Chapter 9
Cell-Specific Aptamers for Disease Profiling and Cell Sorting

Kwame Sefah, Joseph Phillips and Cuichen Wu

Abstract The molecular recognition of medically relevant cell-surface proteins and other biomarkers by molecular probes plays a major role in this current era of molecular medicine. Molecular probes have served as platforms for diagnosis, prognostic indication and targeted radio- or chemotherapy in cancer medicine. Since cancer is generally a heterogeneous disease, the elucidation of new disease specific molecular features will facilitate our understanding of cancer. The development of new molecular probes to detect disease specific features will improve our ability to specifically target and treat cancers. Cell-specific aptamers have emerged as unique candidates for molecular identification of cancer cells. Single runs of cell-SELEX can generate panels of aptamers that target disease specific molecular markers with high affinity and selectivity. We have shown that these panels can be used for molecular profiling of cancer and aid in the diagnosis of cancer. The ability to detect diseased cells in biological fluids is important for early detection, monitoring disease progression or remission, and tracking drug efficacy. Our research has shown that aptamers can be used to purify cells from a flowing suspension of biological fluid. When integrated into microfluidic devices, aptamers can be used for enrichment of rare tumor cells and multiplexed cell sorting of heterogeneous cell mixtures. For these reasons, aptamers have emerged as unique candidates for molecular recognition and cell-isolation and their future contributions will be a key factor in molecular medicine.

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W. Tan and X. Fang (eds.), *Aptamers Selected by Cell-SELEX for Theranostics*,
DOI 10.1007/978-3-662-46226-3_9

Keywords Aptamers · Molecular profiling · Cell isolation · Flow cytometry · Microfluidic channel

9.1 Introduction

The availability of molecular probes for the recognition of medically relevant cell-surface proteins and biomarkers has had and will continue to have a major impact in this era of molecular medicine. Tailored molecular probes have the ability to improve every aspect of medicine, including early detection, diagnostic resolution, and disease treatment options. Generally antibodies have been the probe of choice as they are able to detect molecular markers with high affinity and specificity. In fact, antibodies have been utilized successfully in many aspects of medicine including diagnosis, therapy, and prognosis of diseases. One major challenge for antibody technology is that there are not enough antibodies to target all the necessary disease-specific markers and creating new antibodies is a relatively long, technically cumbersome process involving animals with a generally low success rate. Further, many of the molecular markers that antibodies do recognize are also expressed on healthy cells and this may lead to deleterious effects when implementing such antibody-mediated drugs schemes or interventions. Ideally, technology for generating molecular probes should target disease specific molecular markers, generate high affinity and highly selective probes, be relatively fast with a high success rate, and produce multiple probes simultaneously. One promising technology for producing molecular probes that target diseased cells is the systematic evolution of ligands by exponential enrichment (SELEX) or Cell-SELEX.

Cell-SELEX generates molecular probes called aptamers that can target diseased cells specifically and with high affinity. Aptamers are single-stranded nucleic acids (DNA or RNA) that can bind with high affinity and selectivity to proteins, peptides, and other small molecules [1–3]. The dissociation constants of aptamers to their targets can range from picomolar to micromolar (See section on aptamers for details). Aptamer selection via cell-SELEX uses complex cellular targets, such as live cancer cells, red blood cells, or bacteria surface proteins, has been used to generate useful aptamers for cell recognition. Our research has focused on using cell-SELEX to generate cancer cell-specific aptamers and use them to develop novel technologies in the field of molecular medicine [4–9]. Two major areas that we have applied aptamer technology to are cancer cell-sorting/enrichment and molecular profiling of cancer cells. New technologies in these areas can be directly translated into novel methods for early detection of cancer, cancer diagnostic assays, and monitoring of disease progression and therapeutic response.

9.2 Cell-Sorting/Enrichment

Biological cells are mostly of heterogeneous population. In order to obtain single species for analysis, and obtain accurate biological information, it is important to track and isolate these cells into individual sub-populations based on their unique biological features. Therefore cell sorting of heterogeneous subpopulations of tumor and tumor-associated cells has been a long established strategy in cancer research. This technology has many important applications including detecting circulating tumor cells, separating stem cells from tissues, cell and protein engineering, and diagnosing the hematologic malignancies [10–15]. Sorting can be performed based on a myriad of cell properties, for example, size, shape, surface antigen expression, protein expression, and metabolic activity. Cell size and shape can be roughly discerned using FACS methods and more novel microfluidic methods [16]. Many cell properties can be detected using fluorescent probes and therefore FACS methods are popular. There are important situations in which FACS methods are not possible or efficient, e.g. point-of-care diagnostics, therefore other methods have gained in popularity. Our research has focused on microfluidic methods of tumor cell enrichment and cell sorting based on cell-surface protein/antigen expression, due to the nature of cell-SELEX aptamer selection which targets the surface of live cells.

To show proof-of-concept of tumor cell-enrichment, simple microfluidic channels were constructed by sandwiching parafilm between a cover glass and microscope slide. The channel was then coated with streptavidin by allowing the protein solution to fill the channel by capillary action. Excess streptavidin was then rinsed away by drawing solutions through the channel using filter paper as a wick. Biotinylated aptamers were then introduced and excess rinsed via wicking. Mixtures of fluorescent labeled target and non-target tumor cells were then introduced into the channel via wicking. After a final rinse step, the channel surface was imaged using a fluorescent microscope. Based on image analysis, the percent of target and non-target cells captured and purity of captured cells could be calculated. With this rudimentary device, we achieved 95 % purity and ~15 % efficiency of target cell capture [17]. To further improve the device capabilities, a PDMS version was created in which the channel height was reduced by four times to ~25 μm, on the same order as the cell diameter. The PDMS channel was reversibly attached to a large cover glass and operated via syringe pump. This PDMS device achieved 97 % purity and 80 % efficiency of target cell capture see Figure Enrichment. The increase in efficiency of target cell capture of the PDMS device could not be explained based on the difference in channel geometry compared to the parafilm-based device. One plausible explanation for this effect is based on mathematical modeling of particle velocities in microfluidic flows at low Reynolds number. Modeling predicts that if the particle diameter is on the order of the channel height, then the particle could exhibit significant velocity perpendicular to the fluid flow direction. In this case, we estimated that the tumor cells could be moving at 1–50 μm/s, fast enough to traverse the 25 μm channel height while passing through

the device. This basic research exhibiting the selective and efficient aptamer-based capture of tumor cells from a flowing suspension has been implemented in other microfluidic cell detection systems, including microcapillary [18], paper-based lateral flow devices [19], and Differential Mobility Cytometry [20]. Other research that has improved the efficiency of target cell capture to >90 % includes improving the device architecture by adding arrays of micropillars [21] and implementing multivalent aptamer technology like aptamer-conjugated gold nanoparticles [22] and linear-repeating aptamer arrays generated by rolling circle amplification [23], (Figs. 9.1 and 9.2).

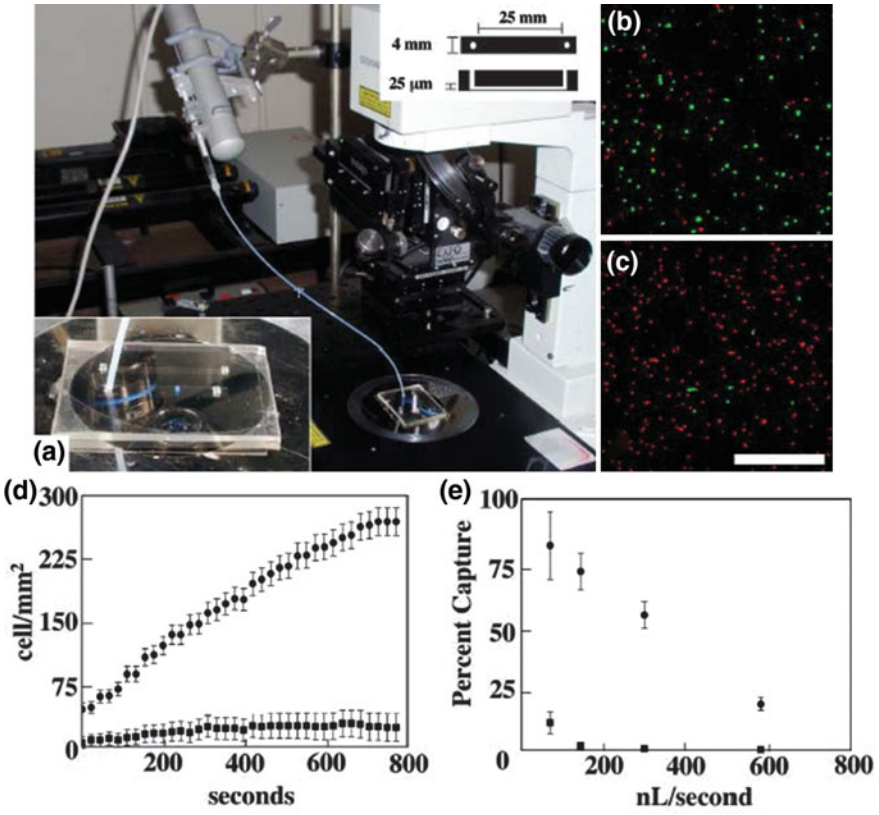

Fig. 9.1 Image of basic PDMS device on confocal microscope (**a**). The *bottom left* inlay shows the device, and the *top right* inlay shows *top-down* and *sideways views* with dimensions. Representative images of original mixture of cells before cell capture assay (**b**) and channel surface after the cell capture assay performed at 154 nL/s flow rate (**c**) with target and control cells stained *red* and *green*, respectively. Cell-surface density measured over the course of the cell capture experiment showing linear increase in target cells captured over time (**d**). Target cell capture efficiency decreases with increased fluid flow rate (**e**). Bar = 500 µm (Reprinted with the permission from Ref. [17], Copyright 2009 American Chemical Society)

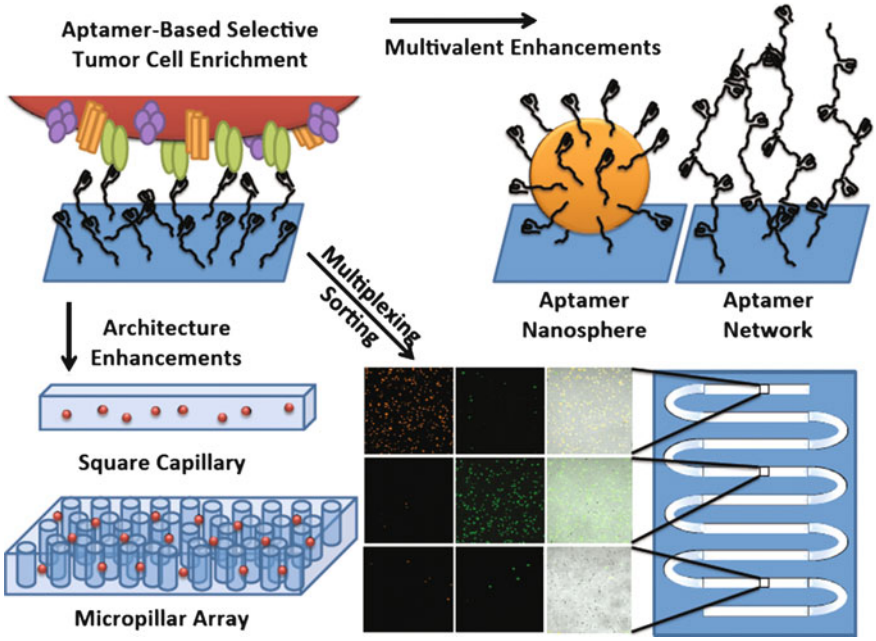

Fig. 9.2 Evolution of aptamer-based microfluidic devices for tumor cell enrichment (Reproduced with the permission from Ref. [18], The Royal Society of Chemistry, Adapted from Refs. [21, 22, 24], Copyright 2009, 2012, 2013 American Chemical Society)

To further demonstrate the capability and feasibility of tumor cell-sorting using aptamer platform, we created an S-shaped PDMS channel with multiple regions for cell-capture. The channel had fluid ports positioned at each bend which allowed us to immobilize one of three different biotinylated aptamers within each long stretch of the channel. We mixed 3 different tumor cell types together and were able to selectively capture each target cell within the region associated with its specific aptamer. We achieved ~97 % purity for two tumor cell lines and ~88 % purity for the third tumor cell line [24]. We also released cells from each region of the device and cultured them for several days. Results from flow cytometry experiments on the cultured cells showed that sorted cells had ~96.5 % purity. The level of multiplex sorting in this type of device is only limited to the surface area of the device.

After the initial proof-of-concept of capturing tumor cells from a flowing suspension using aptamer-based microfluidic devices, improvements in device design and implementation of molecular engineering of multivalent structures have produced devices that can achieve >90 % capture efficiency. These devices can take whole blood as sample matrix and operate at flow rates that are useful for point-of-care applications. Sorting of cells can be achieved without the use of lasers or any other sophisticated equipment. Since aptamers can be created for any diseased cell, we believe that these types of devices should be useful for detecting tumor cells in in various bodily fluids.

9.2.1 Summary

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9.3 Disease (Molecular) Profiling

As personalized medicine is becoming increasingly important in the effective management of diseases, especially cancers, there is the need to intensify efforts to identify unique disease signatures of therapeutic importance. This is necessary because even tumors arising from the same source have varied molecular characteristics as the disease progressed. Therefore molecular profiling of individual disease will allow us to measure the expression of multiple genes on tissues or biological samples and this will present individual molecular portraits of specific disease. This will allow us to capture the biological complexity of diseases more comprehensively, and utilize these features for design of effective therapeutic regimen. Simply put, molecular profiling will allow us to define diseases more carefully, for example; giving a molecular classification between healthy and disease cells (good diagnostic performance), or molecular markers to determine outcome after intervention (predictive performance), or for monitoring prognosis. This type of technology is integral to better understanding the unique molecular characteristics of a patient's disease rather than using the morphological features, which is the common gold standard practice for most tumors. It is anticipated that molecular profiling will become a valuable tool for oncologists when making treatment decisions for patients with difficult-to-treat and/or rare and aggressive cancers.

In theory, the expression of molecular targets of therapeutic and diagnostic importance is not in doubt. Many studies have demonstrated that each disease has unique sets of genes that are expressed and can be targeted for use in diagnosis and therapy. These biomarkers provide unique features about each patient's diseases, from which more tailored treatments can be most effective. Similarly, biomarkers can also provide information about which treatment regimen might not be suitable for a patient's disease based on the molecular profile and thus prevent excessive use of ineffective drugs. This observation of over treatment is well documented in breast cancer [25]. If an exhaustive set of biomarkers were known, physicians could

prescribe more defined treatment options for patients and further monitor these treatments with certainty. For instance, by gene expression patterns, it is now clear that breast cancer can be sub-typed into more than five different diseases [25]. In fact, breast cancer was the first cancer in which molecular profiling was approved for clinical use [26, 27]. Profiles such as the 21-gene recurrence score (Oncotype Dx), 70-gene signature, 76-gene signature, and wound-response gene profile of predicting breast cancer survival have shown great promise [28–31]. Other profiling technology such as Lymphochip has shown great success in treating lymphomas [32, 33]. The potential of molecular profiling is not limited to lymphomas and breast cancer. Progress has been made in others such as acute leukemia, prostate and lung cancers [34–39]. Generally, this technology has been developed using different cellular product platforms including RNA profiling, DNA profiling, and proteomic profiling [40–42]. While RNA and DNA profiling are undoubtedly promising, the scope of this book will only deal with proteomic profiling, and specifically using cell surface expressed proteins.

While there are many gene products that differ between disease state and normal cells and could serve as potential biomarkers for disease management, surface expressed genes are good candidates for effective and reliable targeted therapy. Membrane proteins are uniquely important because they play a critical role in how the cell interacts with its environment. Most FDA-approved clinically proven cancer drugs target cell-surface proteins and inhibit their functions [43]. Discovery of tumor-specific membrane proteins is a significant challenge. Using whole-proteome analysis, the most under-represented group is membrane proteins and roughly 30 % of proteins consist of membrane proteins, but less than 5 % of this total are recognized by mass spectroscopy, a major limitation for drug development [44]. The hydrophobic properties of membrane proteins further complicate their analysis as they are insoluble in non-detergent buffers. In addition, membrane proteins are typically lower in abundance when compared with soluble proteins. Therefore any technology that can overcome these limitations and generate reagents that can target membrane proteins for disease management will have significant impact in biological mechanism studies, biomarker discovery, and drug development.

9.3.1 How Do We Isolate and Identify These Genes?

Generally mass spectroscopy has been used to identify these genes and even predict the importance of these targets in drug development. Methods that can both identify genes and provide probes to target their gene products are ideal. The probes should identify the surface molecules with high specificity and affinity. For targeted therapies, these probes could serve as delivery tools to target these gene products and deliver drug payload capable of killing cancer cells or inhibiting their growth. Traditionally, antibodies have been generated and used to perform these functions and most of the current targeted therapies have antibodies as the central reagent.

While this has been successful, there are many important targets that do not have specific antibodies. Further, most of the antibody tumor targets are also expressed on normal cells, therefore limiting their utility. Thus there is a great need to generate more probes but generating high affinity tumor specific antibodies is not easy. As a result, researchers found different ways to complement antibody-mediated molecular target identification. In the past two decades, attention has been focused on the use of another powerful technology called Systematic Evolution of Ligands by EXponential enrichment (SELEX), which can generate molecular probes called aptamers. Aptamers are short nucleic acid strands that are selected from a library and they identify their targets with high affinity and specificity. It has been clearly demonstrated that aptamers can uniquely identify surface gene products of diagnostic and therapeutic importance [45–47]. By using cell-SELEX, many aptamers have been generated for cell surface molecules [4, 7, 48]. While the identity of some of these molecules are yet to be identified, their unique characteristics and sensitivity to identify specific diseases have adequately been demonstrated. Some of these aptamers have been used as baits for biomarker discovery [49–51], profiling clinical samples [52], and used for diagnostics even though their specific targets may not be known. We believe that cell-SELEX can adequately profile any diseased cell by using multiple aptamers that target cell surface molecules.

9.3.2 Why Is Aptamer-Mediated Molecular Profiling Important?

For molecular profiling to be effective, we must possess many molecular probes that can recognize specific cell surface markers important to disease diagnosis or therapy. With the correct probes, one can fully define the molecular identity of specific diseases and define the prognosis with certainty. The aptamer technology is important for molecular profiling because:

(i) SELEX produces multiple aptamers targeting different expressed genes of importance.
(ii) Negative selection against healthy cells can produce aptamers that are highly selective for diseased cells.
(iii) Aptamers can be engineered to suit specific needs or intended use.

While the molecular profiling procedure has not been well established for aptamers, the potential for aptamers to revolutionize this technology has adequately been demonstrated and the Tan group has played a leading role. Some of these aptamer-based profiles are discussed below.

One of the earliest demonstration of aptamers as potential molecular profiling reagents was reported by Shangguan et al. [52]. In this study, the authors used aptamers that had previously been generated for leukemia cells using CCRF-CEM as the target cell and Ramos as negative cell line [4]. The selection generated a

Table 9.1 Using aptamers to recognize cancer cells [4]

	Cell line	sgc8	sgc3	sgc4	sgd2	sgd3
Cultured cell lines	CCRF-CEM, Pre T ALL	+++	++	++++	++++	++
	Molt-4, pre T ALL	++++	+++	++++	++++	++++
	Sup-T1, Pre-T ALL.	++++	+	++++	++++	++
	Jurkat, Pre-T ALL	++++	+++	++++	++++	++++
	SUP-B15, pre-B ALL, Ph+	+	0	++	+	0
	U266, plasmacytoma	0	0	0	0	0
	Ramos, Burkitt lymphoma	0	0	++++	++++	0
	Toledo, B cell lymphoma	0	0	++++	++++	+
	Mo2058, B cell lymphoma	0	++	++	0	+
	NB-4 (AML, APL)	0	0	+++	++++	0
Cells from Patients	T cell ALL	++	+++	+++	+++	+++
	Large B Cell lymphoma	0	0	0	0	0

Note A threshold based on fluorescence intensity of FITC in the flow-cytometric analysis was chosen so that 99 % of cells incubated with the FITC-labeled unselected DNA library would have fluorescence intensity below it. When the FITC-labeled aptamer was allowed to interact with the cells, the percentage of the cells with fluorescence above the set threshold was used to evaluate the binding capacity of the aptamer to the cells. 0, <10 %; +, 10–35 %; ++, 35–60 %; +++, 60–85 %; ++++, >85 %; *AML* acute myeloid leukemia; *APL* acute promyelocytic leukemia (Reprinted with the permission from Ref. [4], Copyright 2006 National Academy of Sciences, USA)

panel of aptamers that showed specific features unique to individual aptamers. Based on the initial cell culture studies (Table 9.1) and limited clinical samples obtained from the pathology department of Shands hospital at the University of Florida, the authors showed that, these aptamers could be used to profile leukemia clinical samples.

The observation was important since diagnosis of leukemia is commonly based on morphologic evaluation and immunophenotype analysis and not molecular profiling. Current antibodies for leukemia are not specific for only diseased cells and therefore not intended for comprehensive recognition of molecular features of specific disease, especially subtyping. The lack of disease-specific markers is a shortfall not only in leukemia, but also in many other cancers. Thus in subsequent and more comprehensive clinical samples studies, the authors used the leukemia aptamers to profile leukemia patients' samples [52]. The selected aptamers could group real leukemia patient samples into different categories, T-cell acute lymphoblastic leukemia (T-ALL), B-cell acute lymphoblastic leukemia (B-ALL), acute myeloid leukemia (AML), and other lymphomas of mature lymphocytes based on surface markers. These results as shown in Table 9.2, clearly demonstrate an effective detection of targets on the cell membranes by the aptamers. This recognition was not due to non-specific interactions or random binding. All the lymphoma cases showed no or very low binding, in agreement with the fact that the mature lymphoma cells often do not share the same receptors with the immature leukemia cells. Moreover, the aptamers had much stronger binding with the T-ALL cases than others did, an expected outcome since the aptamers were selected to

Table 9.2 Aptamer profiling of cancer cells [52]

Cells lines		sgc8	sgc3	sgc4	sgd2	sgd3	sgd5
Cultured cell lines							
T-ALL	CCRF-CEM	+++	++	++++	++++	++	0
	Molt-4	++++	+++	++++	++++	++++	0
	Sup-T1	++++	+	++++	++++	++	0
	Jurkat	++++	+++	++++	++++	++++	0
B-ALL	SUP-B15	+	0	++	+	0	0
myeloma	U266,	0	0	0	0	0	0
B-cell lymphoma	Ramos	0	0	++++	++++	0	0
	Toledo	0	0	++++	++++	+	++
	UF1[c]	0	0	+	0	0	0
	Mo2058	0	++	++	0	+	0
AML	NB-4 (APL)	+	0	++++	++++	0	0
	Kasumi-1	+++	0	++++	++++	++	0
Cells in normal bone marrow							
CD3 (+) T cells		0	0	0	0	0	0
mature B cells[a]		0	0	0	0	0	0
Immature B cells[b]		0	0	+	+	0	0
Granulocytes		0	0	0	0	0	0
Monocytes		0	0	+	+	0	0
Erythrocytes		0	0	++	++	0	0
Patient's samples							
T ALL 1		++	+++	+++	+++	+++	ND
T ALL 2		++	+	+++	++	+	0
T ALL 3		+	+	++++	+++	+	0
T ALL 4		+	+	++	+++	+	0
T ALL 5		+	+	++	+	+	0
T ALL 6		0	0	+	+	0	0
T ALL 7		0	0	++	++	0	0
T ALL 8		+	+	++	++	+	0
T ALL 9		+	0	+	+	0	0
TALL10		0	+	+	0	+	0
B ALL 1		0	0	++	++	0	0
B ALL 2		0	0	++	++	0	+
B ALL 3		++	0	++	++	0	+
B-ALL 4		0	0	+	+	0	0
AML 1		+	+	++	+	0	0

AML 2	+	0	++	+	0	0
AML 3	+	0	+	+	0	0
AML 4	0	0	++++	++++	0	0
AML 5	0	0	+	0	0	0
AML 6	+	0	0	0	0	0
AML 7	+	0	0	0	0	0
AML 8	+	0	+++	+++	0	0
1, Peripheral T-cell lymphoma	0	0	0	ND	ND	ND
2, follicular lymphoma	0	0	0	0	0	0
3, B-cell lymphoma	0	0	0	0	0	0
4, T-cell lymphoma,	0	0	0	0	0	0
5, B cell lymphoma	0	0	0	0	0	0
6, plasma cell neoplasm	0	0	+	+	0	0
7, follicular lymphoma	0	0	+	0	0	0

0 :< 10% + :10-35% ++ :35-60% +++ :60-85% ++++ :>85%

Note In the flow cytometry analysis, a threshold based on fluorescence intensity of FITC was chosen so that 99 % of cells incubated with the FITC-labeled unselected DNA library would have fluorescence intensity below it. When FITC-labeled aptamer was allowed to interact with the cells, the percentage of the cells with fluorescence above the set threshold was used to evaluate the binding capacity of the aptamer to the cells. 0 for <10 %; + for 10–35 %; ++ for 35–60 %; +++ for 60–85 %; ++++ for >85 % (Reprinted with the permission from Ref. [52], Copyright 2007 American Association for Clinical Chemistry)

target CCRF-CEM cells, a T-ALL cell line. In addition, aptamer binding patterns corresponded well with general categories pre-defined by antibodies.

Since one barrier to developing robust molecular profiling technologies is the use of expensive and difficult to standardize platforms, e.g. microarray technology [25, 53], it is important that this study featured the use of flow cytometry as the detection platform. By using this combination of cell-SELEX and flow cytometry, more labs and research facilities will begin to implement aptamer technology for molecular profiling. Similar to this demonstration of SELEX technology for molecular profiling, other studies have emerged that further support that aptamer-mediated molecular profiling and cancer cell specific recognition can be an essential reagent for personalized medicine [5–8].

In the journal Leukemia, Sefah et al. [7] reported the generation of aptamers that can distinguish between NB4 cells, acute promyelocytic leukemia (APL), and HL60 (AML) cells. This report is important for molecular profiling because there was no known probe that could distinguish between these 2 cell lines prior to this report. In fact, HL60 and NB4 cells are morphologically similar and can both be induced to differentiate toward monocytic and granulocytic pathways depending on the chemical induce. Gene expression profiling studies showed that NB4 and HL60

cell lines had the most closely related profiles of mRNA expression [54]. In this study, in addition to the differential recognition by specific aptamers, two other aptamers could respond to the pattern of differentiation in both cell lines (Figs. 9.3 and 9.4). The targets of these aptamers were down regulated when the cell lines were treated with all-trans retinoic acid (ATRA), which caused cells to differentiate into mature granulocytes. On the other hand targets were up regulated when treated with sodium butyrate, differentiation through the monocytic pathway. The ability to distinguish this off-on switch molecular event is important because these probes could be used to develop targeted therapy based on these markers and simultaneously monitor progress of course of the therapy.

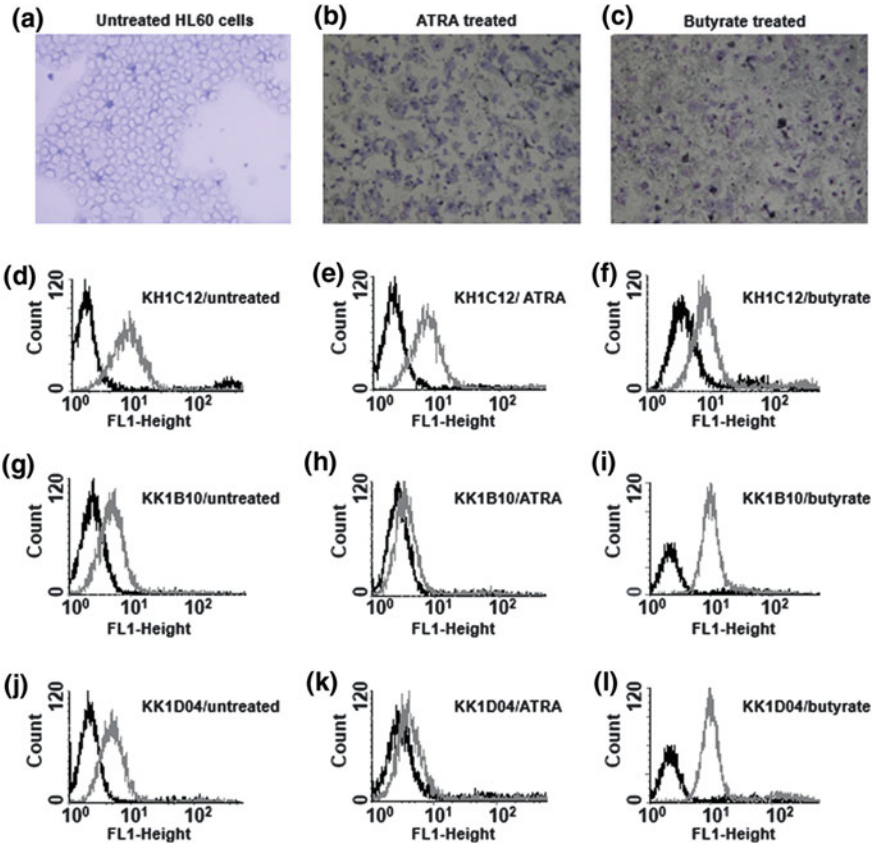

Fig. 9.3 Cytospin preparations followed by Accustain Wright staining of HL60 cells **a** untreated, **b** ATRA-induced differentiation, and **c** sodium butyrate-induced differentiation, showing the formation of formazan deposits (×10 magnification). The FACscan histograms (**d–l**) above show the binding profile of the selected aptamers to the untreated, ATRA-treated and sodium butyrate-treated cells. The *dark* histograms show fluorescence background using the unselected DNA library [7] (Reprinted with the permission from Ref. [7], Copyright 2009 Nature Publishing Group)

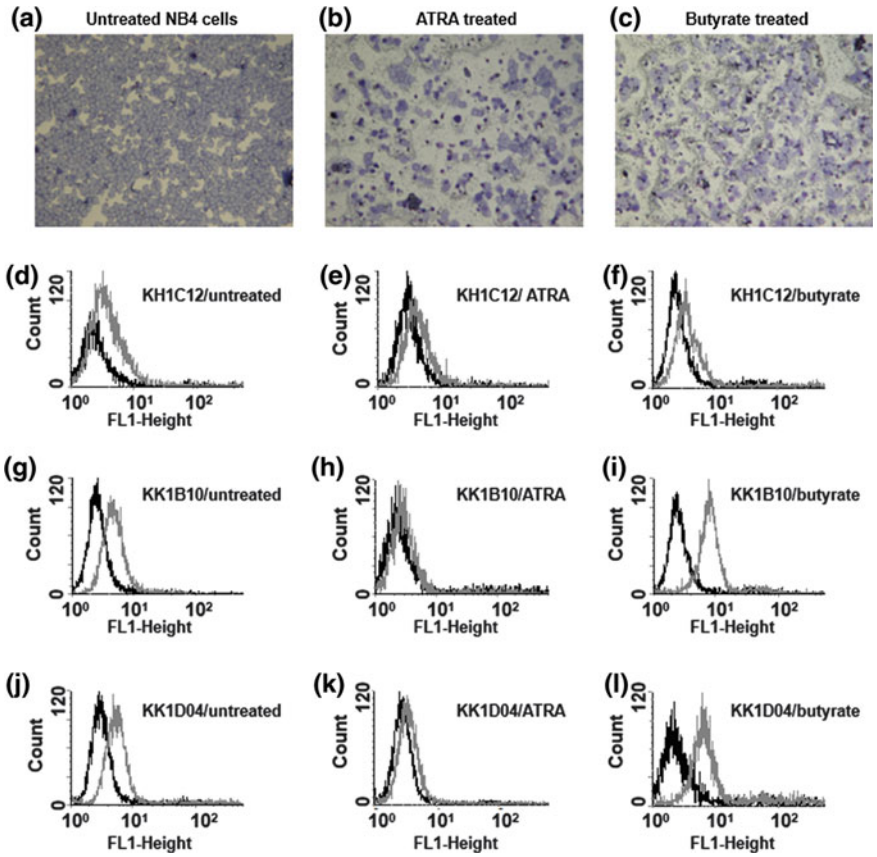

Fig. 9.4 Cytospin preparations followed by Accustain Wright staining of NB4 cells **a** untreated, **b** ATRA-induced differentiation, and **c** sodium butyrate-induced differentiation, showing the formation of formazan deposits (×10 magnification). The FACscan histograms (**d**–**l**) show the binding profile of the selected aptamers to the untreated, ATRA-treated and sodium butyrate-treated cells. The *dark* histograms show fluorescence background using the unselected DNA library (Reprinted with the permission from Ref. [7], Copyright 2009 Nature Publishing Group)

As the significance of these 2 cell lines has been well documented in leukemia research, most recently Yang et al. [55], has also developed aptamers that can differentiate between HL60 and NB4 cell lines. These aptamers could further differentiate between malignant and non-malignant cells (Fig. 9.3). The authors probed three groups of AML clinical samples, AML non-M3 CD34(+), AML non-M3 CD34(−), and 3) AML M3 with these aptamers and tested if the aptamers could differentially recognize any groups of AML cases. As expected, the aptamers showed low levels of reactivity on normal CD34(+) progenitors, but could recognize both CD34(+) and CD34(−) cells of AML non-M3 cases with the median values of fluorescence intensity higher than those of background binding. Also, they further

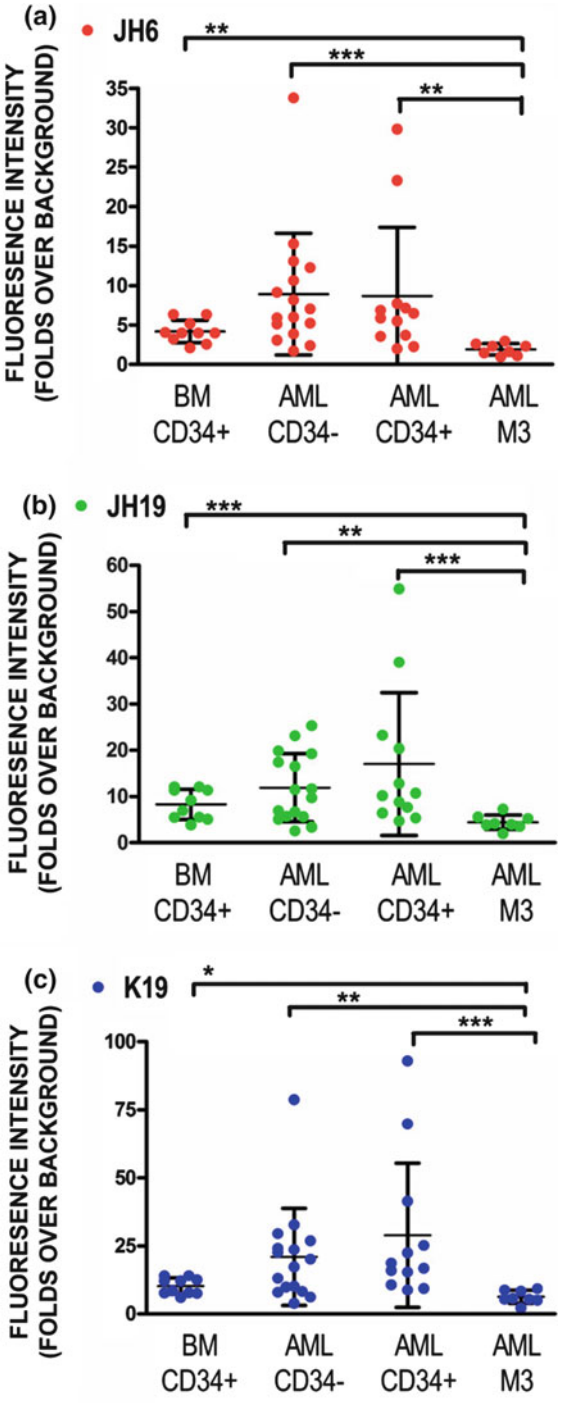

Fig. 9.5 Comparison of aptamer recognition of AML leukemic cells and non-malignant CD34(+) cells. The AML cases were separated into three groups: (1) CD34(+) AML non-M3; (2) CD34(−) AML non-M3; and (3) AML M3. The fluorescence levels of bound aptamers or single-stranded negative control DNA were determined by flow cytometry. The fluorescence intensity levels of bound aptamers (folds over background) were calculated (**a** JH6, **b** JH19, and **c** K19). Individual values for each aptamer bound on each case are shown as individual symbols, and mean ± standard deviation of individual groups are also shown. The P values are given as "*", "**", and "***" representing the P values of <0.05, <0.01, and <0.001, respectively (Reprinted from Ref. [55])

identified the binding molecule of one of the aptamers, called K19, to be Siglec-5, a specific but low expressed marker on NB4 cells. Based on these examples of aptamer technology providing panels of highly selective probes that are useful as molecular profiling reagents, we believe that the implementation of aptamers to manage these cancers is feasible. The unique nature of aptamers, i.e. ease of generation, sensitivity, specificity, ease of chemical modification, non-toxicity, make aptamer technology a solid platform for disease management (Fig. 9.5).

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