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Selection of Aptamers for Molecular Recognition and Characterization of Cancer Cells

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In this paper, we describe a new way to generate molecular probes for specific recognition of cancer cells. Molecular medicine will require a large number of probes for molecular recognition and characterization of a variety of diseased cells. Aptamers, single-stranded DNA/RNA probes, are poised to become a chemist's antibody and have the potential to serve as molecular probes for a variety of biomedical applications. By applying newly developed cell-SELEX (cell-based systematic evolution of ligands by exponential enrichment) against whole living cells, panels of aptamers have been evolved from an initial DNA library to characterize target cells at the molecular level. Ramos cells, a B-cell lymphoma cell line, were used as target cells for the generation of effective molecular probes. By taking advantages of the repetitive and broad enrichment strategy, the selected aptamers could bind to target cells and other closely related cell lines in variant patterns with an equilibrium dissociation constant (Kd) in the nanomolar range. Some aptamers could also specifically recognize the target lymphoma cells mixed with normal human bone marrow aspirates. The cell-based SELEX is simple, fast, and robust. The strategies used here will be highly useful for aptamer selection against complex target samples in order to generate a large number of aptamers in a variety of biomedical and biotechnological applications, paving the way for molecular diagnosis, therapy, and biomarker discovery.

One of the major problems in molecular medicine today is the lack of molecular level understanding of diseased cells. For example, cell membrane proteins play an indispensable role in cell growth, proliferation, and signaling.[1,2] Understanding the molecular basis of a cell's membrane is crucial for uncovering the cellular life processes, as well as the disease mechanisms at the molecular level.[3] Cancers, for example, usually originate from gene mutations. They often cause alterations in the expression level and/or function of cell membrane receptors, leading to a systematic disorder of cellular metabolism, signaling, and proliferation.[4,5] Hence, the bioassay and study of cell membrane receptors provide us with a remarkable opportunity for cancer investigation, diagnosis, and therapy. Unfortunately, the discovery of effective membrane biomarkers targeting a particular cancer remains a tremendous challenge for current technologies, which is reflected by the fact that very few biomarkers are available for effective cancer diagnosis.[6−9] The lack of effective molecular probes recognizing the cell membrane receptors hindered our understanding of the molecular basis of cancers as well as the effective diagnosis of and therapy for cancer.

For example, Burkitt's lymphoma, an acute blood cell cancer, is one of the most progressive of all human cancers.[10] Early diagnosis and targeted therapy are crucial for treating its victims. However, Burkitt's lymphoma is immunophenotypically indistinguishable from many other large B-cell lymphomas and is usually diagnosed by inspecting the morphology of blood cells and the patient's symptoms.[11,12] The lack of effective molecular probes to recognize cell surface biomarkers prevents early diagnosis of Burkitt's lymphoma and makes the study of its developing mechanism difficult or even impossible. In this study, we will develop molecular aptamers for the specific recognition of Burkitt's lymphoma cells.

Molecular level understanding of diseases requires effective molecular probes. Currently, there are not enough usable molecular probes for the study of human diseases. The development of new molecular probes has proven to be a difficult, costly, and time-consuming process. Of the many potential probes, a new class of designer nucleic acids (called aptamers) holds great potential in

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molecular recognition and targeting. In many ways, aptamer is a chemist's antibody. Aptamers emerged as a novel class of molecules that could recognize a variety of molecules ranging from macromolecules such as proteins to small compounds like ATP.[13−15] Aptamers are single-stranded oligonucleotides that can specifically bind to their targets.[16] They are generated through an in vitro selection process termed SELEX (system evolution of ligands by exponential enrichment), which involves repeated competitive binding to target molecules starting from an initial library containing 10^{15} random DNA or RNA sequences.[17,18] There are a few important advantages to use aptamers as molecular probes in molecular medicine research and development. Their advantages include economical and reproducible synthesis, excellent affinity and specificity for molecular recognition, biocompatibility, and flexible modification.[19−21] After more than a decade's development, aptamers have shown ample potential and have a promising future in the fields of chemical biology, biomedicine, and biotechnology.[21−23] Most aptamers reported so far are selected against simple entities, such as a purified protein. Recently, SELEX targeting complex objects, such as blood cell membranes and endothelial cells, was also reported.[24−27] We have just reported a systematic study of using cell-SELEX to select a panel of aptamers for cancer cells.[27] In comparison to current technologies for probe development, the cell-SELEX aptamer selection process has demonstrated its strengths with fast enrichment, easy operation, no requirement that the details of complex targets be known, and the capability of obtaining a group of probes simultaneously. All of these traits render it suitable for use as an efficient way to discover cell membrane biomarkers.[27] In our previous work, a cell-SELEX strategy was developed to produce a panel of aptamers that could characterize the molecular differences between any two types of cell lines. Most of the selected probes showed specific responses to target cells and little or no response to negative control cells. The newly generated probes have been used in molecular profiling of cancer and in cancer cell enrichment.[27−30]

In practice, it would be helpful to use pattern recognition for disease diagnosis and therapy, which require molecular probes comprehensively targeting cell surface receptors. To achieve this goal, the selection should allow the gradual and broad enrichment of aptamers. In this paper, we demonstrated a new SELEX strategy to obtain the aptamers showing various response patterns against relevant cells, which are potentially useful for pattern recognition application. Specifically, we have selected a group of aptamers to recognize Ramos cells, a Burkitt's lymphoma cell line. This cell line is important in lymphoma diagnosis and therapy. A variety of single-stranded DNA aptamers have been generated for Ramos cells with strong affinity, and some of them have excellent specificity. The selected probes should be helpful for understanding the molecular interactions and recognition events on Ramos cells, as well as for the future medical diagnosis and treatment of lymphoma.

MATERIALS AND METHODS

Cell Lines and Reagents. Ramos (CRL-1596, B lymphocyte, human Burkitt's lymphoma), CCRF-CEM (CCL-119, T lymphoblast, human acute lymphoblastic leukemia), and Toledo (CRL-2631, B lymphocyte, human diffuse large cell lymphoma) were obtained from ATCC (American Type Culture collection) and were cultured in RPMI 1640 medium (ATCC) supplemented with 10% fetal bovine serum (FBS) (heat inactivated, GIBCO) and 100 IU/mL penicillin−streptomycin (Cellgro). The wash buffer contained 4.5 g/L glucose and 5 mM $MgCl_2$ in Dulbecco's PBS (Sigma). Binding buffer used for selection was prepared by adding yeast tRNA (0.1 mg/mL) (Sigma) and BSA (1 mg/mL) (Fisher) into the wash buffer to reduce background binding. Antibodies against CD19 and CD45 were purchased from BD Biosciences. Trypsin and proteinase K were purchased from Fisher biotech. The Taq-polymerase and dNTPs used in PCR were obtained from Takara.

SELEX Primers and Library. A fluorescein isothiocyanate (FITC)-labeled 5′-primer (5′-FITC- AAG GAG CAG CGT GGA GGA TA-3′) and a triple biotinylated (trB) 3′-primer (5′-trB- ACC ACG ACG ACA CAC CCT AA-3′) were used in the PCR. The SELEX library consisted of a central randomized sequence of 45 nucleotides (nt) flanked by two 20 nt primer hybridization sites (5′- AAG GAG CAG CGT GGA GGA TA - N_{45} - TTA GGG TGT GTC GTC GTG GT -3′). To minimize the possibility of nonspecific amplification of random library sequences in PCR, the primers and library sequences were carefully optimized by using oligonucleotide prediction software (Oligoanalyzer, Integrated DNA Technologies). After incubation and binding on streptavidin-coated sepharose beads (Amersham Bioscience), the double-stranded PCR product was denatured in alkaline condition (0.2 M NaOH) to separate the FITC-conjugated sense ssDNA strand from the biotinylated antisense ssDNA strand, after which the sense ssDNA strand could be used for next round selection.

SELEX Protocol. The process of whole cell selection was as follows: ssDNA pool (200 pmol) dissolved in 400 μL of binding buffer was denatured by heating at 95 °C for 5 min and cooled on ice for 10 min before binding. Then the ssDNA pool was incubated with $1-2 \times 10^6$ Ramos cells on ice and shaken at 200 rpm for 45 min. After washing, the bound DNAs were eluted by heating at 95 °C for 5 min in 300 μL of binding buffer. After centrifugation, the supernatant was desalted and then amplified by PCR (10−20 cycles of 30 s at 94 °C for denaturing, 30 s at 60 °C for annealing, and 30 s extension at 72 °C, followed by 5 min extension at 72

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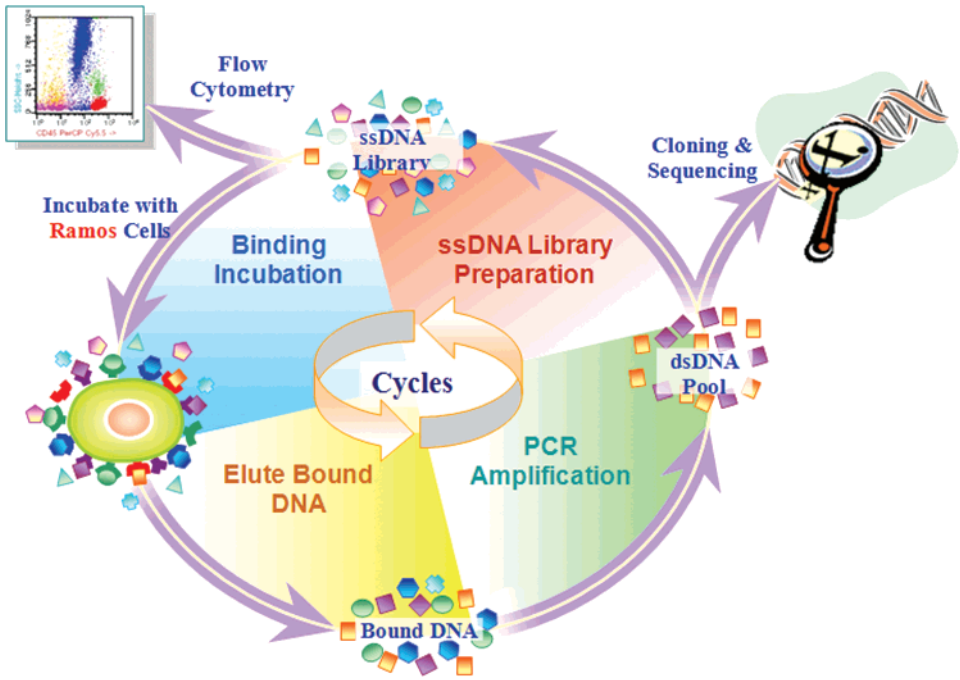

Figure 1. Schematic of the whole living cell-based aptamer selection. The ssDNA pool was incubated with Ramos cells (target cells). After washing, the bound DNAs were eluted by heating to 95 °C for 5 min. The eluted DNAs were amplified by PCR. The double-stranded PCR products were separated into ssDNAs, and the sense strand DNAs were collected for the next round selection or tested by flow cytometry to monitor the SELEX progression. When the selected pool was enriched enough, the PCR product of the evolved pool was cloned and sequenced for aptamer identification.

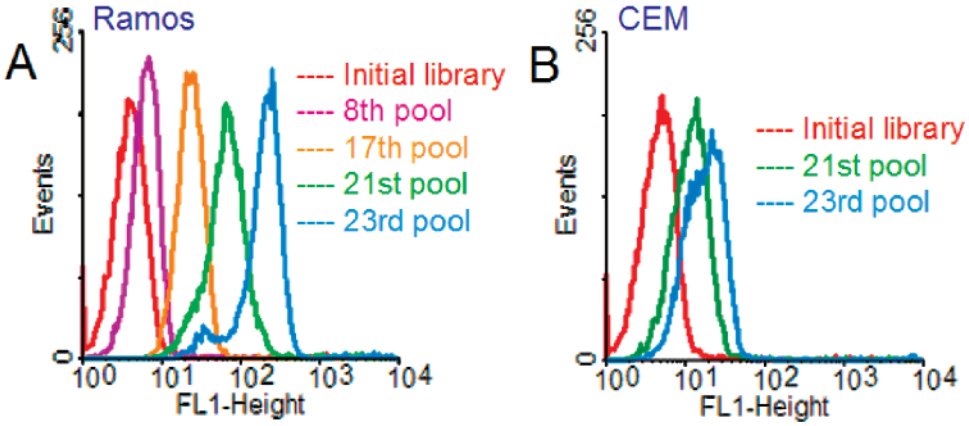

Figure 2. Cytometry results of selected pools with Ramos and CEM cells. The red curve represents the nonspecific binding of the unselected DNA library with Ramos or CEM cells. (A) The fluorescence intensity of Ramos cells binding with selected pools increased gradually as the selection progressed, indicating the enhanced binding affinity of enriched pools. (B) The selected pools also showed increased affinity with CEM cells, but the response was about 1 order of magnitude weaker than with Ramos.

°C). The selected sense ssDNA was separated from the biotinylated antisense ssDNA strand by streptavidin-coated sepharose beads (Amersham Bioscience). The initial library containing 20 nmol of random DNA was dissolved in 1 mL of binding buffer and used for the first round selection. To evolve the aptamers with high affinity and specificity, the wash strength was enhanced gradually by extending the wash time (from 1 to 10 min) and increasing the volume of wash buffer (from 1 to 5 mL) and the number of washes (from 1 to 4 times). Furthermore, 10% FBS and 50−300-fold molar excess genomic DNA were added into the incubation solution to decrease the nonspecific binding. After 23 rounds of selection, the enriched ssDNA pool was PCR-amplified using unmodified primers and cloned into *Escherichia coli* using the TA cloning kit (Invitrogen). The aptamer candidate sequences were obtained from the Genome Sequencing Services Laboratory at the University of Florida.

Flow Cytometric Analysis. To monitor the enrichment of aptamer candidates during selection, FITC-labeled ssDNA pool was incubated with 2×10^5 Ramos cells in 200 μL of binding buffer containing 10% FBS on ice for 30 min. Cells were washed twice with 0.7 mL of binding buffer (with 0.1% NaN_3) and suspended in 0.4 mL of binding buffer (with 0.1% NaN_3). The fluorescence was determined with a FACScan cytometer (Becton Dickinson Immunocytometry Systems, San Jose, CA) by counting 30 000 events. The FITC-labeled initial ssDNA library was used as background sample.

The binding affinity of selected aptamers was measured by incubating Ramos or CCRF-CEM cells (5×10^5) with a series of

Table 1. Response and Kds of Identified Aptamers[a]

aptamer	Ramos	CEM	Toledo	sequences	Kd (nM)
TC01	++++	++++	+++	ACCAAACACAGATGCAACCTGACTTCTAACGTCATTTGGTG	17.8 ± 2.1 (Ramos) 48.5 ± 3.9 (CEM)
TC02	− − −	+++	− − −	AGCATCAACAAGGTCATAAAAACACGTCAGCTCCTTCACATTTGCC	795 ± 260 (CEM)
TD02	+	− − −	− − −		
TD04	− − −	+	− − −		
TD05	++++	− − −	− − −	AACACCGTGGAGGATAGTTCGGTGGCTGTTCAGGGTCTCCTCCCGGTG	74.7 ± 8.7 (Ramos)
TD07	− − −	+	− − −		
TD08	++++	++	++	AAGGAGCAGCGTGGAGGATACTCTAATTGCCGTATAAGGTCAGGGGGTTGGTTGGTTCC-TAGTGCTTAGGGTGTGTCGTCGTGGT	3.26 ± 0.13 (Ramos)
TD09	+	− − −	+	AAGGAGCAGCGTGGAGGATAGCTGTCATCGGCTTATGTGCTTTCCAGGGTCCGTGCTGTC-CGTTGTTAGGGTGTGTCGTCGTGGT	5.15 ± 0.96 (Ramos)
TE01	+	− − −	− − −		
TE02	+++	− − −	− − −	TAGGCAGTGGTTTGACGTCCGCATGTTGGGAATAGCCACGCCT	0.76 ± 0.13 (Ramos)
TE03	+	− − −	− − −		
TE04	+++	+	− − −	TCCTCGATGCACCAGTTCACCTTATTTGCTTCTTCTCTCTGTTCTGACTGGGTGCTGAGGA	207 ± 41 (Ramos)
TE08	+	+	+		
TE13	++++	++++	+++	AGGCCCCCAGGCTCGGTGGATGCAAACACATGACTATGGGCCCGT	202 ± 52 (Ramos) 144 ± 15 (CEM)
TE16	− − −	+	− − −		
TE17	− − −	+++	− − −	CAGCTACGCAATACAAAACTCCGAACACCTGCTTCTGACTGGGTGCTG	675 ± 131 (CEM)
TE18	+	− − −	+		
TE22	+	− − −	+		

[a] The sequences of aptamers showing high response toward cells are listed, in which some sequences have been optimized. A threshold based on fluorescence intensity of FITC in the flow cytometry analysis was set so that 99% of cells incubated with the FITC-labeled unselected DNA library would have fluorescence intensity below it. After binding with FITC-labeled aptamer, the percentage of the cells with fluorescence above the set threshold was used to evaluate the binding capacity of the aptamer to the cells: − − −, <10%; +, 10−35%; ++, 35−60%; +++, 60−85%; ++++, >85%. The cell line used for Kd detection is indicated in the following brackets.

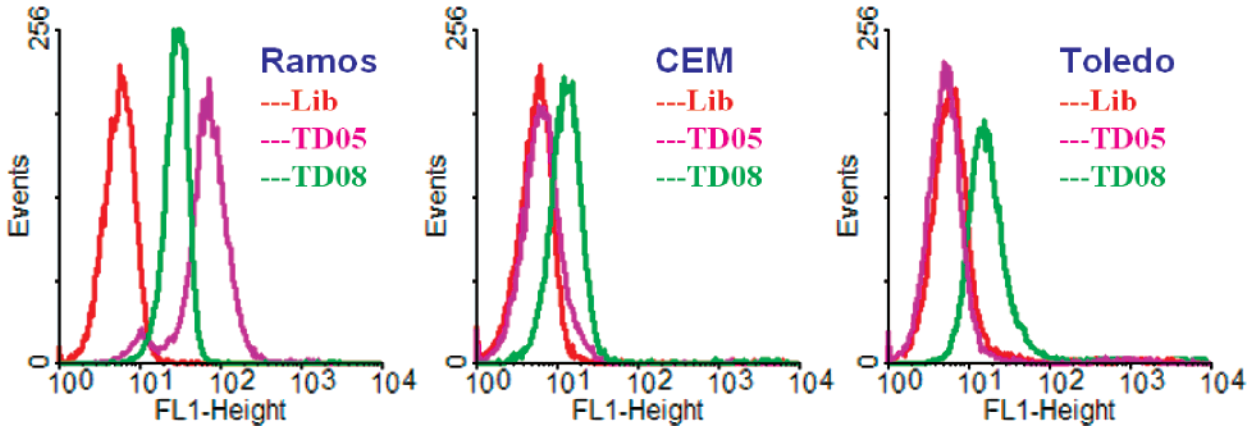

Figure 3. Identification of aptamer candidates. Flow cytometry assay for the binding capacity of the FITC-labeled sequence TD05 and TD08 with Ramos cells, Toledo cells, and CCRF-CEM cells was used. The concentration of the aptamer candidates in the binding buffer was 250 nM.

FITC-labeled aptamers dissolved in a 200 μL volume of binding buffer containing 10% FBS on ice for 30 min. Cells were then washed twice with 0.6 mL binding buffer containing 0.1% sodium azide, after which the cells were suspended in 0.4 mL binding buffer with 0.1% sodium azide and subjected to flow cytometric analysis. The FITC-labeled initial ssDNA library was used as the control sample to determine the nonspecific binding. After subtracting the mean fluorescence value of the control sample, the mean fluorescence intensity of target cells bound with aptamers was used to calculate the equilibrium dissociation constants (Kd) of the aptamer−cell interaction.[29,30] By using the SigmaPlot software (Jandel Scientific, San Rafael, CA), the apparent Kds of the aptamer−cell interaction were evaluated according to the dependence of fluorescence intensity of specific binding on the concentration of the aptamers, which was well-known as the one binding site equation $Y = \mathrm{Bmax}X/(\mathrm{Kd} + X)$, where the Y represents the bound fraction, the Bmax is the saturated binding, and the X is the concentration of ligand.

In the experiments which used aptamers to detect Ramos cells in real biological samples, FITC-labeled aptamers were mixed with PE or PE-Cy5-labeled antibodies of CD19, and CD45, and incubated with 2×10^5 cancer cells and/or 2×10^5 cells in normal human bone marrow aspirates. After incubation and washing, the samples were analyzed with a FACScan cytometer (Becton Dickinson Immunocytometry Systems, San Jose, CA). The compensation of three fluorescence channels was carefully set up each

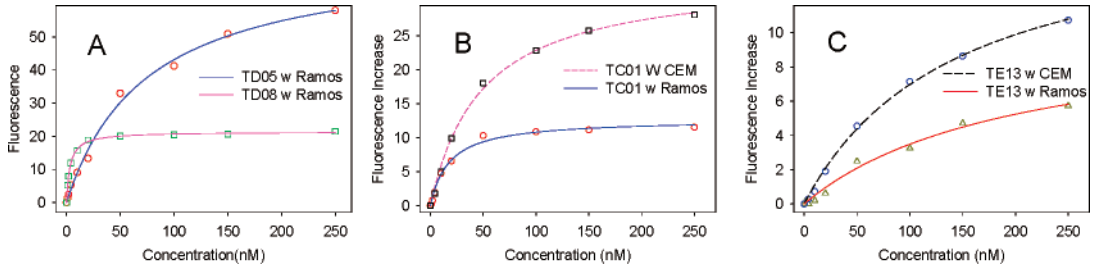

Figure 4. Kd determination of aptamers. Flow cytometry assay was employed for the binding affinity measurement. (A) Binding curves of TD05 and TD08 with Ramos cells; (B) TC01 showed different binding affinities with Ramos and CEM cells; (B) TE13 showed different binding affinities with Ramos and CEM cells.

time to eliminate the signal interferences between three channels.

Proteinase Treatment for Cells. After washing with 2 mL of wash buffer, 5×10^6 of Ramos or RRCF-CEM cells were incubated with 1 mL of 0.05% Trypsin/0.53 mM EDTA in HBSS or 0.1 mg/mL proteinase K in PBS at 37 °C for 2.5 and 11 min. To quench the proteinase digestion, the sample was quickly mixed with 200 μL of FBS and placed on ice. Then the treated cells were washed with 2 mL of binding buffer and used for the aptamer binding assay as described in the aforementioned flow cytometric analysis.

RESULTS AND DISCUSSION

Using Living Cells as Targets for Cell-SELEX to Generate Aptamer Candidates. Figure 1 illustrates the whole cell-SELEX strategy used in our work, whereas the detailed procedures are depicted in the Materials and Methods. To evolve the aptamers broadly recognizing the cell membrane proteins on relevant cells, only target cells are used in this cell-SELEX for positive selection, which is different from our previous study.[27] Ramos cells were used as the target for the generation of molecular probes to recognize B-cell lymphoma. We believe that the multiple round of SELEX will result in the selection of the most effective aptamers without any possibility of missing the important receptors. The potential loss of aptamer candidates in the negative selection is not a concern here. With the results we are going to show below, it is clear that this strategy works for Ramos cells.

A single-stranded DNA library containing a 45 mer random sequence region flanked by two 20 mer PCR primer sequences was used in this selection. After denaturation and being placed on ice, the library was incubated with the target cells to allow DNA sequences to bind to molecules on cell membranes. Then the cells were rinsed and spun to remove supernatant containing unbound DNAs, after which the DNA sequences bound to the cell surface were eluted by heating. The collected DNAs were amplified by PCR for the next round of selection. After multiround selection, the aptamer candidates that bound to the target cells were enriched.

The evolutionary DNA sequences labeled with FITC dye after each selection round were incubated with target cells. Flow cytometry was employed to monitor the fluorescence intensity of labeled cells. The progress of the selection was represented by the binding capacity of the enriched DNA pool to the target cells. With an increasing number of selection rounds, steady enhancement in fluorescence intensity on the Ramos cells was observed as shown in Figure 2, indicating that DNA sequences with increased binding affinity to the target cells were enriched. In contrast, the selected pools' affinities to a control cell line (CCRF-CEM) were also investigated. These showed about 1 order of magnitude lower fluorescence signals than that displayed by the Ramos cells, implying a good selectivity of enriched DNA sequences for Ramos cells.

Identification of Aptamer Candidates. After about 20 rounds of selection, the enriched pool presented a considerable increase in affinity for the target cells compared to the initial DNA library. Then the highly enriched DNA pool was cloned and sequenced using a high-throughput genome-sequencing method. According to the homology of the DNA sequences of individual clones, the candidates were classified into families containing similar DNA sequences. The number of repeats in a sequence family was considered important in preselecting potential aptamers from many different families.

A total of 32 aptamer candidates were chosen for further synthesis and analysis due to their high abundance in the DNA sequence result. The binding abilities of the candidates toward target cells were evaluated using flow cytometry. Three cell lines, Ramos and two other blood cell lines including CCRF-CEM and Toledo, were used to test the binding features of the aptamer candidates. Eighteen aptamer candidates showed significant binding to the target cells, of which eight aptamers had high affinities for the target cells (see Table 1).

Characterization of the Selected Aptamers. The aptamer binding patterns were varied and could be classified into four groups. Some of the aptamers, such as TD05 (see Figure 3), TD02, and TE03, showed specific responses to Ramos cells but no binding to CCRF-CEM or Toledo cells. Some aptamers had a strong response to Ramos cells and a weak response to CCRF-CEM and/or Toledo cells, such as TD08 (see Figure 3), TE01, TE02, and TE04. The third group of sequences, such as TC01, TE08, and TE13, showed equal response to all three cell lines. The most interesting group, including TC02, TD04, TD07, TE16, TE17, and TE22, did not show obvious response to Ramos cells but demonstrated binding capacity with CCRF-CEM and/or Toledo cells. These results indicate that the aptamers can be enriched effectively in spite of their low-level expression on the target cell membrane. This is a reasonable result of the selection applied with gradual enrichment strategy. The binding patterns constructed using multiple aptamers represent molecular signatures on the target cells and provide useful information for understanding the relationship between target cells and relevant cells at the molecular level.

Evolved Aptamers Presented Various Recognition Patterns against Relevant Cell Lines. As aforementioned, the

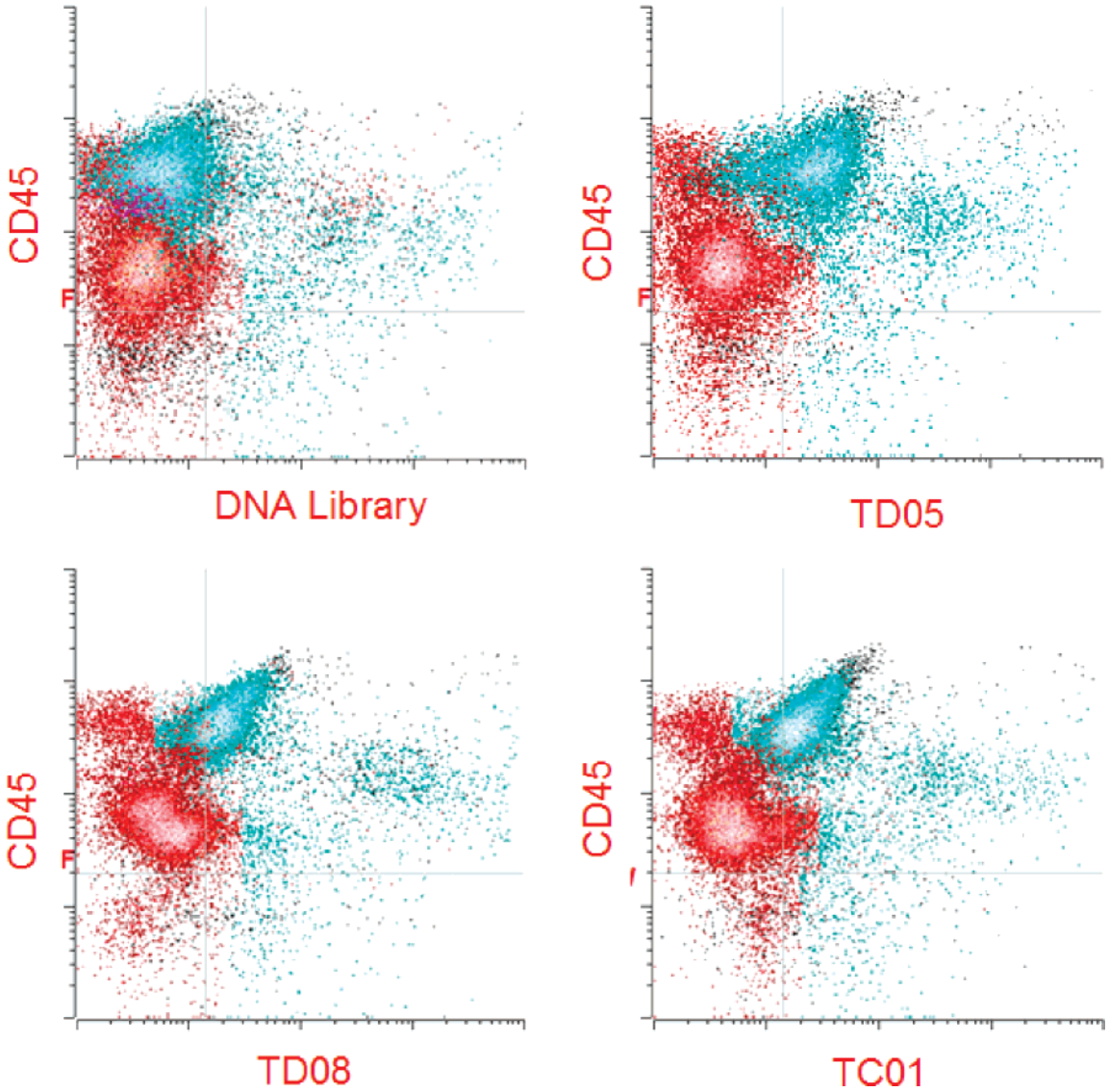

Figure 5. Recognition of Ramos cells in the mixture containing human bone marrow cells. The FITC-labeled aptamer (TD05, TD08, or TC01) and monoclonal antibodies were incubated with Ramos cells and/or bone marrow cells. The Ramos cells were plotted in blue color, whereas the bone marrow cells were in red. TD05, TD08, and TC01 were able to distinguish the Ramos cells specifically when it was mixed with human bone marrow aspirates. CD45 and CD19 were used in these samples to classify the cell groups in cell mixture.

evolved aptamers gave various response patterns toward three relevant cells. It is worth noting that some cloned candidates did not show obvious binding to Ramos cells in flow cytometry but did present obvious binding against CEM and/or Toledo cells. This should not be interpreted as the nonspecific or simply random presence of these sequences in the selected pool. In fact, these candidates may target receptors of low density on the target cell surface so that the binding cannot be detected by conventional flow cytometry. For example, sequence TE17 showed a low response to Ramos cells similar to that of the initial DNA library, but it had a strong interaction with the control CCRF-CEM cells. One explanation could be that the target of TE17 was present on both cells but the expression level on Ramos cells was considerably lower than that of CEM cells. It is thus implied that the gradually and broadly evolving and enriching selection strategy used here might allow the enrichment of aptamer candidates targeting lowly expressed receptors on cell membranes and, thus, could potentially provide a broader molecular spectrum of the target cell membranes.

Evolved Aptamers Show a High Affinity with Target Cells. To evaluate the binding affinities of evolved aptamers, the apparent Kds were measured using flow cytometry. As shown in Figure 4A, aptamers TD05 and TD08 recognize the Ramos cells with apparent Kds of 74.7 ± 8.7 and 3.26 ± 0.13 nM, respectively. Other aptamers possess Kds in the picomolar to nanomolar range (see Table 1). The high affinity of selected aptamers is a result of the stringent washing conditions in the later rounds of selection, which removed aptamer candidates that were weakly bound to their targets. This high affinity of the aptamers for their target cells is absolutely essential if we hope to apply them to disease diagnosis and drug delivery.

Interestingly, some aptamers capable of binding with multiple cell lines showed different Kds with those cell lines. TC01, for instance, had a Kd of 48.5 nM with CEM cells, which was about 3 times of that with Ramos cells at 17.8 nM (Figure 4B). Another aptamer, TE13, had a slight higher Kd with Ramos (202 nM) than its Kd with CEM cells (144 nM) (Figure 4C). These Kd discrepancies are reasonable as there is no negative selection in our cell-

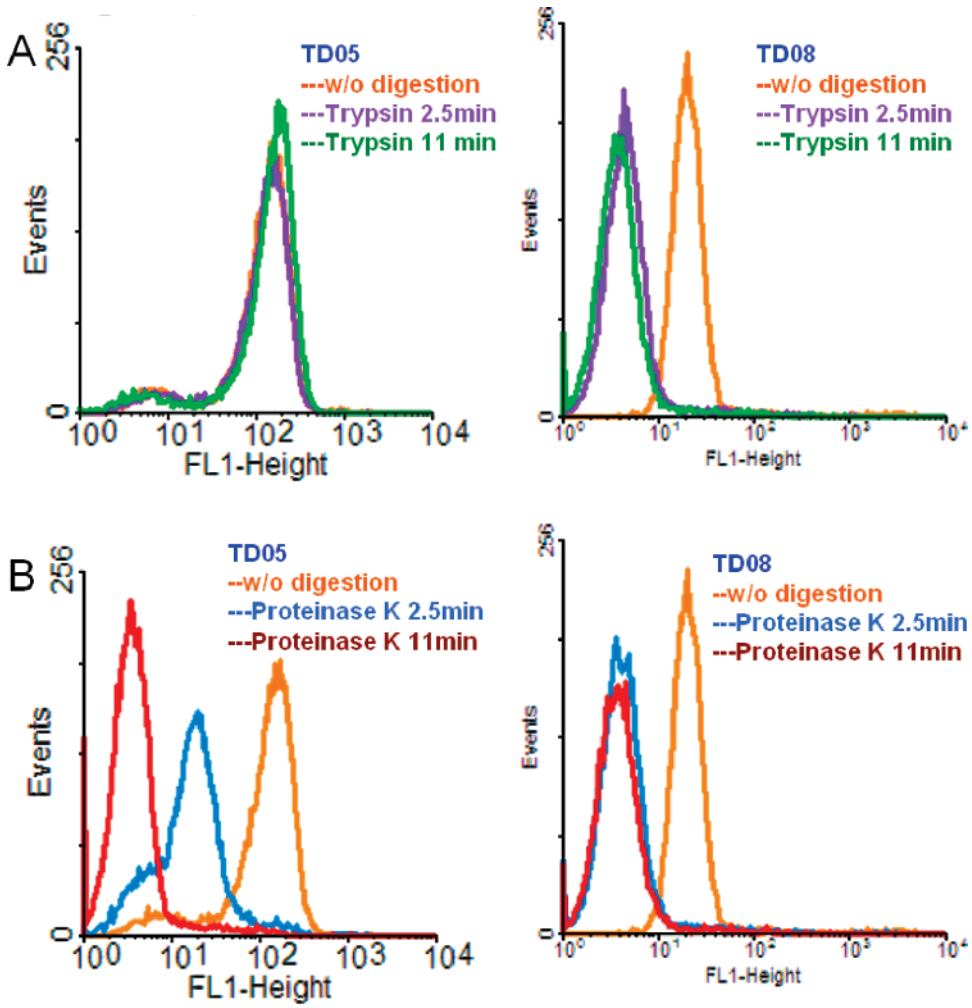

Figure 6. Effects of trypsin (A) and proteinase K (B) digestion on the binding of TD05 and TD08. The Ramos cells were treated with trypsin or proteinase K for 2.5 or 11 min before incubating with aptamers. The binding of TD05 was slightly affected by trypsin digestion but totally quenched by proteinase K.

SELEX. In addition, some of these might be caused by variation in the Kd measurements and the different status of the receptors on the cell surface.

Highly Specific Recognition of Target Cells Mixed with Real Biological Samples. The specific recognition of target cells in real biological samples remains a great challenge for many molecular probes. To determine the capability of the evolved aptamers as probes for specific receptor recognition, FITC-labeled aptamers and monoclonal antibodies were used to detect Ramos lymphoma cells mixed with normal human bone marrow aspirates. TD05, TD08, and TC01 were selected as representatives of our aptamers with three different response patterns. Human bone marrow is a complex mixture consisting of a variety of cells including mature and immature granulocytes, nucleated erythrocytes, monocytes, mature and immature B cells, as well as T cells. The results showed that TD05 only recognized Ramos cells (Figure 5) and did not bind to normal CD19-positive B cells or any other bone marrow cells. Aptamers TD08 and TC01 also showed highly specific recognition of Ramos cultures, distinguishing the Ramos cells from mixed human bone marrow cells. The capacity of the selected aptamer probes to correctly identify the target cells in a real biological sample makes cell-SELEX a promising approach for discovering effective molecular probes for recognition of disease biomarkers and applying them to both clinical practice and medical research.

Receptors of the Aptamers on the Target Cells Are Most Likely Proteins. The aptamer targets could be a variety of molecules including protein, sugars, small organics, and ions. To prove that the aptamers evolved from cell-SELEX bind to cell membrane proteins, Ramos cells were treated with trypsin or proteinase K for several minutes before incubation with the aptamers. As shown in Figure 6, after 11 min of treatment, aptamers TD08, TE02, and TE04 lost their binding to the cells, whereas aptamer TE13 showed gradually reduced binding capacity. Interestingly, TD05 totally lost its binding to the Ramos cells after proteinase K treatment but was not affected by trypsin. The results above clearly suggest that the targets of the aptamers are most likely membrane proteins or tightly associated with them.

In this report, a whole living cell-SELEX strategy for effective and broad enrichment of aptamers has been designed and applied to generate a group of aptamers. These aptamers can be used as molecular probes to characterize target cells at the molecular level. The evolved aptamers demonstrated various response patterns toward target cells and relevant cell lines, portraying the molecular spectra of these cell lines' membranes receptors and potentially providing an effective approach to the discovery of biomarkers

as a molecular signature of diseases. Prior knowledge of specific receptors on the cell surface is not required in our search for the molecular probes for diseased cell recognition. This will significantly simplify the process of molecular probe development and molecular elucidation of biological mechanism studies. The whole cell selection process is simple, rapid, reproducible, and straightforward. Most of the evolved aptamers can bind to target cells with Kds in the nanomolar range. Some aptamers can specifically bind to the target cells and differentiate them from mixtures of normal human bone marrow aspirate. Additional results have suggested that the targets of the selected aptamers are most likely associated with cell membrane proteins, making the aptamers an effective molecular tool for clinically meaningful biomedical studies and biomarker discovery. The use of these aptamers for biomarker discovery has shown very promising preliminary results. The development of a group of molecular probes characterizing target cells via whole living cell-SELEX gives us insight into the molecular signatures existing on the surface of disease cells. The cell-SELEX also shows promise for developing effective molecular tools for molecular level understanding of the diseased cells.

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