ACCOUNTS
of chemical research

Complex Target SELEX

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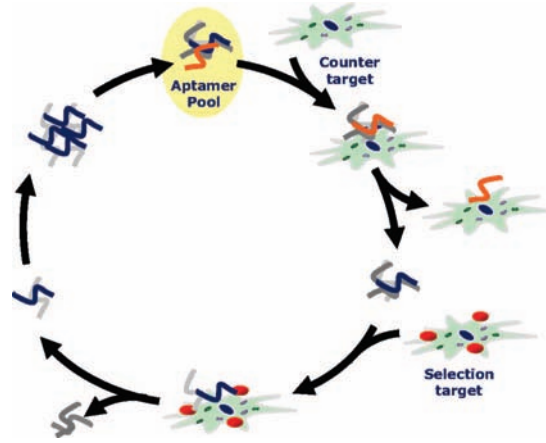
RECEIVED ON JUNE 11, 2007

CONSPECTUS

Aptamers are non-naturally occurring structured oligonucleotides that may bind to small molecules, peptides, and proteins. Typically, aptamers are generated by an *in vitro* selection process referred to as SELEX (systematic evolution of ligands by exponential enrichment). Aptamers that bind with high affinity and specificity to proteins that reside on the cell surface have potential utility as therapeutic antagonists, agonists, and diagnostic agents. When the target protein requires the presence of the cell membrane (e.g., G-protein-coupled receptors, ion channels) or a co-receptor to fold properly, it is difficult or impossible to program the SELEX experiment with purified, soluble protein target. Recent advances in which the useful range of SELEX has been extended from comparatively simple purified forms of soluble proteins to complex mixtures of proteins in membrane preparations or *in situ* on the surfaces of living cells offer the potential to discover aptamers against previously intractable targets. Additionally, in cases in which a cell-type specific diagnostic is sought, the most desirable target on the cell surface may not be known. Successful application of aptamer selection techniques to complex protein mixtures can be performed even in the absence of detailed target knowledge and characterization.



This Account presents a review of recent work in which membrane preparations or whole cells have been utilized to generate aptamers to cell surface targets. SELEX experiments utilizing a range of target "scaffolds" are described, including cell fragments, parasites and bacteria, viruses, and a variety of human cell types including adult mesenchymal stem cells and tumor lines. Complex target SELEX can enable isolation of potent and selective aptamers directed against a variety of cell-surface proteins, including receptors and markers of cellular differentiation, as well as determinants of disease in pathogenic organisms, and as such should have wide therapeutic and diagnostic utility.

Introduction

Aptamers are non-naturally occurring structured oligonucleotides that bind their targets with high affinity and have the capacity to inhibit protein–protein interactions with potencies similar to that observed with antibodies.[1,2] Aptamers are discovered using a process termed SELEX (systematic evolution of ligands by exponential enrichment), in which ligands are isolated from highly diverse (10^{13}–10^{15}) starting pools via rounds of affinity capture and amplification (Figure 1).[3] High-affinity aptamers against over 150 different targets including cytokines,[4,5] growth factors,[6–8] proteases,[9,10] immunoglobulins,[11] and cell adhesion molecules[12,13] have been reported. Recently, aptamers received validation as a therapeutic modality with approval of the anti-VEGF aptamer pegaptanib for the treatment of wet age-related macular degeneration (AMD).[14,15]

The majority of aptamers have been selected using purified, soluble forms of the protein target. In general, the best outcomes from SELEX experiments are obtained when the target protein assumes a stable conformation, allowing for con-

Published on the Web 01/15/2008 www.pubs.acs.org/acr
10.1021/ar700142z CCC: $40.75

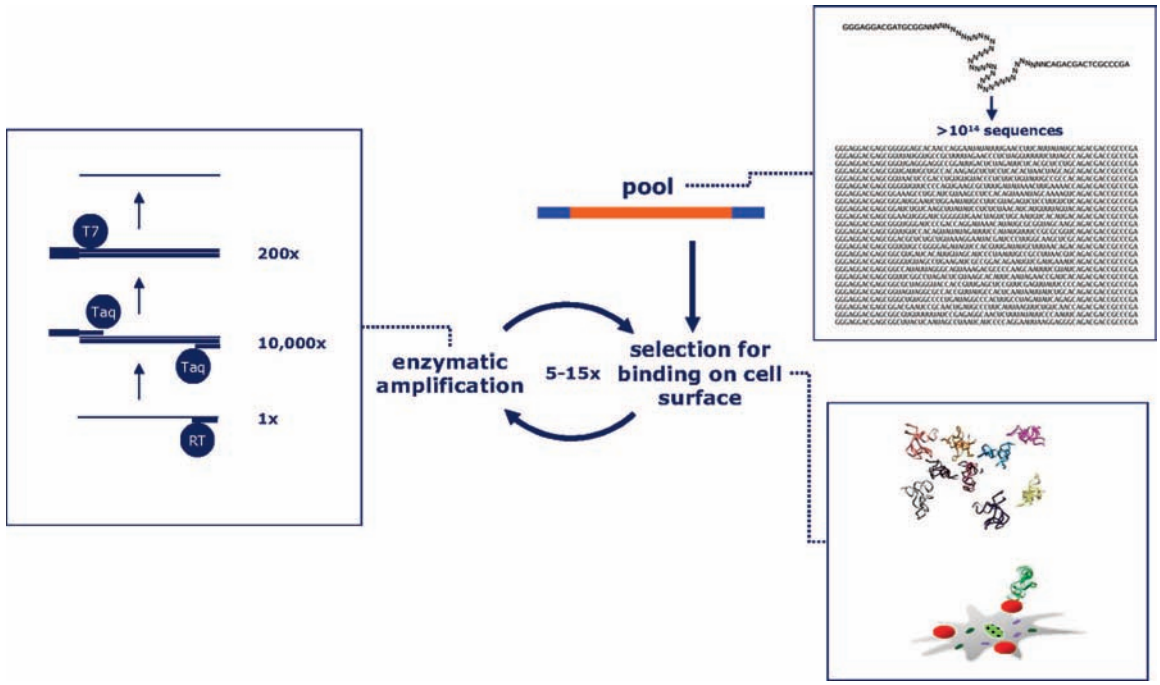


FIGURE 1. SELEX cycle with complex targets.

sistent presentation of structural epitopes from one round of selection to the next. Many interesting therapeutic targets or diagnostic markers are expressed on the cell surface. In addition, many membrane proteins function as coreceptors with other cell surface components. Copresentation of these proteins on the cell surface can provide optimal functional activity and additional epitopes for aptamer binding by shared receptor subunits. However, in such cases it may be difficult or impossible to obtain the relevant active protein(s) in purified form. Furthermore, when seeking a diagnostic marker, it is not always clear which specific protein on the cell surface provides the optimal target. Aptamers to targets expressed on cell surfaces can be generated by conducting SELEX against soluble membrane target ectodomains or against targets presented in complex mixtures such as membrane preparations or the surfaces of intact cells (Table 1).[16] This Account summarizes the emerging field of complex target SELEX and highlights the varying approaches that have been used to obtain aptamers against cell surface targets.

Perhaps the most significant challenge encountered when performing SELEX against complex target mixtures is deriving aptamers that are specific for the intended target. In conventional SELEX formats, a single purified protein provides the "bait" for recovery of target-specific aptamers. Various techniques that allow for facile partitioning of aptamers from nonbinders may be used, including affinity beads, hydrophobic plates, or nitrocellulose filters. Figure 2 illustrates various strategies that can be applied during SELEX to drive target specificity during selection. Counter-SELEX or "negative selection" may reduce the possibility of evolving nonspecific binders or improve the odds of recovering aptamers specific for one target over another. Counterselection techniques have been used to great effect with small molecules;[17] recently an aptamer

TABLE 1. Complex Target SELEX Examples

target type	target ID	ref
Cell Fragments/Membrane Preparations		
red blood cell ghosts	not identified	19
Torpedo californica electroplax membrane preparations	nicotinic acetylcholine receptor	21
Parasites/Bacteria		
Trypanosoma brucei	not identified	22
Trypanosoma cruzi	not identified	25
Mycobacterium tuberculosis	not identified	26
Viruses		
Rous sarcoma virus	not identified	28
influenza A virus	hemagglutinin	29
Mammalian Cells		
U251 glioblastoma cell line	tenascin-C	35
U251 glioblastoma cell line	tenascin-C	36
CCRF-CEM lymphoblastic leukemia cell line	not identified	37
YPEN-1 endothelial cell line	pigpen	39
Burkitt's lymphoma cell line (Ramos cells)	Ig heavy mu chain	32
NGF-differentiated pheochromocytoma (PC12) cell line	not identified	41
adult mesenchymal stem cells	not identified	42
Chinese hamster ovary (CHO) cell line expressing recombinant transforming growth factor-β type III receptor (TGFβRIII)	TGFβRIII	43
PC12 cell line expressing recombinant MEN2A mutant of rearranged during transfection (RET) receptor	MEN2A mutant RET	44

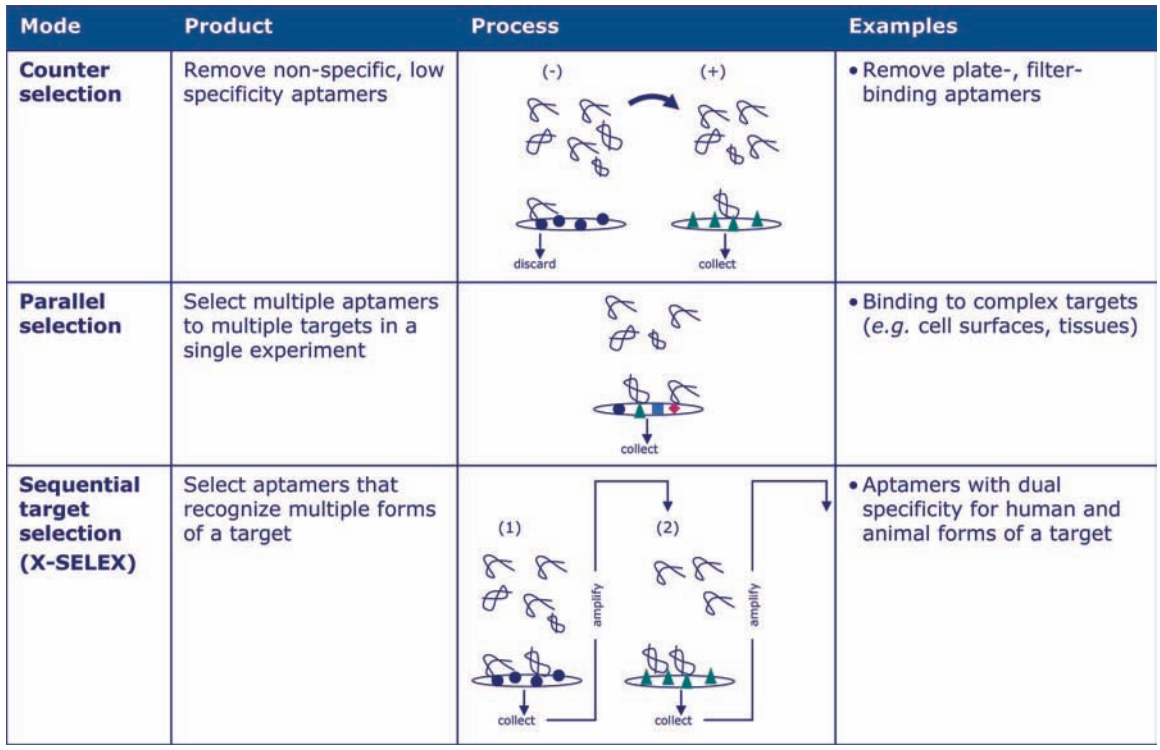


FIGURE 2. SELEX configurations.

highly specific for ADP vs ATP was generated through use of counterselection against ATP binding.[18] Similar principles are applicable to complex targets, allowing identification of aptamers that bind to a specific cell surface target or to a specific cell population. In either case, counterselection strategies are critical given the multitude of potential "aptogenic" epitopes, that is, epitopes against which aptamers can be generated readily that are common to the surface of many cell types. The presentation of a complex target during SELEX *without* counterselection measures allows for the parallel selection of aptamers to multiple targets in a single selection. Aptamers that recognize multiple forms of a specific target can also be obtained by sequential target selection. Several SELEX strategies used in the pursuit of aptamers to targets presented in a complex milieu are highlighted in this Account.

Cell Fragments/Membrane Preparations

In one of the first examples in which SELEX was employed successfully against membrane fractions derived from intact cells, Morris et al.[19] used human red blood cell (RBC) membrane preparations, or RBC "ghosts", as a SELEX target. The aptamer binding, partitioning, and recovery methods were similar to those used for pure protein targets;[3] however, there were some important differences in the selection results. While SELEX against pure targets ordinarily yields pools of converged sequence families by round 8−12, RBC ghost SELEX was carried forward for 25 rounds yet still yielded pools with high sequence complexity. Very few repeated sequences were identified although two recurring sequence motifs were identified in a total of approximately 25% of the clones. When clones containing either motif were conjugated to chemical photo-cross-linkers and light-activated in the presence of RBC ghosts, protein complexes of distinct size were identified by SDS−PAGE. Cross-competition using aptamers lacking the photo-cross-linker confirmed the evolution of two distinct sequence families that bind distinct RBC targets with high affinity. This study provided the first clear indication that nucleic acid libraries could be used to isolate aptamers against target preparations derived from cell surfaces.

Whereas the selection of nucleic acid ligands against RBC ghosts was "open-ended" in that no particular cell surface protein was strategically targeted, there are examples in which the intended target was presented in an enriched membrane preparation. The electric organ of the ray *Torpedo californica* is a rich source of acetylcholine receptor (AChR), containing approximately 0.7 nmol/mg membrane protein.[20] Neutralizing aptamers were desired, and since inhibitors typically bind poorly to AChR when presented in a purified soluble form, electroplax membrane preparations of *T. californica* were used for SELEX to ensure proper target presentation.[21] Two different partitioning strategies, nitrocellulose filtration and native

gel mobility shift, were employed to drive the selection toward AChR-binding aptamers. Despite the high density of AChR in the electroplax membrane preparations, there remained the possibility that aptamers against other cell surface targets would evolve if counterselection were not employed. Thus, the AChR inhibitor phencyclidine (PCP) was used in each round of nitrocellulose filter selection to specifically elute aptamers bound to the ligand-binding domain of AChR. Two different classes of AChR-binding aptamers were identified, one of which blocked the binding of PCP and cocaine to the AChR without inhibiting receptor function. Such aptamers could be effective at reducing the toxicity associated with addiction to these abused drugs.[21] This study highlights a useful strategy to circumvent a key challenge in complex target SELEX, that is, the use of competitive ligands to displace, elute, and enrich specific target-bound aptamers in the context of myriad cell surface proteins.

Parasites and Bacteria

African trypanosomes are protozoan parasites that cause sleeping sickness in many mammals including humans by multiplying within the peripheral blood and capillary beds of infected hosts. Homann and Goringer[22] have utilized live trypanosomes (*Trypanosoma brucei*) as targets for SELEX to identify multiple aptamer classes that bind the parasites. SELEX against live parasites may offer therapeutic potential, because the complexity of the parasite surface in its endogenous state allows for multiple potential targets to drive the selection process.

SELEX with preparations of two different strains of live *Tr. brucei* was carried out by incubating pools with bloodstream-stage trypanosomes and partitioning bound from free aptamer by centrifugation washes. Approximately 70% of the sequences obtained from enriched pools were identical; no clone was capable of discriminating between the two trypanosome strains, suggesting the convergence of aptamers against invariant surface targets shared by both strains. All of the selected aptamers were specific for bloodstream-stage parasites since they failed to bind the surface of trypanosomes in the insect stage. Thus, the use of live trypanosomes in SELEX enabled the identification of aptamers that bind invariant targets of stage-specific parasites. Such aptamers could be valuable in directing a host immune response to infection that would not be susceptible to variation in parasite surface glycoproteins.[23]

Another protozoan parasite, *Tr. cruzi*, which is responsible for Chagas' disease, has been employed as a live target for SELEX. In its trypomastigote stage, *Tr. cruzi* contacts and invades host cells by penetrating a network of extracellular matrix (ECM) proteins. A specific glycoprotein on *Tr. cruzi* trypomastigotes, tc85-11, interacts with multiple host cell matrix proteins thereby enabling the parasite to enter cells.[24] Thus, an aptamer that binds to tc85-11 and neutralizes its ECM protein binding capability could represent a promising therapeutic option for Chagas' disease. Ulrich et al.[25] presented live *Tr. cruzi* trypomastigotes isolated from infected monkey kidney epithelial cells as targets for SELEX. In contrast to the example described above, the SELEX efforts with *Tr. cruzi* were designed to isolate aptamers against specific ECM receptors on the parasite surface. This was accomplished by a competitive ligand elution strategy similar to that utilized to generate a competitive aptamer antagonist to AChR.[21] In each round of selection, aptamers that were bound to *Tr. cruzi* surfaces were eluted by incubation with a combination of soluble ECM components including laminin, fibronectin, heparin sulfate, and thrombospondin. The use of ECM proteins to displace bound aptamers applies a selective pressure during SELEX for the enrichment of aptamers that can block *Tr. cruzi*/ECM interactions. Whereas aptamers against *Tr. brucei* bound parasite in a stage-specific fashion, *Tr. cruzi* aptamers from round 5 SELEX pools displayed binding activity against both infective stage trypomastigotes and noninfective epimastigotes. Therefore, beginning in round 6, a counterselection paradigm was introduced into the SELEX cycle wherein aptamer pools were precleared of epimastigote-binding aptamers. Pools were incubated with epimastigotes and, following a 1-h contact step, nonbinding aptamers in the supernatant were isolated away from epimastigotes and carried forward into the positive selection step with *Tr. cruzi* trypanomastigotes. The combination of competitive ligand elution and stage-specific counterselection resulted in the identification of trypanomastigote-specific aptamers that were capable of partially blocking host cell invasion.

Bacterial cells have also been used as SELEX targets for the purpose of developing aptamers as antibacterial agents.[26] Tuberculosis (TB) remains a serious health concern for millions worldwide in part because of the prevalence of multidrug resistant strains of *Mycobacterium tuberculosis* (MTB), the etiologic agent of TB. New therapeutic modalities that lack cross-resistance with existing antimycobacterial drugs would constitute valuable treatment alternatives for TB. SELEX conducted against live MTB yielded aptamers that modestly enhance the survival rate of mice infected with the virulent strain H37Rv.[26] Selections were designed to generate H37Rv-specific aptamers by incorporating a counterselection step against an attenuated mycobacterial species (*Mycobacterium bovis* BCG) in every round. A resulting aptamer (NK2) prefer-

entially binds H37Rv, increases the levels of interferon-γ in $CD4^+$ T cells, and reduces bacterial counts in the spleens of infected mice. Although neither the bacterial surface target nor the mechanism of protection has been elucidated, the authors suggest that NK2 likely binds to and interferes with a self-protective H37Rv membrane protein, thus leading to more effective cell-mediated immunity. As described above, the NK2 aptamer might now be used as a purification probe to identify a surface target specific to virulent strains of MTB and to develop more effective treatment strategies.

Viruses

Blockade of viral entry into host cells is a potentially effective mechanism of action for antiviral therapies. Cell entry is typically mediated by interactions between pathogen surface proteins and receptors on the extracellular surface of host cells. Identification of viral surface proteins involved in cell entry has facilitated the use of purified proteins in SELEX to isolate aptamers that can block host cell infection.[27] However, several groups have also adopted the strategy of utilizing intact viruses as SELEX targets and have been successful in isolating antiviral aptamers with high affinity and specificity that neutralize viral infection.

Rous sarcoma virus (RSV) is an enveloped avian retrovirus with surface glycoproteins that are required for host cell binding and entry. Pan et al.[28] sought to isolate aptamers that bind to the RSV surface. A pool of $\sim 10^{15}$ oligonucleotides were contacted with sucrose-gradient-purified Prague A strain RSV particles, and bound aptamers were separated from nonbinding oligonucleotides by either nitrocellulose filtration or high-speed centrifugation. After 12 rounds of selection, neutralization of viral infection by selected pools was detected and sequence analysis indicated convergence to multiple sequence families, five of which displayed distinctive RSV neutralizing activity and some of which could also neutralize multiple RSV strains. In this study, SELEX using intact RSV particles enabled the isolation of neutralizing aptamers without requiring a full understanding of the viral surface components involved in host entry and infection, highlighting an advantage of this approach.

The human influenza virus exists as multiple closely related strains, and most commercially available monoclonal antibodies are incapable of discriminating among the different strains. For genotyping and, ultimately, for diagnostic purposes, it would be advantageous to have reagents that could bind to and distinguish closely related pathogenic strains. In contrast to RSV, the surface of the human influenza virus is well characterized, consisting of two glycoproteins, hemagluttinin (HA) and neuraminidase (NA) in a 3:1 ratio.[29] Although HA or NA can be prepared in a soluble purified form for use as a protein SELEX target,[30] the use of live virus as a SELEX target is an attractive alternative given the opportunity to present the viral surface proteins in their native conformations. Gopinath et al.[29] have recently subjected intact live influenza A viruses to SELEX and identified aptamers that can distinguish closely related influenza strains and block HA-mediated membrane fusion with host cells. Influenza virus binders were partitioned from nonbinders using nitrocellulose filtration with progressive increases in selection stringency by sequentially reducing both pool and virus concentration, as well as by incorporating competitor oligonucleotide (tRNA) in later rounds. SELEX resulted in the isolation of two distinct aptamer sequences capable of binding to influenza A Panama virus but not influenza A Aichi virus, despite the 84% amino acid sequence similarity shared by the two strains. The aptamers were shown to specifically bind HA on Panama strain viruses and inhibit membrane fusion with red blood cell ghosts, indicating neutralization of the virus' ability to infect host cells.

Cell Surface Marker Identification

The use of complex cells as targets can enable identification of aptamers to cell surface-specific markers for the purposes of target identification, differential cell targeting and purification, and diagnostics. In oncology, one of the keys to developing effective anticancer therapeutics is identifying specific tumor cell or tumor vasculature markers that can be used for targeted cell neutralization or destruction. SELEX against tumor cells provides a means to isolate aptamers that bind to tumors or infiltrating endothelial cells. The resulting aptamers can then be used in reverse chromatography strategies to purify and identify the specific tumor cell marker. SELEX against live Ramos cells, a Burkitt's lymphoma cell line, led to the identification of DNA aptamers that bind to Ramos cells but not to other T and B lymphocyte lines.[31] One of the selected aptamers was then covalently bound to Ramos cells via a modified uracil derivative and used to purify the target protein by streptavidin-mediated magnetic extraction.[32] Mass spectroscopy analysis identified the target protein as the immunoglobulin heavy mu chain, a major component of the B-cell receptor complex and potential marker for Burkitt's lymphomas.[33,34]

Daniels et al.[35] utilized a similar approach to identify aptamers and the tumor-associated proteins to which they bind in cultures of the glioblastoma cell line U251. SELEX was performed by contacting pools with U251 cell monolayers fol-

lowed by cell washing, trypsinization, and phenol extraction for aptamer recovery. After 21 rounds of selection, one clone representing 10% of the sequenced pool was found to bind tightly to U251 cells at 4 °C and was used in an affinity purification procedure to identify the known tumor cell line associated protein, tenascin-C. The selections were carried out at 4 °C in an effort to minimize cellular internalization and degradation of aptamers during the SELEX process and, interestingly, yielded temperature-sensitive aptamers with reduced target-binding affinity at 37 °C.

Given the temperature sensitivity of the tenascin-C aptamers derived from 4 °C selections, a second independent group sought to isolate more stable tenascin-C aptamers by performing three parallel selections at 37 °C.[36] In addition to one branch of selection against plates coated with purified tenascin-C protein, a second branch was conducted against intact U251 cells, and a third consisted of a cross-over approach with pools resulting from round 9 of selection against U251 cells carried forward two additional rounds of selection against purified protein. All three selection strategies yielded tenascin-C aptamers, and the sequences grouped into three distinct families. Although the cell SELEX round 9 pools displayed moderate to weak target-binding affinities, addition of just two further rounds of cross-over SELEX against purified tenascin-C dramatically improved pool target-binding affinities, highlighting the utility of this approach in cases where aptamers against a specific cell-associated target are desired.

U251 cells express an abundance of tenascin-C in the extracellular matrix, and thus, it is not surprising that enriched pools from two independent selections against these cells were dominated by the presence of tenascin-C aptamers. Cell SELEX has also been utilized to identify aptamers to lower abundance tumor-specific targets through the use of counterselection approaches directed against distinct tumor types or nontumorigenic control cells. Shangguan et al.[37] devised a counterselection strategy to select aptamers that can bind to a cultured T-cell acute lymphoblastic leukemia cell line, CCRF-CEM, but not Ramos cells. The selections consisted of iterative cycles of initial pool contact with cultures of CCRF-CEM cells followed by heat elution, subsequent contact with the counterselection Ramos cell line, and collection of nonbinders from the cell media. Thus, only aptamers that bind CCRF-CEM cells but not Ramos cells were selected and amplified. The results of the selection were consistent with expectations in that high-affinity aptamers against CCRF-CEM cells were identified with no detectable binding to the surface of Ramos cells. Furthermore, these aptamers were capable of discriminating CCRF-CEM cells mixed with normal human bone marrow aspirates and have more recently been used in the rapid collection and detection of acute leukemia cells from whole blood[38] suggesting that the counterselection strategy was successful in yielding tumor-cell-specific aptamers.

A counterselection strategy has also been implemented to isolate aptamers that could specifically bind brain tumor vasculature but not the vessels of normal brain. Blank et al.[39] conducted SELEX against a transformed endothelial cell line (YPEN-1 cells) and used N9 microglial cells as a counterselection target to subtract aptamers that could bind other cell types from the pool. Individual sequences were screened for cell-specific binding (YPEN-1 vs N9 cells) and histological staining of pathological neovasculature. Sixteen different aptamers with YPEN-1-specific binding and staining activity on tumor vasculature were identified. The strongest binder of these was biotinylated, coupled to streptavidin-coated magnetic beads, and used to purify its cognate target protein from solubilized YPEN-1 cells. Peptide fingerprinting and sequencing led to the identification of the protein as the rat homolog of the endothelial cell protein pigpen. Thus, whereas cell SELEX has been used to identify aptamers against previously known tumor cell markers (as with tenascin-C), SELEX against live tumor cell lines can also be applied to identify novel tumor targets. Aptamers against cell surface tumor targets could be valuable reagents for diagnostic imaging of disease or stage-specific markers, for delivery of conjugated toxins to specifically kill tumor cell populations, or as antagonists to block the proliferative or metastatic-inducing activities of cell surface receptors.

In fact, counterselection strategies can be used to take advantage of the molecular differences between any two closely related cell populations in the pursuit of aptamers that can bind to and discriminate distinct cell types. Since cellular differentiation generally leads to the induction of specific cell-surface markers, it is possible to use cell SELEX approaches to identify aptamers that can bind discriminately to differentiated cell types. For example, the pheochromocytoma-derived PC12 cell line differentiates into neuronal-like cells upon treatment with nerve growth factor (NGF).[40] Using undifferentiated PC12 cells in a primary subtraction step followed by pool contact with NGF-differentiated cells, Wang et al.[41] selected DNA aptamers that can distinguish differentiated PC12 cells from their undifferentiated counterparts. Aptamers specific for undifferentiated cell types such as stem cells may be valuable as cell enrichment and purification reagents in the emerging field of regenerative medicine. Guo et al.[42] have identified aptamers capable of binding adult mesenchymal stem cells

(aMSCs), leading to an improved technique for the isolation and enrichment of these stem cells from whole bone marrow. Although no counterselection approach was incorporated into the selection scheme, aptamers with specificity against aMSCs were isolated and used to fish out aMSCs from bone marrow when coupled to magnetic microbeads. The cells displayed surface markers consistent with established aMSC cultures and were capable of differentiating into osteogenic and adipogenic lineages with appropriate morphological characteristics. Thus, cell SELEX can provide an effective approach for the identification of aptamers that distinguish the differentiated states of cell populations. Such aptamers may then be used to isolate specific cells as part of a therapeutic regime.

Neutralizing Aptamers to Cell Surface Receptors

Cell surface receptors are attractive therapeutic targets for many clinical indications and can be neutralized by aptamers that block ligand-induced activation. Through the use of engineered cell lines expressing recombinant receptor targets, SELEX has been applied by several groups to identify receptor-neutralizing aptamers. Despite the high levels of target expression achievable by standard transfection protocols, counterselection strategies are usually necessary to drive selection toward target-specific aptamers and away from nonspecific cell-surface binders. Cell lines that lack endogenous target expression can be used as host cells in which to express a recombinant target and serve as the ideal counterselection tool. This approach has recently been applied to identify neutralizing aptamers to the transforming growth factor-β type III receptor (TGFβRIII) ectopically expressed on the surface of Chinese hamster ovary (CHO) cells.[43] The oligonucleotide pool was contacted with mock-transfected CHO cells to remove unwanted nonspecific binders. This study highlights a number of ways in which increased stringency can be introduced in progressive SELEX rounds. In the early rounds of SELEX (rounds 1 and 2), no negative selection steps were applied; that is, aptamer pools were contacted only with target-expressing cells. Negative selection commenced in rounds 3−5 with one parental cell subtraction per round and then increased to two subtractions for rounds 6−8 followed by three subtractions for rounds 9−11. In addition, the number of washing steps was progressively increased from two washes at round 1 to five washes by round 11. After 11 rounds of selection, several aptamer families were enriched, and one was identified that displayed target-cell-specific binding activity. This aptamer also disrupted TGFβ/TGFβRIII complexes *in vitro* suggesting that it could be used to neutralize TGFβRIII-mediated signaling events *in vivo*.

Using creative counterselection cell SELEX strategies, Cerchia et al.[44] identified aptamers that bind to and neutralize a cell surface receptor only when presented in a specific conformation that recapitulates a form of inherited neoplasia. The rearranged during transfection (RET) receptor tyrosine kinase has been linked to the regulation of cell proliferation in several cancers.[45] Two different germline mutations in the *RET*-gene lead to neoplastic syndromes by distinct mechanisms, one (MEN2A) involving a mutation in the extracellular domain that leads to constitutive receptor dimerization and activation and a second germline mutation (MEN2B) in the intracellular domain of RET that also activates the receptor constitutively but via a different mechanism that does not involve receptor dimerization. Three different cell lines were utilized in a selection scheme designed to identify aptamers that were specific for the MEN2A form of RET.[44] PC12 cells were used as a host cell line in which the RET isoforms corresponding to the MEN2A and MEN2B mutations were expressed. To avoid selecting aptamers that would bind to unintended targets on the cell surface, in every round of selection the pool was contacted with parental PC12 cells first and unbound aptamers collected by centrifugation. The pools subtracted for nonspecific binding were next incubated on PC12 cells expressing the MEN2B monomeric isoform of RET, and unbound aptamers were partitioned and collected. Recovered aptamers were then exposed to PC12 cells expressing the constitutively dimerized MEN2A isoform of RET, and following stringent washes, bound aptamers were captured and amplified. This selection scheme led to the identification of an aptamer that could inhibit constitutively dimerized (MEN2A) RET as well as ligand-induced dimerization of wild-type RET but not dimerization of the constitutively activated MEN2B isoform of RET. Thus, in addition to eliminating nonspecific cell surface binders from aptamer pools, counterselection strategies in cell SELEX can also apply stringent selective pressure for the enrichment of target-conformation-specific aptamers. Interestingly, aptamers selected against RET on the cell surface of PC12 cells could not bind soluble RET ectodomain, and aptamers selected against soluble RET could not bind to RET-expressing PC12 cells. This result illustrates an advantage of conducting SELEX against receptors presented on the cell surface because the method ensures that selected aptamers bind to epitopes present in the native target conformation.

Conclusion

Recent advances in which the useful range of SELEX has been extended from comparatively simple purified forms of soluble proteins to complex mixtures of proteins in membrane preparations or *in situ* on the surfaces of living cells offer the potential to discover aptamers against previously intractable targets. Proteins on cell surfaces or in membranes present target epitopes in biologically relevant conformations and are amenable to counterselective measures designed to promote recovery of aptamers that bind with high affinity and specificity and possess the desired biological activity. Furthermore, successful application of aptamer selection techniques to complex protein mixtures can be performed even in the absence of detailed target knowledge and characterization. Complex target SELEX can enable isolation of potent and selective aptamers directed against a variety of cell-surface proteins, including receptors and markers of cellular differentiation, as well as determinants of disease in pathogenic organisms, and as such should have wide therapeutic and diagnostic utility.

BIOGRAPHICAL INFORMATION

Steven Shamah is a Principal Investigator at Archemix Corp. in Cambridge, Massachusetts. Dr. Shamah was trained as a cell biologist and currently focuses on the identification and characterization of therapeutic aptamers.

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Sharon Cload was trained in bio-organic chemistry and throughout her career in biotechnology has focused on therapeutics targeting RNA, RNA-based biosensors, and aptamer-based therapeutics. Dr. Cload is currently Sr. Director, Aptamer Discovery at Archemix Corp.

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