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In vitro selection of CD70 binding aptamer and its application in a biosensor design for sensitive detection of SKOV-3 ovarian cells

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ABSTRACT

Single-stranded DNA aptamers recognizing CD70 molecule overexpressed in some tumor cell lines was isolated by means of systematic evolution of ligands by exponential enrichment (SELEX). Both purified CD70 protein and CD70-expressing cells were used to isolate target-specific aptamer. After certain rounds of protein-based SELEX and cell-SELEX (ten and seven rounds, respectively), Aptamer 928 (hereafter Apt928) with high affinity and specificity for CD70 was obtained. The specific binding of the aptamer to its target could block interaction of CD70 with CD27 (known as CD70 receptor). Dissociation constant of Apt928 was calculated to be 66 nmol L-1. Moreover, ATTO 674N-labeled Apt928 was applied as a fluorescent aptasensor for rapid and sensitive detection of SKOV-3 cells as CD70-positive cells. The detection limit of this aptasensor was 14 cells mL-1.

Keywords: SELEX; DNA aptamer; CD70; Biomarker; Aptasensor; Cancer

1. Introduction

Nucleic acid aptamers are short single-stranded oligonucleotides which have high affinity and specificity for their targets. They are screened from DNA/RNA libraries containing 10^{14} to 10^{15} random sequences via a method called SELEX (Systematic Evolution of Ligands by EXponential enrichment). In this method, a chemically synthesized oligonucleotide library is incubated with target molecules. After removing unbound sequences, desired sequences with high affinity toward their target are amplified by polymerase chain reaction (PCR) and additional rounds of selection are performed to enrich the initial pool of oligonucleotides [1, 2]. Aptamers are known as suitable substitution for antibodies because of their advantages over antibodies, including higher stability, easier synthesis and modification and lower immunogenicity [3, 4]. Additionally, aptamers show high affinity and specificity for a wide range of molecules such as metal ions [5], small molecules [6] and organic dyes [7]. However, these molecules do not elicit a strong immune response, thus it is difficult to identify and generate antibodies against all of these molecules. Furthermore, small size of aptamers (20-80 nucleotides in length) enables them to penetrate into their targets more efficiently than antibodies [8]. On account of all these excellent characteristics, aptamers have been broadly employed in preclinical and clinical cancer research in the fields of sensors [9], therapeutics [10, 11] and targeted drug delivery [12].

The noninvasive early detection of cancer can result in better prognosis and increase life expectancy of patients. Both proteomics and bioinformatics have contributed to the discovery of an increasing number of tumor cells biomarkers [13]. After tumor cell biomarkers discovery, a sensitive and selective method is needed to recognize the biomarkers. Although antibodies have been extensively used to achieve this goal for decades, they exhibit drawbacks in biomedical and biological applications. For instance, antibodies undergo irreversible denaturation following exposure to temperature changes [14]. Hence, there has been a propensity for introducing alternative probes. Aptamers with unique properties mentioned above have been proposed as promising probes for various biomarker recognition-based applications.

CD70, a type II transmembrane protein, is a member of the tumor necrosis factor (TNF) superfamily whose expression is tightly regulated and has not been reported on any normal non-hematopoietic cells until now. Among the hematopoietic lineages, CD70 expression is only transiently restricted to activated T and B cells as well as on mature dendritic cells (DCs) [15, 16]. Interaction of CD70 with its receptor (CD27) can promote the accumulation of cytotoxic effector T cells, memory T cell formation and plasma cell differentiation [17, 18]. In pathologic conditions, CD70 is overexpressed in a variety of tumor cells including ovarian carcinoma cell lines [19], renal cell carcinoma [20], glioblastoma [21] and malignant B

cells [22]. Therefore, the design of sensing agents that are able to recognize and bind to this tumor marker can assist cancer diagnosis.

In the present study, we used bead-based protein SELEX method to isolate a new aptamer that specifically binds to CD70 with high affinity. Cells overexpressing CD70 on their surfaces were used as target in cell-SELEX to enrich the selected aptamers. The employment of both purified protein and cells which is called hybrid-SELEX, has been previously introduced to develop Tenascin-C specific RNA aptamers [23]. Moreover, the screened aptamer (Apt928) was conjugated with a fluorescent dye to generate a fluorescent aptasensor for the detection of CD70 positive tumor cells.

2. Materials and methods

2.1. Chemicals and materials

Human recombinant protein CD70-his Tag (Cat No = P6144) was purchased from Abnova (Taiwan). Human recombinant protein CD27-Fc-His Tag (Cat No = 10039-H03H) was obtained from SinoBiological (China). Human recombinant programmed death-ligand 1 (PD-L1/CD274) His-tagged protein (Cat No = 10084-H08H) and human recombinant IL2Ra/CD25 protein-His Tag (10165-H08H) were purchased from Sinobiological (China). Anti-CD70 FITC-conjugated antibody (sc65271) and PE-labeled anti-human Fc antibody (Cat No = 409303) were purchased from Santa Cruz and Biolegend, respectively. The synthetic single-stranded DNA (ssDNA) library containing a sequence with 43 random nucleotides (5′-GCTGTGTGACTCCTGCAA-N43-GCAGCTGTATCTTGTCTCC-3′) and both forward and reverse primers (5′-GCTGTGTGACTCCTGCAA-3′ and 5′phosphate-GGAGACAAGATACAGCTGC-3′, respectively) [24, 25] were purchased from Bioneer Company (South Korea). ATTO 647N-labeled oligonucleotides were provided by Microsynth Company (Switzerland). PCR reagents were ordered from SinaClon (Iran). Lambda exonuclease (#EN0562), Gene JET Plasmid MiniPrep Kit (#K0502) and InsTAclone PCR Cloning kit (#k1214) were obtained from Thermo Fisher Scientific (USA). NucleoSpin PCR clean-up and gel extraction kit was bought from Macherey-Nagel (Germany). Magnetic beads (#36111) and nickel-nitrilotriacetic acid (Ni-NTA) HisSorb Stripes (#35023) were ordered from Qiagen Company (Germany). All other chemicals, such as NaCl, KCl, Na_2HPO_4 and K_2HPO_4 were supplied by Merck Company (Germany).

2.2. Cell Lines

SKOV-3 cell line (human ovarian carcinoma) was purchased from the Pasteur Institute of Tehran, Iran. A2780 cell line (human ovarian carcinoma) was kindly provided by Dr. F. H. Shirazi, Shaheed Beheshti Pharmacy School, Tehran, Iran. All cell lines were cultured in RPMI 1640 medium (Biosera,

Inc) supplemented with 10% fetal bovine serum (Gibco) and 100 U mL-1 penicillin-streptomycin (Invitrogen). Cell lines were incubated at 37°C under a 5% CO_2 atmosphere.

2.3. Immobilization of human recombinant CD70-his tag protein

Homogeneous Ni-NTA magnetic beads (20 µL) were washed twice with 50 µL of phosphate buffered saline with tween (PBS-T) (50 mmol L-1 K_2HPO_4, 150 mmol L-1 NaCl and 0.05% Tween-20, pH 7.5). Then, beads were resuspended in PBS-T (90 µL) and CD70-his tag protein (5 µg) was added and incubated in shaker for 30 min at 4°C. The complex of protein CD70-bead was then washed three times with 200 µL of PBS-T, and stored at 4°C until use.

2.4. In vitro aptamer selection (ligand SELEX)

In the first round of selection, 1 nmol of initiating ssDNA library was diluted into 100 µL of PBS-T containing 1 mg mL-1 bovine serum albumin (BSA) and heated to 95°C for 5 min. Then, the heated library was immediately placed in an ice bucket for 20 min and diluted in 5 mL binding buffer (50 mmol L-1 K_2HPO_4, 150 mmol L-1 NaCl, 0.05% Tween-20, BSA 1 µg mL-1, 0.1 mg mL-1 yeast tRNA, pH 7.5). CD70-bead conjugate was then added to this mixture and incubated at room temperature for 45 min with gentle shaking. In order to remove the unbound sequences, tube was placed under a magnetic field (Dexter) and beads were washed three times by 1 mL of PBS-T. Proteins and bound aptamers were eluted from the Ni-NTA magnetic beads by adding 10 µL of elution buffer (20 mmol L-1 Tris pH 7.5 containing 500 mmol L-1 imidazole). The eluted oligonucleotides were amplified by PCR (5′-phosphorylated reverse primer was used at PCR mixture). The amplification steps were as follows: pre-denaturation at 94°C for 5 min, denaturation at 94°C (30 s), annealing at 60°C (30 s) and elongation at 72°C (30 s), for the final extension, the reaction was done at 72°C for 3 min. To determine the optimum cycle of PCR with no non-specific bonds and primer-dimer products, all amplicons were analyzed by agarose gel electrophoresis. The collected ssDNA from the first round of selection was amplified at the optimum cycle of PCR and purified by NucleoSpin PCR clean-up purification kit (Macherey-Nagel, Germany), according to the manufacturer's instructions. All purified double-stranded DNAs (dsDNAs) were converted to ssDNAs upon digestion of 5′-phosphorylated anti-sense strand by means of Lambda exonuclease enzyme. The ssDNA was then purified by use of NucleoSpin PCR clean-up purification kit (Macherey-Nagel, Germany) and ssDNA concentration was measured using a NanoDrop spectrophotometer (NanoDrop UV-Vis spectrophotometer, USA). The purified ssDNA was applied for the next round of SELEX. By decreasing the amount of target protein, bead and the incubation time to 20 min on one hand and increasing the washing number and time, on the other hand, more stringent conditions were imposed on selection process to isolate aptamers with high affinity and specificity in the next rounds. To remove Ni-

NTA magnetic beads binding aptamers, counter SELEX was performed after third round of selection. In brief, 20 μL of magnetic beads were added to 1 mL of eluted ssDNA in PBS-T and incubated for 45 min at room temperature and the supernatant was then collected for the next round.

2.5. Aptamer enrichment assay

After 10 rounds of selection, the degree of enrichment of obtained ssDNAs for binding CD70 was evaluated by flow cytometry analysis. Amplification of DNA library and sequences of 3rd, 9th and 10th rounds were done using ATTO 647N-labeled forward primer and 5′-phosphorylated reverse primer. All resulting dsDNAs were treated with lambda exonuclease enzyme to generate ssDNAs. 1500 ng of ssDNA from each round was added to CD70-bead conjugates (1 μg of protein) and incubated at room temperature for 30 min with mild shaking. After incubation, all tubes were washed twice with washing buffer (50 mmol L-1 K_2HPO_4, 150 mmol L-1 NaCl, 0.05% Tween-20, pH 7.5) and resuspended in it. After that, the bead-protein complexes were subjected to flow cytometry to select the round for which the highest fluorescence intensity was obtained. The best round in terms of fluorescence intensity was selected for cell-SELEX.

2.6. Cell-SELEX

This process was performed on SKOV-3 cell monolayers at 4°C (to minimize the aptamer internalization and enzymatic digestion). SKOV-3 cell line was cultured in a culture dish 100 mm × 20 mm (Thermo Scientific Nunc Cell Culture Dishes) and allowed to form a monolayer at 37°C in RPMI medium 1640 (Biosera, Inc) containing 10% FBS and 100 U mL-1 penicillin-streptomycin. Cells were washed twice with cold phosphate buffered saline (PBS) before adding the ssDNAs obtained from the 9th round. The sequences (1500 ng) were heated and cooled under the same condition as applied in ligand SELEX. The monolayers cultured cells with at least 90% confluency were incubated with prepared oligonucleotides along with yeast tRNA and sonicated salmon sperm DNA at 4°C for 45 min with continuous mild shaking. After incubation, the cells were washed twice with washing buffer (cold PBS). The cells were detached by a cell scraper (SPL Life Sciences) and transferred into a 1.5 mL tube. Cell suspension was heated at 95°C for 10 min and oligonucleotides were recovered by centrifugation at 13000g for 5 min. The obtained DNA pool was amplified using PCR and employed for the next round of cell-SELEX. Seven rounds of cell-SELEX were performed, followed by flow cytometry to evaluate the enrichment of obtained ssDNAs. Accordingly, DNA library and 1st, 4th and 7th rounds were amplified and prepared under similar conditions used in the enrichment assay. SKOV-3 cells were cultured in a 12-well plate (SPL Life Sciences) to reach at least 90% confluency. The cells were washed twice with cold PBS and incubated with oligonucleotides at 4°C for 30 min. Each well was then washed with cold PBS and

scraped. The scraped cells were transferred into the flow cytometry tubes and signal intensity of each tube was measured by flow cytometer.

2.7. Cloning and sequencing

The cloning was carried out using an InsTAclone PCR Cloning kit according to the manufacturer's instruction (Fermentas, WI, USA) in *Escherichia coli* Top 10. Fifty colonies were selected and plasmids were extracted and purified by Gene JET Plasmid Miniprep kit (Bioneer, South-Korea). Purified plasmids were sequenced by Bioneer Company (South-Korea). Using DNAMAN software, analysis of sequenced aptamers was performed. In addition, secondary structures of selected aptamers were drawn by the M-fold web-based software.

2.8. Aptamer binding to CD70-expressing cell lines

Two cell lines including SKOV-3 and A2780 were washed with PBS. Staining was performed by adding ATTO 647N-labeled Apt928 to these cells. After incubation at 37ºC for 30 min, the cells were washed with PBS and analyzed using flow cytometry.

2.9. Specificity evaluation of the selected aptamer

To assess the specific binding of the selected aptamer (Apt928) to its target (CD70), the ATTO 647N–labeled Apt928 was separately incubated with CD70, PD-L1 and CD25-coated magnetic beads (the final concentration of each protein was 100 nmol L-1). The beads were washed twice with 500 µL of washing buffer and suspended in 300 µL of washing buffer. The signal intensity of each tube was analyzed by flow cytometry.

2.10. Blocking assay

To further confirm the Apt928 specific binding to CD70, the target cells (SKOV-3) were incubated with aptamer (100 nmol L-1) for 30 min at 37ºC. Then, 100 ng of Fc-tagged CD27 human recombinant protein was added to the cells and incubated for 30 min. The cells were then washed and subjected to PE-conjugated anti-Fc antibody for 30 min. In parallel, CD27-Fc protein was directly added to the cells followed by washing steps and antibody exposure without treatment with Apt928. The cells were washed and resuspended in 400 µL of washing buffer. The fluorescence intensity of aptamer-treated cells was compared with those without aptamer treatment.

2.11. Determination of dissociation constant of aptamer

Binding affinity of Apt928 to CD70 was measured by incubation of CD70 protein (375 ng)-bead conjugates with different concentrations of aptamer in 250 µL of binding buffer at 22ºC for 30 min. Beads were then washed twice with 500 µL of washing buffer, resuspended in 300 µL of washing buffer and subjected to flow cytometry analysis. The equilibrium dissociation constants (K_d) for Apt928-CD70 interaction was obtained by fitting the dependence of fluorescence intensity of specific binding (Y) on the aptamer concentration (X) using the equation ($Y = B\ max\ X/(K_d + X)$).

2.12. Aptamer-based detection of tumor cells

For biosensing of SKOV-3 cells by ATTO 647N–labeled Apt928, varying numbers of SKOV-3 cells from 10 cells up to 10^5 cells were incubated with ATTO647N–labeled Apt928 (final concentration of 50 nmol L-1) at 37ºC for 1 h. After washing, the cells were suspended in washing buffer and analyzed using flow cytometry.

3. Results and discussion

3.1. Aptamer isolation and characterization

Isolation of ssDNA aptamers with the capability of recognizing and binding to CD70 molecule was performed using protein-based SELEX followed by cell-SELEX from a pool of about 10^{15} different sequences each comprising 43-base random region flanked by two constant primer-binding regions. In protein-based SELEX with the aid of His tag/Ni-NTA interaction, His tag-CD70 recombinant protein was covalently conjugated to Ni-NTA magnetic agarose beads. After immobilization, synthetic library was incubated with bead-protein complexes and bound sequences were separated from either weakly bound or unbound ones by multiple washing steps. The bound aptamers were then eluted by adding imidazole and amplified by PCR to obtain an enriched pool used for the next round of selection (Fig. 1). After purification of the PCR products, double-stranded sequences were converted to ssDNAs using lambda exonuclease. Selection pressure was gradually enhanced through consecutive selection rounds by adding salmon sperm DNA, decreasing protein concentration and incubation time with the target, increasing washing time and steps and increasing beads concentration during counter SELEX rounds [24]. The progress of aptamer enrichment during SELEX was monitored using flow cytometry. Fluorescence intensities of control, library and rounds 3, 9 and 10 were measured. Since aptamer enrichment began to decrease at 10^{th} round, the selection process was discontinued and round 9 was chosen for cell-SELEX (Fig. 2). The significant decrease in binding of 10^{th} round sequences to CD70 protein can be contributed to PCR bias during PCR amplification, resulting in loss of high-affinity and high-specificity sequences [26]. Previous studies demonstrated that CD70 is highly expressed by SKOV-3 and to a lesser extent by A2780 cell lines [19]. Therefore, SKOV-3 cell was chosen as a CD70-overexpressing cell line for cell-

SELEX process wherein SKOV-3 cells were incubated with all obtained ssDNAs of 9th round of ligand SELEX. This process was repeated up to 7th round until the enrichment reached a plateau (Fig. 3). Then, the sequences of round 7 of cell-SELEX were cloned. Fifty clones were selected for sequencing. The sequences were analyzed by DNAMAN software and according to their homology and phylogenic tree, three sequences were chosen. The selected sequences were synthesized and conjugated with ATTO 674N fluorescent dye. Fluorescently labeled aptamers were tested in terms of their binding ability to CD70 molecule.

3.2. Evaluation of aptamer binding and specificity to target

One sequence out of three synthesized sequences namely Apt928 (5′ GCTGTGTGACTCCTGCAAGCGGGAAGAGGGCAGGGGAGGGAGGGTGACGCGGAAGAGGCA AGCAGCTGTATCTTGTCTCC 3′) showed the best performance in recognizing and binding to CD70-expressing cells. SKOV-3 and A2780 cells were subjected to flow cytometry analysis. As shown in Fig. 4 A and B, Apt928 could detect and bind to CD70 expressed on the surfaces of two mentioned cell lines with higher affinity compared to library sequences. Considering that the level of CD70 is higher in SKOV-3 compared to A2780 on one hand, and higher binding affinity of Apt928 to SKOV-3 compared to A2780 cells on the other hand, we concluded that the target of Apt928 on SKOV-3 cells surface was CD70 molecule.

To determine the specificity of Apt928, its binding strength to CD70 and non-target recombinant proteins including CD25 and PD-L1 was investigated using fluorescent assay. The flow cytometry results indicated that Apt928 possessed higher affinity toward CD70 in comparison with CD25 and PD-L1 proteins (Fig. 5). Blocking assay results provided further confirmation of aptamer specificity to target. Pre-incubation of CD70 positive cells (SKOV-3) with aptamer led to lesser binding of Fc-tagged CD27 (as a receptor for CD70) to CD70, resulting in lower fluorescence intensity upon addition of anti-Fc PE-conjugated antibody compared to the situation where the cells were not treated with Apt928 (Fig. 6). These results verified that Apt928 could specifically bind to CD70 and inhibit CD70-CD27 interaction as well.

3.3. K_d determination of aptamer

Dissociation constant (K_d) of isolated aptamer for CD70 protein was determined by incubation of varying concentrations of fluorescently labeled Apt928 with bead-protein conjugates. Using non-linear regression analysis, the K_d value was determined to be 66 nmol L-1 at 22ºC (Fig. 7).

3.4. Tumor cells detection ability of Apt928

Early detection of cancer can improve clinical outcome for many tumors. Hence, many efforts have been made to develop early detection strategies. To date, various tumor cell detection methods including PCR-based methods, cytometric methods and cell enrichment methods have been introduced. Although these methods take advantages of high sensitivity and broad applicability, they suffer from their own limitations such as long time of detection/processing, need for sophisticated instrument and high expenses [27]. Thus, the need for an inexpensive, rapid and simple tool with high sensitivity and specificity for detection of cancer cells is inevitable. Aptamers can fulfill all these requirements. The ease of production and implementation, high sensitivity and specificity, low toxicity and immunogenicity and versatility in application are the advantages of aptamers compared to other molecular probes [4, 28]. In this study, various number of target cells (SKOV-3) were incubated with a fixed concentration of aptamer (50 nmol L-1). The mean fluorescence intensity of each condition was measured and accordingly, an appropriate linear range from 10 to 10^5 cells per 100 µL was observed (Fig. 8). The limit of detection (LOD) of sensor was 14 cells mL-1 (3σ/slope, where σ was the standard deviation of blank response, n = 3). In comparison with other detection methods listed in Table 1, the robustness of Apt928 (detection of SKOV-3 cells as low as 14 cells mL-1) introduces it as a potential probe for the development of different aptasensors for detection of CD70 positive tumor cells.

Conclusion

In the present study, a novel ssDNA aptamer (Apt928) with high affinity for CD70 was successfully isolated using protein-SELEX followed by cell-SELEX. The selected aptamer possessed high affinity (K_d value of 66 nmol L-1) for target molecule. Not only Apt928 could specifically bind to CD70, but it could also block the interaction of CD70 with respective receptor (CD27). Furthermore, the results indicated that the aptamer was able to detect tumor cells with detection limit of 14 cells mL-1. All these findings signify applicability of Apt928 as an aptasensor for cancer cell detection.

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Conflicts of Interest

The authors declare no conflict of interest.

References

[1] C. Tuerk, L. Gold, Systematic evolution of ligands by exponential enrichment: RNA ligands to bacteriophage T4 DNA polymerase, Science 249 (1990) 505-510.

[2] A.D. Ellington, J.W. Szostak, In vitro selection of RNA molecules that bind specific ligands, Nature 346 (1990) 818-822.

[3] S.D. Jayasena, Aptamers: an emerging class of molecules that rival antibodies in diagnostics, Clin. Chem. 45 (1999) 1628-1650.

[4] C.R. Ireson, L.R. Kelland, Discovery and development of anticancer aptamers, Mol. Cancer Ther. 5 (2006) 2957-2962.

[5] J. Ciesiolka, J. Gorski, M. Yarus, Selection of an RNA domain that binds Zn^{2+}, Rna 1 (1995) 538-550.

[6] D. Mann, C. Reinemann, R. Stoltenburg, B. Strehlitz, In vitro selection of DNA aptamers binding ethanolamine, Biochem. Biophys. Res. Commun. 338 (2005) 1928-1934.

[7] J.R. Babendure, S.R. Adams, R.Y. Tsien, Aptamers switch on fluorescence of triphenylmethane dyes, J. Am. Chem. Soc. 125 (2003) 14716-14717.

[8] E. Levy-Nissenbaum, A.F. Radovic-Moreno, A.Z. Wang, R. Langer, O.C. Farokhzad, Nanotechnology and aptamers: applications in drug delivery, Trends Biotechnol. 26 (2008) 442-449.

[9] N.M. Danesh, M. Ramezani, A.S. Emrani, K. Abnous, S.M. Taghdisi, A novel electrochemical aptasensor based on arch-shape structure of aptamer-complimentary strand conjugate and exonuclease I for sensitive detection of streptomycin, Biosens. Bioelectron. 75 (2016) 123-128.

[10] S.M. Taghdisi, P. Lavaee, M. Ramezani, K. Abnous, Reversible targeting and controlled release delivery of daunorubicin to cancer cells by aptamer-wrapped carbon nanotubes, Eur. J. Pharm. Biopharm. 77 (2011) 200-206.

[11] A.D. Keefe, S. Pai, A. Ellington, Aptamers as therapeutics, Nat. Rev. Drug Discov. 9 (2010) 537-550.

[12] S.M. Taghdisi, N.M. Danesh, A. Sarreshtehdar Emrani, K. Tabrizian, M. ZandKarimi, M. Ramezani, K. Abnous, Targeted delivery of Epirubicin to cancer cells by PEGylated A10 aptamer, J. Drug Target. 21 (2013) 739-744.

[13] M.S. Boguski, M.W. McIntosh, Biomedical informatics for proteomics, Nature 422 (2003) 233-237.

[14] A.W. Vermeer, W. Norde, The thermal stability of immunoglobulin: unfolding and aggregation of a multi-domain protein, Biophys. J. 78 (2000) 394-404.

[15] M. Croft, Costimulation of T cells by OX40, 4-1BB, and CD27, Cytokine Growth Factor Rev. 14 (2003) 265-273.

[16] J. Borst, J. Hendriks, Y. Xiao, CD27 and CD70 in T cell and B cell activation, Curr. Opin. Immunol. 17 (2005) 275-281.

[17] J. Kober, J. Leitner, C. Klauser, R. Woitek, O. Majdic, J. Stöckl, D. Herndler-Brandstetter, B. Grubeck-Loebenstein, B.M. Reipert, W.F. Pickl, The capacity of the TNF family members 4-1BBL, OX40L, CD70, GITRL, CD30L and LIGHT to costimulate human T cells, Eur. J. Immunol. 38 (2008) 2678-2688.

[18] S. Morimoto, Y. Kanno, Y. Tanaka, Y. Tokano, H. Hashimoto, S. Jacquot, C. Morimoto, S.F. Schlossman, H. Yagita, K. Okumura, CD134L engagement enhances human B cell Ig production: CD154/CD40, CD70/CD27, and CD134/CD134L interactions coordinately regulate T cell-dependent B cell responses, J. Immunol. 164 (2000) 4097-4104.

[19] S. Aggarwal, T. He, W. FitzHugh, K. Rosenthal, B. Feild, J. Heidbrink, D. Mesmer, S.M. Ruben, P.A. Moore, Immune modulator CD70 as a potential cisplatin resistance predictive marker in ovarian cancer, Gynecol. Oncol. 115 (2009) 430-437.

[20] J. Diegmann, K. Junker, B. Gerstmayer, A. Bosio, W. Hindermann, J. Rosenhahn, F. von Eggeling, Identification of CD70 as a diagnostic biomarker for clear cell renal cell carcinoma by gene expression profiling, real-time RT-PCR and immunohistochemistry, Eur. J. Cancer 41 (2005) 1794-1801.

[21] J. Wischhusen, G. Jung, I. Radovanovic, C. Beier, J.P. Steinbach, A. Rimner, H. Huang, J.B. Schulz, H. Ohgaki, A. Aguzzi, Identification of CD70-mediated apoptosis of immune effector cells as a novel immune escape pathway of human glioblastoma, Cancer Res. 62 (2002) 2592-2599.

[22] S. Lens, P. Drillenburg, B.F. Den Drijver, G. Van Schijndel, S.T. Pals, R.A. Van Lier, M.H. Van Oers, Aberrant expression and reverse signalling of CD70 on malignant B cells, Br. J. Haematol. 106 (1999) 491-503.

[23] B.J. Hicke, C. Marion, Y.-F. Chang, T. Gould, C.K. Lynott, D. Parma, P.G. Schmidt, S. Warren, Tenascin-C aptamers are generated using tumor cells and purified protein, J. Biol. Chem. 276 (2001) 48644-48654.

[24] P. Dua, S. Kim, D.-k. Lee, Nucleic acid aptamers targeting cell-surface proteins, Methods 54 (2011) 215-225.

[25] G. Mayer, M.-S.L. Ahmed, A. Dolf, E. Endl, P.A. Knolle, M. Famulok, Fluorescence-activated cell sorting for aptamer SELEX with cell mixtures, Nat. Protoc. 5 (2010) 1993-2004.

[26] L. Wang, R. Wang, F. Chen, T. Jiang, H. Wang, M. Slavik, H. Wei, Y. Li, QCM-based aptamer selection and detection of *Salmonella typhimurium*, Food Chem. 221 (2017) 776-782.

[27] D.D. Dickey, P.H. Giangrande, Oligonucleotide aptamers: A next-generation technology for the capture and detection of circulating tumor cells, Methods 97 (2016) 94-103.

[28] G. Liu, X. Mao, J.A. Phillips, H. Xu, W. Tan, L. Zeng, Aptamer−nanoparticle strip biosensor for sensitive detection of cancer cells, Anal. Chem. 81 (2009) 10013-10018.

[29] L. Feng, Y. Chen, J. Ren, X. Qu, A graphene functionalized electrochemical aptasensor for selective label-free detection of cancer cells, Biomaterials 32 (2011) 2930-2937.

[30] J. Zhao, L. Zhang, C. Chen, J. Jiang, R. Yu, A novel sensing platform using aptamer and RNA polymerase-based amplification for detection of cancer cells, Anal. Chim. Acta 745 (2012) 106-111.

[31] M. Su, H. Liu, L. Ge, Y. Wang, S. Ge, J. Yu, M. Yan, Aptamer-Based electrochemiluminescent detection of MCF-7 cancer cells based on carbon quantum dots coated mesoporous silica nanoparticles, Electrochim. Acta 146 (2014) 262-269.

[32] X. Chen, Y. Pan, H. Liu, X. Bai, N. Wang, B. Zhang, Label-free detection of liver cancer cells by aptamer-based microcantilever biosensor, Biosens. Bioelectron. 79 (2016) 353-358.

[33] M. Yan, G. Sun, F. Liu, J. Lu, J. Yu, X. Song, An aptasensor for sensitive detection of human breast cancer cells by using porous GO/Au composites and porous PtFe alloy as effective sensing platform and signal amplification labels, Anal. Chim. Acta 798 (2013) 33-39.

[34] Y. Li, Y. Zhang, M. Zhao, Q. Zhou, L. Wang, H. Wang, X. Wang, L. Zhan, A simple aptamer-functionalized gold nanorods based biosensor for the sensitive detection of MCF-7 breast cancer cells, Chem. Commun. 52 (2016) 3959-3961.

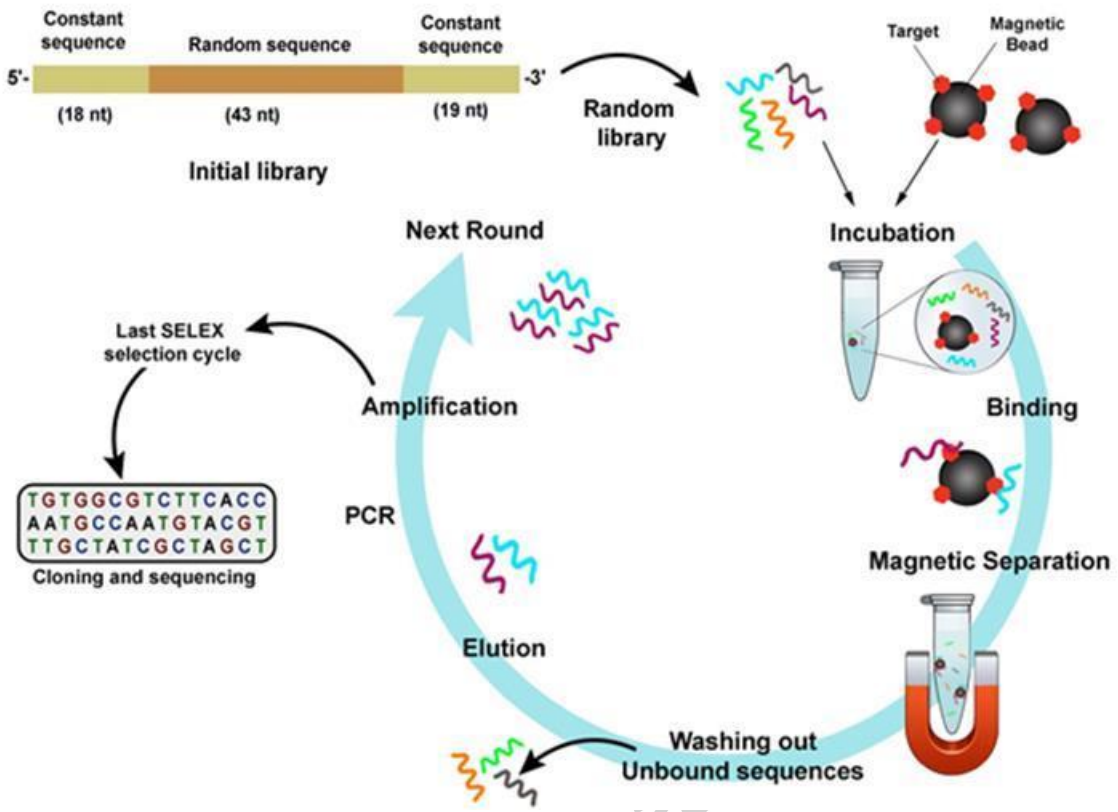


Fig. 1. Schematic illustration of protein-magnetic bead-based SELEX. The DNA aptamer library consists of a central region of random sequence (43 nucleotides in length) flanked by two fixed sequences (18 and 19 nucleotides in length) which enable primer annealing during amplification step of PCR. The SELEX procedure is performed by the repetition of consecutive steps of incubation, binding, partitioning, elution and amplification. In the first round of SELEX the library and magnetic bead-target complexes are incubated for binding. The bound sequences are separated from unbound ones by a magnetic field. The obtained oligonucleotides are collected and amplified using PCR to enrich the aptamer pool. The amplified sequences are then used for the next round of selection. After confirmation of aptamer enrichment for binding to target, cloning and sequencing steps are carried out.

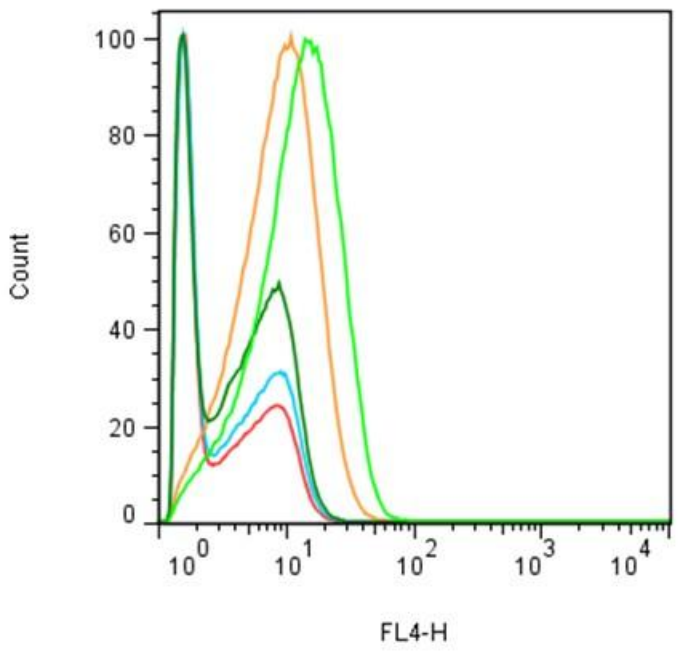


Fig. 2. Evaluation of aptamer enrichment for binding to protein CD70 after 10 rounds of protein-based SELEX. All sequences were labeled with ATTO 647N. Red: control, blue: library, dark green: 10^{th} round ssDNAs, orange: 3^{rd} round ssDNAs, light green: 9^{th} round ssDNAs.

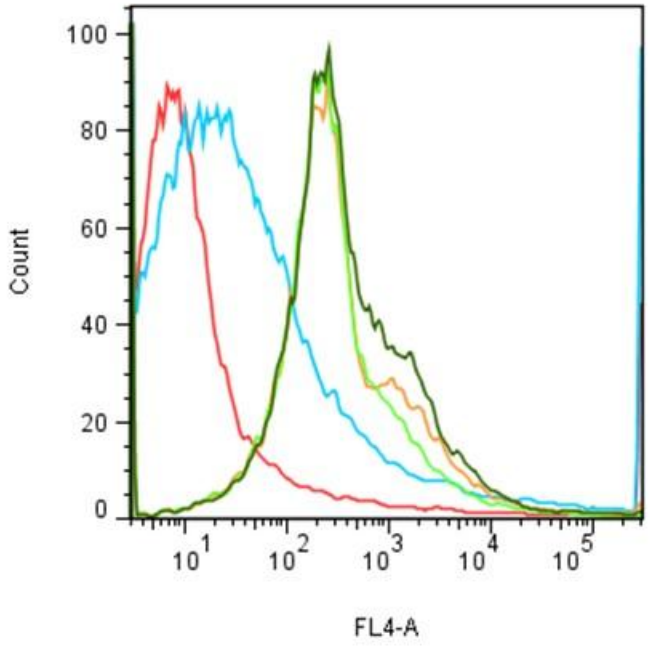


Fig. 3. Evaluation of aptamer enrichment for binding to CD70 at the surface of SKOV-3 cell using cell-SELEX. Red: control, blue: library, orange: 1st round, light green: 4th round, dark green: 7th round.

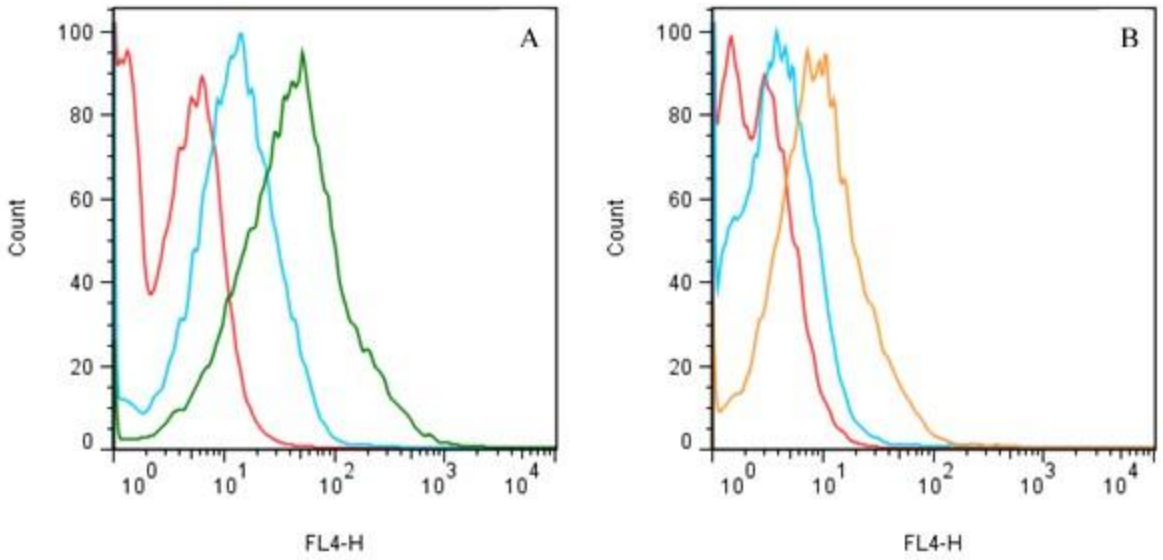


Fig. 4. (A) Binding of Apt928 to CD70 expressed on the surface of SKOV-3 cell line. Red: control, blue: library, green: Apt928. (B) Binding of Apt928 to CD70 expressed on the surface of A2780 cell line. Red: control, blue: library, orange: Apt928.

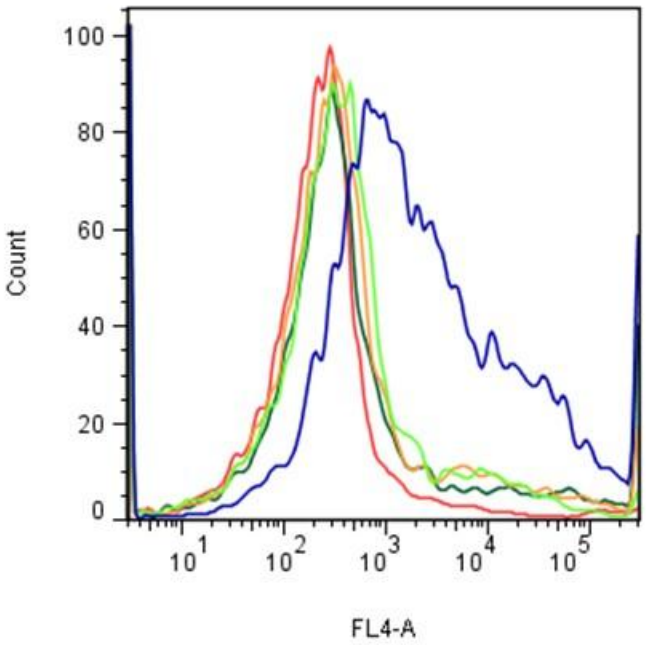


Fig. 5. Specificity evaluation of Apt928 for CD70 protein. Red: control (bead alone), Dark green: Bead-Aptamer, Orange: Bead-Protein PD-L1-Aptamer, Light green: Bead-Protein CD25-Aptamer, Dark blue: Bead-Protein CD70-Aptamer.

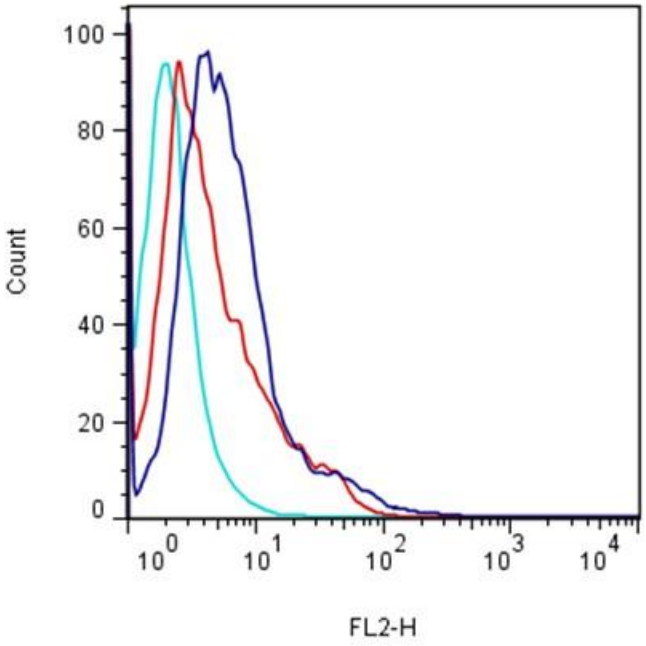


Fig. 6. Further confirmation of specific binding of Apt928 to CD70 through blocking assay. Pre-incubation of SKOV-3 with aptamer could prevent CD27 binding (CD70 receptor) to CD70. Light blue: unstained SKOV-3 cells, red: SKOV-3 cells pre-incubated with Apt928, dark blue: SKOV-3 cells without Apt928 treatment.

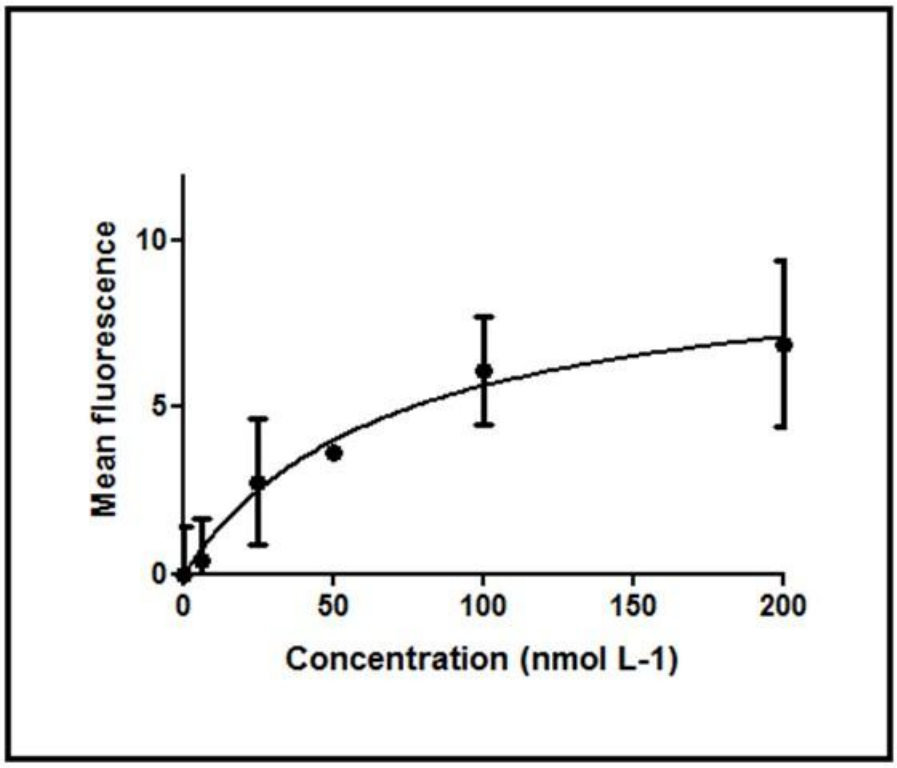


Fig. 7. K_d determination through binding analysis of Apt928 to beads coated with CD70 protein using flow cytometry. Based on non-linear regression analysis (the equation Y = B max X/(K_d + X)) the K_d value of Apt928 was calculated to be 66 nmol L-1 (n = 2).

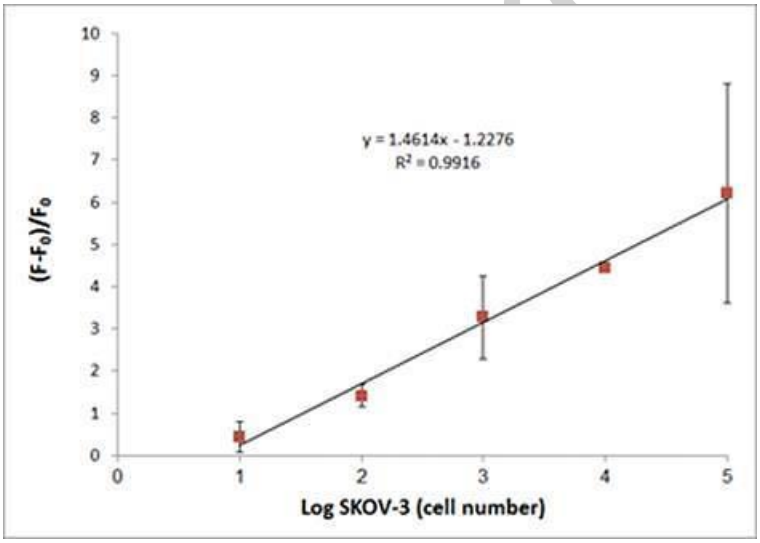
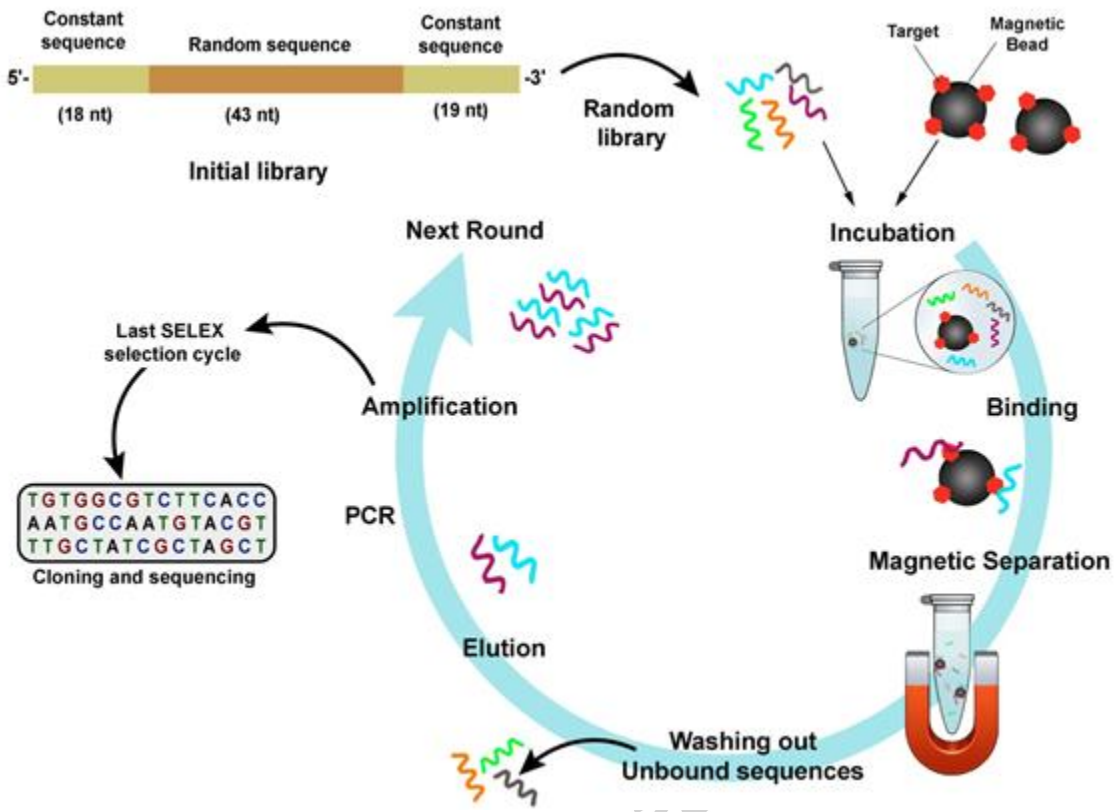


Fig. 8. Relative fluorescence intensity of ATTO 647N–labeled Apt928 after incubation of a fixed concentration of aptamer with varying numbers of SKOV-3 cells. F = the average of mean fluorescence intensity of tumor cells treated with aptamer, F_0 = the average of mean fluorescence intensity of the tumor cells without aptamer treatment (n = 3).



Graphical Abstract

Table 1. Comparison of different cancer cell detection methods.

Methods	Materials	Aptamer(s)	Target cells	Detection limit	References
Electrochemical impedance spectroscopy (EIS)	Graphene	AS1411	HeLa	794 cells mL-1	[29]
Aptamer-based sandwich structure	ssT7RNA polymerase promoter sequences	MUC- 1 Aptamer	MCF-7	500 cells mL-1	[30]
Electrochemiluminescence (ECL)	AuNP graphene complex	MUC-1 Aptamer	MCF-7	230 cells mL-1	[31]
Aptamer-based microcantilever biosensor	microcantilever array	TLS11a Aptamer	HepG2	300 cells mL-1	[32]
ECL	Graphene oxide/Au composites	MUC-1 Aptamer	MCF-7	38 cells mL-1	[33]
Localized Surface Plasmon Resonance (LSPR)	Gold nanorods	MUC-1 Aptamer	MCF-7	100 cells mL-1	[34]
aptamer-nanoparticle strip biosensor (ANSB)	gold nanoparticles (Au-NPs)	TD05 & TE02 Aptamer	Ramos	800 cells mL-1	[28]
Flow cytometry	Without any additional material	Apt928	SKOV-3	14 cells mL-1	This study

Highlights

- A CD70-binding aptamer was isolated by protein-based SELEX followed by cell-based SELEX
- The isolated aptamer was able to bind to CD70 with high affinity and high specificity
- Interaction of CD70 with its receptor (CD27) was inhibited by CD70-binding aptamer
- The obtained aptamer could detect SKOV-3 cell line as low as 14 cells mL-1