biology and molecular medicine. In this regard, aptamers represent a very attractive and innovative class of ligand molecules. Aptamers are generated by a reiterated in vitro procedure, named SELEX (Systematic Evolution of Ligands by Exponential enrichment). In order to generate aptamers for heavily modified cell surface-bound proteins and transmembrane receptors, the SELEX procedure has been recently adapted to the use of living cells as complex targets (referred as "cell-SELEX").

Here we give an overview on the most recent advances in the field of cell-SELEX technology, providing a detailed description of the differential cell-SELEX approach that has been developed in our laboratory to identify specific signatures for human malignant glioma and non-small-cell lung cancer. The procedures used for the evaluation of binding specificity and for the preliminary identification of potential target receptors will be also described.

Key words Cell-SELEX, Aptamer, NSCLC, Glioma, RTK

1 Introduction

1.1 Cell-Based SELEX

Several important human diseases, including cancer and metabolic diseases are characterized by the presence of various alterations in cell-surface proteins. Changes in the expression level, localization or structural changes are frequently the cause of abnormal intracellular signaling ultimately determining the pathological states. Therefore, the specific targeting of disease-associated membrane proteins has recently become a challenge for the development of new therapeutic or diagnostic tools.

In this perspective, nucleic acid aptamers are revealing highly promising tools to identify and target cell-surface proteins [1]. Aptamers are short single stranded DNA/RNA molecules that resemble antibodies in many ways. By folding into complex tertiary structures, these oligonucleotides bind with high affinity and specificity their target proteins, thus often leading to the modulation of their activity [2].

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Aptamers show many advantages over proteins or antibodies: (1) they are chemically synthesized avoiding the use of animal cells, thus providing high batch-to-batch fidelity; (2) they can be easily modified to enhance their stability, bioavailability and pharmacokinetics; (3) depending on their formulation, aptamers are usually poorly or not immunogenic [3].

Different approaches have been adopted to generate aptamers for cell surface molecular targets. In many cases, soluble purified cell surface proteins have been used as targets for aptamer selection in vitro (protein-SELEX) [4–7]. Although this approach shows the advantage to be conducted under well-controlled conditions, the selection is performed in a non-physiological context, leading to the possibility that the selected aptamer might not recognize the same target in its native conformation.

The application of the SELEX technology using whole living cells (cell-SELEX) as complex target allows to overcome this problem by selecting aptamers under native conditions in a physiological context. Globally three variants of cell-SELEX have been adopted that permit to achieve different objectives (Fig. 1).

A first strategy (Fig. 1a) allows to select aptamers against a previously identified target protein, by using in the positive selection step an engineered cell line forced to express the recombinant tar-

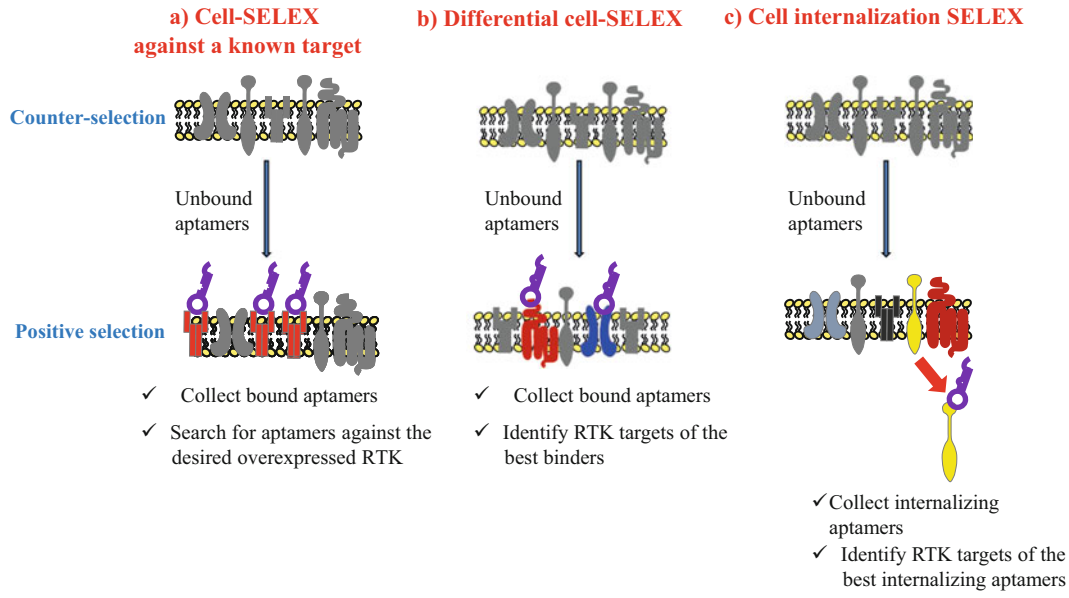

Fig. 1 Schematic representation of three variant of cell-based SELEX proposed to achieve different objectives: (**a**) protocol to select aptamers against a known target; (**b**) protocol to identify a specific cell signature without a prior knowledge of the target protein; (**c**) protocol to specifically select cell internalizing aptamers

get protein. To avoid the parallel enrichment of aptamers for unwanted targets, the introduction at each round of one (or more) counter-selection steps with parental cells is required. To date, several groups have successfully adopted this strategy to generate aptamers that specifically bind cell surface receptors.

An innovative cell-SELEX strategy has been successfully applied to develop aptamers that specifically bind the transforming growth factor-β type III receptor (TGFβRIII) [8], ectopically expressed on the surface of Chinese hamster ovary (CHO) cells, as well as to generate a high-affinity ssDNA aptamer that specifically binds to the HCV E2 envelope glycoprotein [9]. This technique offers the possibility to favor the selection of target-specific aptamers since the selective pressure is increased by the overexpression of the target protein coupled to the use of an appropriate counter-selection procedure.

An alternative strategy (referred to as "differential cell-SELEX") (Fig. 1b) allows targeting of a specific cell type without any prior knowledge of the target protein, thus leading to the identification of multiple ligands able to discriminate among even closely related cell phenotypes. In this case, two different cell lines, or the same cell line under different growth conditions or insults, are used in the positive and in the counter-selection steps. The enrichment for nonspecific ligands can be avoided by modulating the stringency at each SELEX round in different ways, including the use of suitable competitors during the incubation step, the reduction of target concentration and the increase in the number of washings after incubation.

Shangguan et al. applied such approach to identify ssDNA aptamers that by specifically binding T-cell acute lymphocytic leukemia (ALL) cells are able to distinguish them from B-cell lymphoma cells [10]. Moreover, in the same laboratory Chen et al. developed a panel of aptamers specific for small lung cancer (SCLC) that is the most aggressive subtype of lung cancer with a very short life expectancy. They isolated a set of oligonucleotides able to discriminate small lung cancer cells versus large cell lung cancer, providing a specific tumor signature for early diagnosis of SCLC [11]. A similar approach has been used by Sefah et al. to identify aptamers specific for colorectal cancer versus normal tissue or other cancer cells [12].

The specific protocols adopted by different laboratories may largely vary in terms of the specific scheme of SELEX applied (for example the alternation of the selection and counter-selection steps), the cell types used in the positive and counter-selection steps, the time and the temperature of incubation of the library with the cells and the stringency applied through the SELEX rounds.

In order to develop more effective delivery tools for therapeutics, a variant of the two cell-based SELEX strategies (Fig. 1a, b) has been introduced in order to enrich for aptamers capable of selective internalization (Fig. 1c). The protocol (referred as "cell internalization SELEX") includes ice-cold 0.5 M NaCl in Dulbecco's Phosphate-Buffered Saline (DPBS) washings (High Salt Wash) to remove surface-bound aptamers that do not internalize into target cells [13, 14].

1.2 Differential Cell-SELEX on Glioma and Lung Cancer Cells

In our laboratory, we adopted differential cell-SELEX to generate a panel of high affinity RNA aptamers directed against human malignant glioma [15] and non-small-cell lung cancer (NSCLC) cells surface antigens [16].

Gliomas are the most common primary malignant brain tumors characterized by variable grade of malignancy and histological features [17–22]. With the intention to identify new specific molecular markers preferentially expressed on the surface of the more malignant glioma phenotype, we used human U87MG glioma cell line as target for the selection step and the less aggressive human T98G glioma cells for the counter-selection steps [15]. These two glioma cell lines differ for the potential to form tumors in nude mice, being highly tumorigenic and poorly tumorigenic respectively. We used a library of 2'-fluoro pyrimidines (2′-F)-RNAs and performed 14 rounds of SELEX, progressively increasing the selective pressure by changing both incubation and washing conditions. Finally, we identified ten families of highly related aptamers that cover more than 46 % of all individual sequences obtained. Among them, we selected a panel of eight aptamers displaying high binding affinity for U87MG glioma cells (K_D-values ranging between 33 and 700 nM) and no or low affinity for T98G glioma cells and other human cancer cell types, including neuroblastoma, lung, and breast. Five of these aptamers showed biological activity as well, inducing time-dependent down-regulation of extracellular regulated kinase (ERK) and cyclin D1 phosphorylation, thus indicating that they may act as inhibitory ligands for critical cell surface proteins. In addition, one of the functional selected aptamers, named GL21.T, has been further characterized [23].

As a first attempt to identify its molecular target, we performed a phospho-tyrosine kinase receptor (RTK) array analysis that provided us a clear, even if preliminary, indication that GL21.T specifically binds and inhibits Axl RTK that is overexpressed in U87MG glioma cells. This result has been validated and the functional activity of the aptamer has been characterized in detail [23].

Gl21.T revealed strong inhibition of cell migration and invasion in vitro as well as tumor growth in vivo. Moreover, we demonstrated that GL21.T is also able to internalize into target cells in a receptor-dependent manner, thus resulting an interesting cargo for tissue specific internalization of therapeutic agents [24].

More recently, with the aim to discriminate between the chemo-resistant and chemo-sensitive phenotype in NSCLC, we applied a similar approach by using A549 cells in the positive selection step and the more sensitive H460 cells in the counter-selection step [16]. We selected a set of five families of 2′-F-RNA aptamers able to distinguish between the two cell types. Among these, we identified the aptamer CL4 employing phospho-RTK array analysis that binds the epidermal growth factor receptor (EGFR). We demonstrated that CL4 inhibits EGFR-mediated signaling in vitro and tumor growth in vivo.

In conclusion, the identification of the molecular signature of cancer cells is essential for an accurate and early diagnosis and the generation of molecular probes for molecular analysis represents a major goal in oncology. In this respect, differential cell-SELEX strategy offers a great promise for cancer biomarker discovery and therapeutic molecule identification.

In this chapter, we provide a detailed description of the differential cell-SELEX approach that has a general applicability to obtain a molecular signature of cell state in several cancer types.

2 Materials

2.1 In Vitro RNA Transcription

1. Transcription buffer (5×): 30 mM $MgCl_2$, 50 mM NaCl, 200 mM Tris–HCl, pH 7.5 and 10 mM spermidine.
2. To prepare transcription reaction mix add: 1× Transcription buffer, 1 mM 2′-F-2′-dCTP and 2′-F-2′-dUTP, 1 mM ATP, 1 mM GTP, 1 U/ml inorganic pyrophosphatase, 0.5 U/μl RNAse inhibitors, 10 mM dithiothreitol (DTT), 10 μCi/μl 32P-αUTP (3000 Ci/mmol), 2.5 U/μl of $T7^{Y639F}$ RNA polymerase, and 1 pmol/μl DNA in RNAse-free water to a final volume of 600 μl.
3. DNase treatment: DNase I.
4. Denaturing polyacrylamide gel 8 %: dissolve 60 ml 40 % acrylamide–bis solution (37.5:1) and 126 g urea (*see* **Note 1**) in 30 ml of TBE10× [Tris (0.89 M)–borate (0.89 M)-EDTA (0.025 M)].

   Add water to a final volume of 300 ml. To polymerize the gel add 43 μl *N*,*N*,*N*′,*N*′-tetramethyl-ethylendiamine (TEMED) and 450 μl of 10 % ammonium persulfate solution (APS) (*see* **Note 2**) for 50 ml of mix.
5. Gel loading buffer: 480 μl of formamide, 10 μl 0.5 M ethylenediamine tetraacetic acid (EDTA), 10 μl water, and bromophenol Blue.
6. Buffer to elute RNA from gel: 300 mM NaAc with 200 mM EDTA.

2.2 Counter-selection and Selection Steps

1. Buffer used for incubation during both steps: RPMI or Dulbecco's modified Eagle medium (DMEM) serum-free.
2. Washing buffer: RPMI or DMEM serum-free.
3. Nonspecific competitor: polyinosinic acid (Poly-I).
4. RNA extraction: kit used from Ambion Inc.

2.3 Reverse-Transcription Polymerase Chain Reaction (RT-PCR)

1. Reverse-transcription reaction mix: 1× buffer, 1 mM dATP, 1 mM dGTP, 1 mM dCTP, 1 mM dTTP, 1 μM reverse-primer, 100 μl RNA template (*see* **Note 3**), and 2 U M-MuLV RT.
2. PCR buffer (10×): 100 mM Tris–HCl (pH 8.3), 15 mM $MgCl_2$, 500 mM KCl.
3. PCR mix: 1× buffer, 1 mM dATP, 1 mM dGTP, 1 mM dCTP, 2 μM primers, 6 mM $MgCl_2$ (*see* **Note 4**), reverse-transcribed template, and Taq polymerase (0.02 U/μl).

2.4 TOPA-TA Cloning

1. PCR buffer (10×): 100 mM Tris–HCl (pH 8.3), 15 mM $MgCl_2$, 500 mM KCl.
2. To prepare PCR reaction mix add: PCR buffer (1×), 200 μM dATP, 200 μM dGTP, 200 μM dCTP, 200 μM dTTP, 2 μM primers (forward and reverse), DNA template from the final cycle of SELEX, and Taq polymerase (0.02 U/μl) (*see* **Note 5**).
3. TOPO-TA Cloning kit from Invitrogen.
4. TOPO cloning reaction: 4 μl PCR product, 1 μl pCR™2.1-TOPO, 1 μl Salt Solution: 200 mM NaCl, and 10 mM $MgCl_2$.
5. DH5α™ competent cells.
6. LB plates containing 50 μg/ml ampicillin or 50 μg/ml kanamycin.
7. 40 mg/ml X-gaL in dimethylformamide (DMF).

2.5 Binding Assay

1. Buffer for dephosphorylation (10×): 1 mM EDTA, 500 mM Tris–HCl, pH 8.5.
2. Enzyme for dephosphorylation: phosphatase alkaline (PA) 1 U/μl.
3. Inactivation of PA: 200 mM ethylene glycol tetraacetic acid (EGTA).
4. Buffer for phosphorylation (10×): 100 mM $MgCl_2$, 500 mM Tris–HCl, pH 8.2, 50 mM DTT, 1 mM EDTA, 1 mM spermidine.
5. Enzyme for phosphorylation: T4 Polynucleotide Kinase 10 U/μl.
6. Incubation buffer for treatment: culture medium serum-free.
7. Washing buffer: culture medium serum-free.

8. Recovering buffer: 0.6 % sodium dodecyl sulfate (SDS).
9. [γ-^{32}P]ATP (6000 Ci/mmol).
10. Nonspecific competitor: polyinosinic acid (Poly-I).

2.6 Restriction Fragment Length Polymorphism (RFLP) Analysis

1. PCR buffer (10×): 100 mM Tris–HCl, pH 8.3, 500 mM KCl, 15 mM $MgCl_2$.
2. To prepare PCR reaction mix add: PCR buffer (1×), 200 μM dATP, 200 μM dGTP, 200 μM dCTP, 200 μM dTTP, 2 μM primers (forward and reverse), 0.02 U/μl Taq polymerase, and DNA template.
3. [γ-^{32}P]ATP (3000 Ci/mmol).
4. Buffer React 1 (10×) for digestion: 500 mM Tris–HCl, pH 8.0, 500 mM NaCl, 100 mM $MgCl_2$.
5. Restriction enzymes: RsaI, AluI, HaeIII, HhaI (Invitrogen).
6. Denaturing polyacrylamide gel 6 %: dissolve 45 ml 40 % acrylamide–bis solution (37.5:1) and 126 g urea (*see* **Note 1**) in 30 ml of TBE10×. Add water to a final volume of 300 ml. To polymerize the gel add 43 μl TEMED and 450 μl of 10 % APS for 50 ml of mix.

2.7 Phospho-RTK Array Analysis

1. Block buffer: 2 ml of Array Buffer 1 (R&D Systems).
2. Incubation mix: dilute 200-300 μg of cell lysate in 1.5 ml of Array Buffer 1 (R&D Systems).
3. Washing buffer: 20 ml of 1× Wash Buffer. Wash Buffer stock solution is 25× concentrated (R&D Systems) (*see* **Note 6**). Dilute 40 ml of 25× Wash Buffer into 960 ml of deionized or distilled water.
4. Detection antibody: Anti-Phospho-Tyrosine-HRP Detection Antibody (R&D Systems) diluted 1:5000 in 1× Array Buffer 2 (*see* **Note 7**).
5. Chemi Reagent Mix: mix Chemi Reagents 1 and 2 (R&D Systems) in equal volumes within 15 min of use. 1 ml of Chemi Reagent Mix is required per membrane (*see* **Note 8**).

3 Methods

3.1 Generation of the Starting RNA Library

1. The first step is the PCR amplification of the starting library that is a high complexity DNA pool (10^{14} members) containing a 45 nt random sequence, flanked by two fixed regions for PCR amplification. The primers used are:

   P20: 5′-TCCTGTTGTGAGCCTCCTGTCGAA-3′

   P10: 5′-TAATACGACTCACTATAGGGAGACAAGAATAAA CGCTCAA-3′

2. Transcription of the amplified pool is performed at 37 °C overnight in a mix containing 10 μCi/μl 32P-αUTP (3000 Ci/mmol) and 2.5 U/μl of $T7^{Y639F}$ RNA polymerase (*see* **Note 9**).
3. Transcribed RNA is treated with DNase I (0.2 U/μl) for 30 min at 37 °C.
4. RNA is extracted with phenol/chloroform/isoamyl alcohol (25:24:1) and precipitated with 0.3 M NaAc and 3× volumes of ice cold ethanol in the presence of 0.1 mg/ml of linear acrylamide. To precipitate RNA a centrifugation at 13,200 rpm for 30 min at 4 °C is required.
5. Obtained pellet is loaded on 8 % denaturing polyacrylamide gel.
6. After electrophoresis RNA is eluted from gel with NaAc/EDTA at 42 °C for 2 h.
7. RNA is finally resuspended in sterile water and its concentration is evaluated by absorption measurement.

3.2 Differential Cell-SELEX Protocol

The general protocol of differential cell-SELEX includes repeated cycles of five steps (Fig. 2): (1) incubation of the library with non-target cells (counter-selection step); (2) Recovering of the unbound aptamers and incubation with target cells (positive selection step); (3) partitioning of unbound oligonucleotides from those that specifically bind target cells; (4) recovery of aptamers bound to target cells; (5) reverse transcription and amplification of the nucleic acid pool enriched for specific ligands. The introduction of a counter-selection step promotes the isolation of aptamers that specifically bind to the surface of a desired cell phenotype. After reiterated steps of selection and counter-selection, the resulting oligonucleotides are cloned and sequenced. Obtained sequences are screened for conserved structural elements suggestive of potential binding domains and then tested for the ability to specifically bind to target cells.

In our protocol at each round of SELEX, one or two counter-selection steps on non-target cells preceded the positive selection step on target cells.

1. Before treatment, the 2′-F-RNA pool (800–300 pmol) is subjected to a short denaturation–renaturation step in 1.5 ml of serum-free medium (85 °C for 5 min, snap-cooled on ice for 2 min and allowed to warm up to 37 °C).
2. Following the denaturation–renaturation step, 13.5 ml of serum-free medium are added to the RNA to have a final volume of 15 ml for treatment of cells plated in 150 mm dishes.
3. The 2′-F-RNA pool is first incubated at 37 °C for 30 min with non-target cells (counter-selection step).
4. Unbound sequences are then recovered and incubated at 37 °C for 30 or 15 min with target cells (selection step).

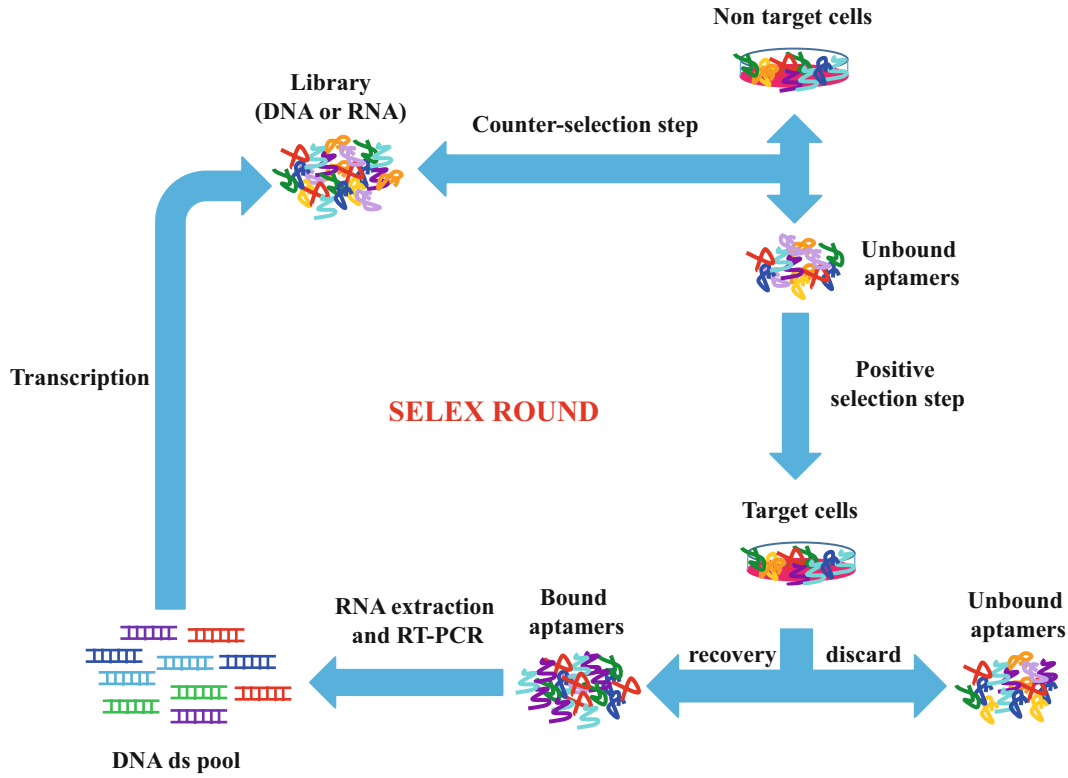

Fig. 2 Schematic representation of differential cell-SELEX technology

5. Finally, following several washings (up to five) with 5 ml of serum-free medium (to discard unbound sequences), bound aptamers are recovered by total RNA extraction.

   During the selection procedure, changing the number of final washings, the incubation time on target cells and the number of counter-selection steps progressively increases the selection-pressure. Exact SELEX conditions used for NSCLC and glioma are shown in Fig. 3.

6. To monitor the enrichment of the selected RNA pool we performed RFLP analysis and observed the occurrence of four-base restriction sites in the population, indicating the presence of distinct aptamer families.

3.3 RFLP Analysis

1. The PCR product (about 500 ng) is first end-labeled with [γ-^{32}P] ATP.
2. Labeled PCR product is digested with a mix of four restriction enzymes (RsaI, AluI, HaeIII, HhaI) in the buffer ReactI for 1 h at 37 °C.
3. Digested samples are loaded onto 6 % denaturing polyacrylamide gel and analyzed by autoradiography.

a **NSCLC**

SELEX condition

Cell number (x10^6)	2	2	2	2	2	2	2	2	2	2	2	2	2	2
RNA amount (x100pmol)	8	8	8	8	8	8	8	7	7	7	8	7	7	6
Incubation time (min)	30	30	30	30	30	15	15	15	15	15	15	15	15	15
Counter-selection number	1	1	1	1	2	2	2	2	2	2	2	2	2	2
Washing number	3	3	3	3	3	3	3	3	3	5	5	5	5	5
PolyI (10ng/ul)	-	-	-	-	-	-	-	-	-	-	-	-	+	+

b **GLIOMA**

SELEX condition

Cell number (x10^7)	10	10	10	10	10	10	10	10	10	10	10	10	10	10
RNA amount (x100pmol)	5	8	8	3	3	3	3	3	3	3	3	3	3	3
Incubation time (min)	30	30	30	30	30	30	30	30	30	15	15	15	15	15
Counter-selection number	1	1	1	1	1	1	1	1	1	1	2	2	2	2
Washing number	1	2	3	3	4	4	5	5	5	5	5	5	5	5

Fig. 3 Experimental conditions used for differential cell-SELEX on NSCLC (**a**) and glioma cells (**b**)

3.4 Cloning and Sequencing of Selected Aptamers

1. After 14 rounds of SELEX, selected oligonucleotides are cloned in TOPO-TA Cloning Kit according to the manufacturer's protocol and about 100 clones are sequenced.
2. Obtained sequences are analyzed by using bioinformatics alignment tools to identify and score sequence patterns present in aptamers. This approach allows to recognize conserved and variable regions and to regroup sequences into quasi-phylogenetic families. Usually, conserved motifs are indicative of specific target recognition domains.

3.5 Binding Assay

Binding of the final pool, individual aptamers or starting pool (used as a control) to target cells is performed in 24-well plates in triplicate.

1. Aptamers are first dephosphorylated with PA at 37 °C for 1 h.
2. Following dephosphorylation, aptamers are end-labeled at 5′ by using T4 polynucleotide kinase in the presence of [γ-^{32}P] ATP at 37 °C for 30 min.
3. 3.5×10^4 cells per well are incubated with various concentrations of pools or individual aptamers in 200 μl of serum-free medium for 20 min at room temperature in the presence of 0.1 mg/ml Poly-I as a nonspecific competitor.
4. After five washings with 500 μl of serum-free medium, bound sequences are recovered with 300 μl of SDS 0.6 % and the amount of radioactivity is measured at the beta counter.

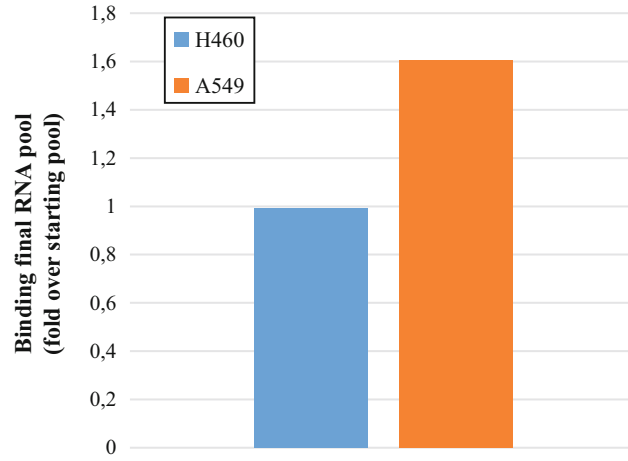

Fig. 4 Binding analyses of the final RNA pool to H460 (non-target) and A549 (target) NSCLC cells. RNA was 5′-[^{32}P]-labeled and incubated at 50 nM with cells. The results are expressed as fold over the binding detected with the starting pool

5. The background value obtained with the starting pool is subtracted from the value obtained with the specific aptamers or final pools.
6. K_D values for each aptamer are determined by Linewaver Burk analysis according to the equation:

   $$1/[\text{complex}] = K_D/[\text{Cmax}] \times 1/[\text{aptamer}] + 1/[\text{Cmax}].$$

   An example of the binding result obtained with final pool following differential cell-SELEX on NSCLC is reported in Fig. 4.

3.6 Target Identification

Since the binding of an aptamer to cell surface receptors often results in the inhibition of the target receptor and its intracellular signaling, to identify the putative functional targets of aptamers with the best binding properties one possibility is to perform a phospho-RTK array analysis.

1. Target cells are serum starved overnight, pretreated with 200 nmol/l aptamer for 3 h and then stimulated with 20 % FBS either alone or in presence of the aptamer.
2. Cell lysates are incubated overnight with the phospho-RTK array and, following three washings with 1× Wash buffer, incubated with anti-Phospho-Tyrosine-HRP Detection Antibody for 2 h.
3. Chemiluminescent signal is detected using Chemi Reagent Mix.

This approach appears to be very useful and convenient as it is informative but very easy and fast to perform.

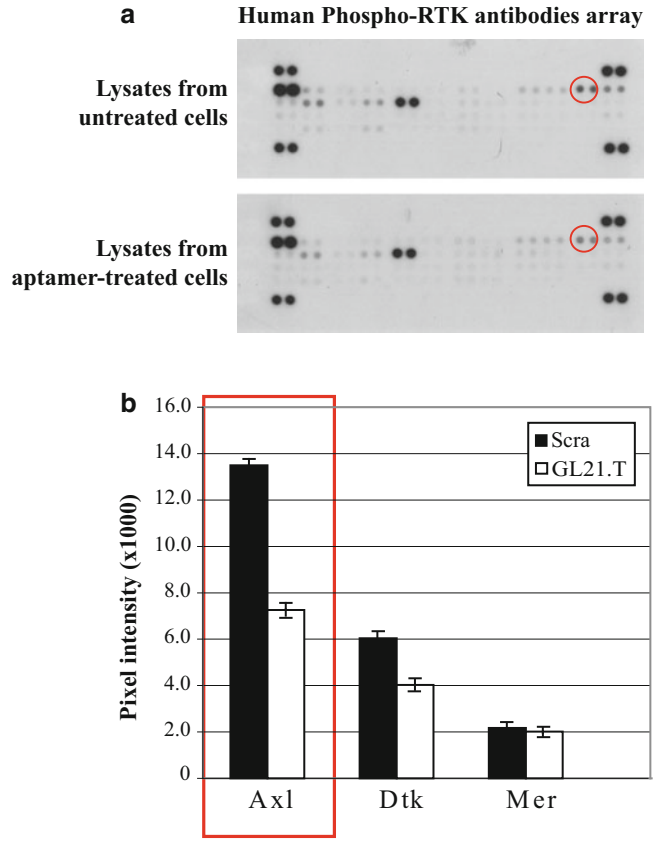

Fig. 5 (**a**) Lysates from untreated (*upper panel*) or GL21.T treated (*lower panel*) U87MG glioma cells were incubated with Human Phospho-RTK array (R&D Systems). The *red circles* indicate the signal coming from hybridization with an anti-Axl. Intensity of signals was measured for Axl and other two members (Dtk and Mer) belonging to the same receptor family. Pixel intensity is reported in the graph (**b**). A clear reduction of Axl phosphorylation was observed. Figure partially reproduced from Ref. [23]

A representative result obtained for the GL21.T aptamer by phospho-RTK array analysis is shown in Fig. 5 (adapted from Ref. [23]). Gl21.T aptamer shows to inhibit Axl phosphorylation. This circumstance has been further validated in our laboratory by different approaches [23].

4 Notes

1. Dissolve urea by adding it slowly. If urea precipitates in the denaturing polyacrylamide solution, warm it to 37 °C to redissolve prior to prepare the gel.
2. Prepare 10 % APS solution in water and immediately freeze it in aliquots at −20 °C.

3. Once extracted, RNA must be resuspended in 100 μl sterile water.
4. The concentration of $MgCl_2$ takes into account that 1× PCR buffer contains 1.5 mM $MgCl_2$. The total final concentration of $MgCl_2$ must be 7.5 mM.
5. It is important to include a 15 min of extension at 72 °C after the last cycle to ensure that all PCR products are full length and 3′-adenylated.
6. If crystals have formed in the concentrated solution, warm the bottle to room temperature and mix gently until the crystals have completely dissolved.
7. To obtain 1× Array Buffer 2, add 2 ml of Array Buffer 2 Concentrate 5× to 8 ml of deionized or distilled water.
8. Protect Chemi Reagents 1 and 2 and Chemi Reagents Mix from light.
9. Mutant $T7Y^{639F}$ RNA polymerase has the ability to efficiently incorporate 2′-modified-ribonucleotides. 2′F- modification of the RNAs increases resistance to nucleases.

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