Cell-Specific Aptamer-Mediated Targeted Drug Delivery

Jiehua Zhou¹ and John J. Rossi^{1,2}

Nucleic acid aptamers are *in vitro*-selected small, single-stranded DNA or RNA oligonucleotides that can specifically recognize their target on the basis of their unique 3-dimensional structures. Recent advances in the development of escort aptamers to deliver and enhance the efficacy of other therapeutic agents have drawn enthusiasm in exploiting cell-type-specific aptamers as drug delivery vehicles. This review mainly focuses on the recent developments of aptamer-mediated targeted delivery systems. We also place particular emphasis on aptamers evolved against cell membrane receptors and possibilities for translation to clinical applications.

Introduction

IN 1990, TWO RESEARCH GROUPS (Ellington and Szostak, 1990; Tuerk and Gold, 1990) independently described the selection of small nucleic acids with selective ligand binding properties (aptamers) from combinatorial nucleic acid libraries under a defined experimental condition/process, which was termed systematic enrichment of ligands by exponential enrichment (SELEX) (Tuerk and Gold, 1990). Inspired by these pioneering discoveries, numerous nucleic-acid aptamers have been raised against an extremely wide variety of targets over the past 20 years (Hesselberth et al., 2000; Lee et al., 2006; Famulok et al., 2007; Mayer, 2009), including small molecules (dyes, metal ions, amino acids, and short peptides), biomacromolecules (nucleic acids and proteins), molecular complexes, viruses (Tang et al., 2009), or even live cells (Blank et al., 2001; Shangguan et al., 2006) and whole organisms (Lorger et al., 2003). The low nanomolar binding affinities and exquisite specificity of aptamers for their targets make them versatile tools for diagnostics, *in vivo* imaging, and targeted therapeutics (Nimjee et al., 2005; Levy-Nissenbaum et al., 2008; Thiel and Giangrande, 2009).

As a new facet of aptamer applications, aptamers raised against membrane receptors have been exploited as carriers and targeting agents for delivery of a variety of reagents to specific cell populations or tissues (Hicke and Stephens, 2000; Yan and Levy, 2009; Zhou and Rossi, 2009). Through specific interaction between the aptamer and its cellular membrane receptor, aptamers actively enhance the accumulation or retention of therapeutic agents. Further, internalization of the aptamer enables the cellular uptake *via* receptor-mediated endocytosis, thereby increasing the drugs' local concentration in the targeted cells/tissues. Although entrapment in endocytic vesicles and subsequent endosomal escape/release is

one of the major limitations for the application of aptamers as drug delivery vehicles, extensive attempts are being conducted to develop aptamer compatible endosomal escape strategies to improve the efficiency of intracellular delivery. For example, aptamers functionalized as pH- or environment-sensitive nanocarriers could facilitate cellular uptake and enhance endosomal release.

The development of new DNA or RNA aptamers specifically targeting membrane receptors and their adoption as drug delivery vehicles has progressed rapidly (Table 1). Recent investigations have succeeded in achieving the cell-type-specific delivery of various molecules of interest that include small interfering RNAs (Guo, 2005; Guo et al., 2005; Chu et al., 2006b; McNamara et al., 2006; Shaw et al., 2008; Wullner et al., 2008; Zhou et al., 2008; Dassie et al., 2009; Zhou et al., 2009), toxins (Chu et al., 2006a), chemotherapeutic agents (Bagalkot et al., 2006; Huang et al., 2009; Taghdisi et al., 2009), anticancer drug-encapsulated polymers (Farokhzad et al., 2004, 2006; Dhar et al., 2008; Gu et al., 2008) or liposome nanoparticles (Cao et al., 2009; Kang et al., 2010), radionuclides (Hicke et al., 2006), a viral capsid (Tong et al., 2009), enzymes (Chen et al., 2008), nano-carriers (Zhang et al., 2007; Huang et al., 2008; Javier et al., 2008; Wang et al., 2008; Li et al., 2010), photodynamic therapeutic agents (Ferreira et al., 2009), etc. Aptamers have also been used as multifunctional targeting delivery devices. In the present review, we discuss the most recent advances in the selection of cell-specific aptamers and the newer aptamer-mediated delivery systems.

The Overview of *In Vitro* SELEX Process

Aptamers are single-stranded RNA or DNA molecules evolved *in vitro* to specifically recognize and tightly bind cognate targets by means of well-defined secondary and

¹Division of Molecular and Cellular Biology and ²Trell and Manella Graduate School of Biological Sciences, Beckman Research Institute of City of Hope, Duarte, California.

TABLE 1. CELL-SPECIFIC APTAMERS FOR TARGETED DELIVERY

Component	Target	Selection strategy (in vitro SELEX)	Aptamer-mediated targeted delivery system in vitro or/and in vivo (ref.)
2'-Fluoro RNA	Prostate-specific membrane antigen	Magnetic beads-based SELEX with the purified fusion protein containing a modified extracellular form of prostate-specific membrane antigen	(1) siRNA: noncovalent aptamer: lamin A/C or GAPDH siRNA conjugates (2) siRNA: covalent aptamer-PLK1 or BCL2 siRNA chimeras (3) siRNA: bivalent-aptamer:EEF2 siRNA conjugates (4) Toxin: covalent aptamer:gelonin conjugates (5) Chemotherapeutic agents encapsulated nanoparticles: Dextran, Docetaxel, Pt(IV), or Doxorubicin-encapsulated nanoparticle-aptamer bioconjugates (6) Anthracycline drugs: aptamer-Doxorubicin physical conjugates siRNA and fluorescent molecules: noncovalent chimeric phi29 RNA dimer or trimer harboring aptamer and siRNAs (survivin, BIM, CD4, BAD siRNAs) and FITC or Rhodamine siRNA: covalent aptamer-HIV <i>tat/rev</i> siRNA chimeras; noncovalent aptamer-sticky bridge-siRNA conjugates (HIV-1 <i>tat/rev</i> , CD4 and TNPO3 siRNAs) Radionuclide and fluorescent agents: Covalent aptamer-99mTc or fluorescent agents conjugates
2'-Fluoro RNA	CD4	Sepharose-beads-based SELEX with the recombinant soluble CD4 antigen	LNcaP cells (Chu et al., 2006b) LNcaP cells; athymic nude mice (McNamara et al., 2006; Dassie et al., 2009) LNcaP cells (Wullner et al., 2008) LNcaP cells (Chu et al., 2006a) LNcaP cells; BALB/c nude mice (Farokhzad et al., 2004 2006; Zhang et al., 2007; Dhar et al., 2008; Gu et al., 2008; Wang et al., 2008) LNcaP cells (Bagalkot et al., 2006) CD4 overexpressing T-cells (Guo, 2005; Guo et al., 2005; Khaled et al., 2005)
2'-Fluoro RNA	HIV-1 gp120	(1) BIAcore biosensor system or (2) nitrocellulose filter-based SELEX with recombinant gp120 protein	HIV-1-infected CEM cells or PBMCs; Rag-Hu mice (Zhou et al., 2008, 2009)
2'-Fluoro RNA (2'-OMe purine substitutions)	TN-C	(1) 96-well Lumino Plate-based SELEX with purified TN-C protein; (2) cell-based SELEX with TN-C-expressing U251 glioblastoma cells; or (3) a crossover SELEX using tumor cells and purified TN-C protein	Glioblastoma tumor cells; nude mice (Hicke et al., 2001, 2006)
2'-Fluoro RNA	Epidermal growth factor receptor	Cellulose filter-based SELEX with purified extracellular domain of human epidermal growth factor receptor protein	A431 cells (Li et al., 2010)
DNA	Protein tyrosine kinase 7	Cells-based SELEX with CCRF-CEM cells	CCRF-CEM cells (Huang et al., 2009) CCRF-CEM cells (Taghdisi et al., 2009; Kang et al., 2010) CCRF-CEM cells (Huang et al., 2008) Jurkat T cells (Tong et al., 2009)
DNA RNA	Transferrin receptor	Filter binding and affinity spin column-based SELEX with the recombinant extracellular domain of the mouse transferrin receptor	Mouse Idua ^{-/-} fibroblasts; LtK ⁻ cells (Chen et al., 2008)
DNA	Nucleolin	AS1411 or AGR100: a guanosine-rich oligonucleotide, which functions as an aptamer, but was not selected by SELEX.	MCF-7 cells (Cao et al., 2009)
DNA	MUC1	96-well polystyrene plate-based SELEX with the recombinant deglycosylated peptides representing five MUC1 tandem repeats	Epithelial cancer cells (Ferreira et al., 2009)

The RNA or RNA aptamers used as delivery ligands or vehicles for various cargoes are listed in the table.

SELEX, systematic enrichment of ligands by exponential enrichment; siRNA, small interfering RNA; TN-C, Tenascin-C; LNcaP, a line of human cells commonly used in the field of oncology; CEM or CCRF-CEM, human T cell lymphoblast-like cell line.

3-dimensional structures. They can be routinely identified through iterative rounds of *in vitro* selection, or SELEX (Tuerk and Gold, 1990) against a wide variety of targets. Basically, an initial combinatorial oligonucleotide library is used for selection that contains a central region with a 25–60 nt random sequence flanked by 2 fixed sequences. The fixed sequences are necessary for library amplification during selection. Random sequences with at least 10^{11} members (Sassanfar and Szostak, 1993) are assumed to be required for high molecular complexity and structural diversity, thereby guaranteeing the presence of active structures with high binding affinity to the target. A typical selection round consists of 3 steps: (1) binding to the target; (2) isolation of target-bound sequences; (3) recovery and reamplification of recovered sequences. The key step in aptamer selection is the isolation of target bound from unbound species. Recently, the development of some powerful tools for isolation, including flow cytometry, surface plasmon resonance (Sayer et al., 2002), capillary gel electrophoresis (Berezovski et al., 2005, 2006; Mosing et al., 2005; Drabovich et al., 2006; Mallikaratchy et al., 2006), and microfluidic devices (Farokhzad et al., 2005; Xu et al., 2009), greatly simplify the processes, thereby reducing the processing time and accelerating identification of potent aptamers.

In general, 5–15 rounds of iterative selection are sufficient to achieve enrichment of the library with predominant sequences exhibiting picomolar or nanomolar dissociation constants with the target. The number of selection rounds depends upon the length of the randomized sequence, characteristics of the target and the enrichment strategy. By carefully adjusting the stringency parameters of *in vitro* selection such as the oligonucleotide length, selection buffer pH, lowering the ionic strength, and adjusting the library and ligand concentrations, the inherent properties of selected aptamers can be finely tuned for different purposes. As nucleic acid entities, various backbone chemical modifications that are compatible with the enzymatic steps of the *in vitro* selection procedure can be introduced into the aptamer selection process to increase their stability in cells and *in vivo* (Keefe and Cload, 2008; Mayer, 2009). For example, the most popular modification of aptamers are the derivatives of the 2'-ribose, such as 2'-fluoro-, 2-amino-methyl, and 2'-O-methyl derivatives. Recently, locked-nucleic acid triphosphates have been adapted into the PCR amplification and *in vitro* transcription steps, allowing isolation of locked-nucleic acid containing aptamers (Veedu et al., 2009). Thus, aptamers can be precisely engineered to achieve additional functions and novel applications *via* site-specific chemical modifications.

The Generation of Cell-Specific Aptamers

To date, an increasing number of aptamers that target a specific cell type or subpopulation of malignant cells have been isolated and characterized through either traditional purified membrane protein-based SELEX or intact cell-based SELEX processes (Table 1). Several basic strategies are employed for the selection of protein-specific aptamers. In the traditional approach, purified proteins are generally immobilized on an appropriate affinity sorbent (bead, resin, column, membrane, chip, or plate), or the complexes of proteins and library are loaded onto nitrocellulose filters. Through simple washing steps with a buffer solution, unbound oligonucleotides are removed from the complexes and

the bound species are subsequently recovered and reamplified for next selection cycle. Thus far, most of the cell-specific aptamers for targeted delivery have been evolved by using the purified protein-based SELEX method. Despite these successes, efficient generation of new aptamers as cell-specific homing agents still poses a significant challenge when performing selection with recombinant proteins due to unavailability of the desired receptor species, labile native conformations (exchange of redox-status or multivalent-monovalent proteins), and problematic biochemical or physical properties (insolubility, instability, heavy glycosylation, etc.).

In this regard, whole cell-based SELEX provides a promising alternative for the generation of aptamers that can bind specifically to a particular target cell population (Phillips et al., 2008; Fang and Tan, 2009). In the cell-based selection, specific cell-surface molecules or even unknown membrane receptors can be directly targeted within their native environment, allowing a straight-forward enrichment of cell-specific aptamers. Cell-based SELEX generally consists of 2 procedures: positive selection with the target cells and counter selection with nontargeted cells. Therefore, the specificity and affinity of aptamers essentially relies upon the differences between 2 types of cells or different states of a cell, also making it possible to simultaneously enrich for aptamers against several membrane receptors. Using this approach, some aptamers have been isolated that are capable of recognizing different cell populations. For example, Black et al. showed that a cell-based DNA aptamer could distinguish rat glioblastoma from microglial cells (Blank et al., 2001). The Tan group also distinguished T-cells and B-cells in patient samples using DNA aptamers (Shangguan et al., 2006, 2007). However, nucleic acids can incur binding to dead cells, which can cause Cell-SELEX to fail. Moreover, in contrast with the traditional purified protein-based SELEX, the complexity and diversity of the cell surface ligands for aptamer binding also contributes to the difficulties in successful Cell-SELEX attempts.

The Development of Cell-Specific Aptamers for Targeted Delivery

Cell-type-specific aptamers targeting cell surface biomarkers or receptors have been used for targeted delivery of various entities (Table 1 and Fig. 1). Decoration of DNA or RNA aptamers on delivery vehicles confers selectivity in cell type interactions and can result in cellular internalization in the target cells, thereby increasing the therapeutic efficacy and reducing potential toxicities of the payloads. These aptamers constitute a mechanism for targeted delivery of various drugs or therapeutic oligonucleotides as discussed below.

Antiprostata-specific membrane antigen aptamers-mediated delivery

Since Lupold et al. (2002) first isolated the 2'-fluoro-modified RNA aptamers A-9 and A-10 against the prostate-specific membrane antigen (PSMA), a transmembrane protein that is highly expressed in human prostate cancer and the vascular endothelium (Liu et al., 1998; Tasch et al., 2001), an increasing number of studies have been conducted to incorporate these aptamers on various molecules or nano-carriers for selective delivery of therapeutics to PSMA-positive cells. Cell-type-specific anti-PSMA aptamer-mediated small

FIG. 1. Aptamer-mediated cell-type-specific drug delivery. Cell surface molecules such as receptors are present on the cell surface. (A) Schematic aptamer–streptavidin–small interfering RNA (siRNA) conjugates. (B) Schematic aptamer–siRNA chimeras. (C) Schematic aptamer–protein (toxin or enzyme) chemical conjugates. (D) Schematic aptamer–nanoparticle–drug conjugates and aptamer–drug physical conjugates. (E) Schematic aptamer–stick–siRNA conjugates. (F) Schematic aptamer-mediated others drug conjugates.

interfering RNA (siRNAs) delivery has been demonstrated by 3 independent research groups. In the first report of aptamer-targeted RNA interference (Chu et al., 2006b), a 27-mer siRNA targeting lamin A/C or GAPDH was delivered to PSMA-positive cells with the A-9 aptamer. Two biotinylated aptamers and siRNAs were noncovalently assembled on a Streptavidin scaffold (Fig. 1A), allowing effective PSMA-mediated cell uptake along with siRNA-mediated target gene silencing. Giangrande and colleagues developed a simple approach to covalently append the A-10 PSMA aptamer to the sense strand of a 21-mer siRNA (Fig. 1B) (McNamara et al., 2006). The resulting aptamer–PLK1 or BCL2 siRNA chimeras induced apoptosis in LNCaP cells and tumor regression *in vivo* after intratumoral delivery. Most recently, the same group optimized their previous chimeric design through rational modifications of the siRNA portion (eg, addition of a 2-nucleotide overhang at the 3'-end, swap of the guide strand and passenger strand, and PEGylation of the passenger strand), thereby prolonging silencing and enhancing the therapeutic efficacy after systemic administration (Dassie et al., 2009). In another effort, Wullner et al. designed 2 bivalent anti-PSMA aptamer–EEF2 siRNA chimeras in which the siRNA itself was used as linker to join the 2 aptamers (Wullner et al., 2008). The multivalent constructs promoted up to 4 times more cellular uptake than their monovalent counterparts, resulting in in-

creased EEF2-siRNA-induced cytotoxicity effects. Although the above reports provided proof of concept, further validation and investigation into the mechanism of this siRNA delivery scheme is required for its general utility for siRNA delivery. For example, the method for conjugation of the siRNA portion to the aptamer and the types of cells used for the siRNA mediated inhibition can influence the results of aptamer mediated delivery of siRNAs. In addition to therapeutic siRNAs, toxins and chemotherapeutic agents have been assembled with aptamers through various strategies. Such aptamer-mediated delivery systems are expected to achieve selective uptake into target cells, thereby minimizing unwanted side effects of the drugs in nontarget cells. The A-9 PSMA aptamer has been employed as a carrier to deliver a gelonin toxin (rGel) into PSMA receptor-expressing cells (Fig. 1C) (Chu et al., 2006a). After modification of the SPDP reagent, the 5'-amino end of the A-9 molecule was covalently conjugated with the surface cysteines of recombinant gelonin to yield aptamer–gelonin conjugates. As anticipated, the conjugates not only showed specific internalization into target cells, but also decreased the toxicity of gelonin by preventing its uptake in nontargeted cells.

Applications of antitumor drugs (such as docetaxel, dextran, cisplatin, daunorubicin, and doxorubicin) are limited due to their side effects/toxicity. The first report of targeted

drug delivery with nanoparticle-aptamer bioconjugates was described by Farokhzad et al. in 2004 (Fig. 1D) (Farokhzad et al., 2004). They first encapsulated rhodamine-labeled dextran within PEGylated PLA nanoparticles (PLA-PEG-COOH) containing a terminal carboxylic acid functional group attached by a nano-precipitation technique, and subsequently covalently inserted amine-modified A-10 PSMA aptamers on the surface of nanoparticles *via* a carbodiimide coupling approach. The resulting bioconjugate was shown to efficiently and selectively deliver drugs to the prostate LNCaP epithelial cells, achieving increased binding over the control, non-aptamer-based delivery. In an extension of this study, the same group employed a xenograft nude mouse model to evaluate the *in vivo* efficacy of the formulation (Dtxl-NP-Apt: Docetaxel-encapsulated PLGA-b-PEG nanoparticles-A-10 aptamer conjugates) (Farokhzad et al., 2006). When compared with nontargeted nanoparticles that lacked the anti-PSMA aptamer, such Dtxl-NP-Apt conjugates mediated targeted uptake and controlled release of drugs, also resulting in potent efficacy and reduced toxicity after intratumoral injection. Further investigations conducted by the Farokhzad group (Cheng et al., 2007; Farokhzad et al., 2005; Gu et al., 2008) have demonstrated that the nanoparticle-aptamer bioconjugates can be precisely engineered and formulated by controlling size, composition, polydispersity, aptamer density, and drug loading, thereby leading to the desired functions and biodistribution required for further clinical development.

Instead of chemically decorating aptamers on the nanoparticle surface, a simple physical conjugation strategy was utilized to facilitate drug delivery in which anthracycline drugs were allowed to noncovalently intercalate into double-strand regions of the aptamer and form a physical complex (Bagalkot et al., 2006). As shown in Fig. 1D, doxorubicin (Dox, represented by the pink globule) intercalated within the helical strand of the anti-PSMA A-10 aptamer *via* the flat aromatic ring and formed stable physical conjugates, specifically recognizing the PSMA-positive LNCaP cells. Thereafter, the Farokhzad group combined the properties of the above 2 delivery systems to engineer a multifunctional nanoparticle-aptamer bioconjugate capable of simultaneously codelivering 2 distinct drugs (Zhang et al., 2007). In this codelivery system, a preformed Dox-A-10 aptamer physical conjugate was chemically assembled on the surface of Dtxl-NP-Apt bioconjugates through an 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride/N-hydroxysuccinimide (NHS) approach, thereby allowing selective codelivery of Dtxl and Dox to the target LNCaP cells. In a similar manner, a multifunctional superparamagnetic iron oxide nanoparticle-aptamer bioconjugate was formulated for targeted delivery of doxorubicin and prostate cancer imaging (Wang et al., 2008). Recently, anti-PSMA aptamer (A-9)-targeted gold nanoparticles were demonstrated as molecular-specific contrast agents for reflectance imaging. An oligonucleotide extension was added to the aptamer to serve as a hybridization site for complementary oligonucleotide-coated gold nanoparticles (Javier et al., 2008).

Anti-CD4 aptamers as delivery vehicles

A CD4-specific RNA aptamer generated by Kraus et al. (1998) also has been exploited to specifically deliver siRNA into CD4-overexpressing T cells. The Guo lab took the advantage of the self-assembling ability of bacteriophage phi29

RNA (pRNA) to fabricate a multifunctional nano-device using receptor-mediated endocytosis of an siRNA and a fluorescent tag (Guo et al., 2005). For example, pRNA molecules, containing right- or left-hand loops capable of polymerization *via* interlocking loop-loop interaction, were, respectively, fused with the CD4-specific aptamer, siRNAs, or a fluorescent dye (Khaled et al., 2005). Under specialized conditions divalent ion and salt concentrations, the resulting chimeric pRNA-aptamer and pRNA-siRNA subsequently form into dimers or trimers ~25–40 nm in diameter. When this nano-scale RNA dimer was applied to a T-cell line engineered to overexpress the CD4 receptor, specific internalization and silencing of the target gene were achieved.

Anti-HIV gp120 aptamer mediated siRNA delivery

2'-F-modified RNA aptamers that bind the HIV-1 envelope glycoprotein gp120 were demonstrated to neutralize HIV-1 (Sayer et al., 2002; Khati et al., 2003; Dey et al., 2005; Zhou et al., 2009). Using the aptamer-siRNA fusion approach described by McNamara et al. (Fig. 1B), a dual-action anti-gp120 aptamer-siRNA chimera containing a 27-mer anti-*tat/rev* duplex RNA was constructed. The chimeras were selectively taken up by HIV-1-infected cells and the siRNA component triggered a downregulation of the targeted HIV *tat/rev* transcripts (Zhou et al., 2008). Recently, we tested the gp120-*tat/rev* siRNA chimera in a HIV-1-infected humanized RAG-Hu mouse model. We observed that the aptamer-siRNA chimeras provided several logs of inhibition of HIV-1 replication with a single injection, and multiple injections completely prevented the CD4⁺ T-cells depletion normally mediated by viral infection (P. Neff et al., manuscript submitted). Importantly, specific knockdown of the target gene and detection of the siRNA was only detectable in gp120 aptamer-siRNA chimera-treated mice, but not in animals treated with uncomplexed siRNAs or a mutant aptamer-siRNA chimera, thus validating gp120 aptamer-targeted siRNA delivery.

Although siRNAs hold therapeutic promise for the treatment HIV-1 infection, rapid emergence of viral escape mutants often abrogate the efficacy of RNAi. In this regard, a combinatorial multitargeting RNAi therapeutics represents an attractive HIV-1 treatment strategy for averting viral escape mutants. We therefore exploited anti-gp120 aptamer for combinatorial siRNA delivery through a sticky bridge strategy (Zhou et al., 2009). As illustrated in Fig. 1E, 1 pair of GC rich complementary sticky sequences (represented by the string of black and gray symbols) were chemically appended to the 3'-terminal of the aptamer and either the guide strand or passenger strand of the various 27-mer siRNAs. Moreover, a flexible 3-carbon atom hinge (represented by string of green symbols) was added between the aptamer RNA and the sticky sequence, thereby minimizing a potential steric hindrance between aptamer and siRNA, and allowing efficient Dicer processing of the siRNA portion.

In our example, a single gp120 aptamer species was used in combination with 3 different siRNAs targeting HIV-1 *tat/rev* transcripts and HIV-1 host dependency factors (CD4 and TNPO3). Despite some competition between siRNA duplexes for RISC entry, the resulting aptamer-stick-cocktail siRNA conjugates markedly suppressed viral loads *in vivo* and preserved CD4⁺ T-cells levels (Zhou et al., unpublished results).

Anti-Tenascin-C aptamer

Because of the abundance of Tenascin-C (TN-C) in tumor stroma and its association with angiogenesis, high-affinity TN-C ligands may be clinically useful tumor-targeting agents. TN-C is a very large hexameric glycoprotein ($>10^6$ Da) that is overexpressed during tissue remodeling processes, including tumor growth and angiogenesis (Erickson and Bourdon, 1989). TN-C levels in tumors are significantly higher than in normal tissue, making TN-C as a promising target that is directly accessible to circulating aptamers. In 2001, Hicke et al. (2001) performed a crossover *in vitro* SELEX procedure to effectively identify 2 high-affinity RNA aptamers, using both the TN-C protein and TN-C-expressing U251 glioblastoma cells. To minimize nuclease degradation, 1 size-minimized, 2'-fluoro-modified RNA aptamer TTA1 was modified post-SELEX by substituting 2'-O-methyl-purine nucleoside for ribose containing nucleotides. It has been demonstrated that TTA1 was able to effectively target and be taken up by a variety of solid tumors, including breast, glioblastoma, lung, and colon. Moreover, a correlation between levels of human TN-C expression and uptake of the aptamer was demonstrated. By the use of site-specific modifications, aptamer properties can be fine-tuned for different purposes. For example, the TTA1 aptamer containing 5'-amine was radio-labeled with ^{99m}Tc and was shown to image TN-C expressing tumors in a xenograph mouse model (Hicke et al., 2006). Although the current work focused on TTA1-radioisotopes or fluorescent dye conjugates for tumor imaging, the same aptamers could be assembled with other therapeutic agents for potential clinical applications.

Anti-epidermal growth factor receptor aptamers

The epidermal growth factor receptor (EGFR), a cell-surface receptor for members of the EGF-family of extracellular protein ligands, has been shown to be involved in many types of human cancers and undergoes endocytotic internalization (Singh and Harris, 2005). Most recently, the Ellington group succeeded in identifying RNA aptamers against the purified extracellular domain of human EGFR through cellulose filter-based *in vitro* selection process (Li et al., 2010). The anti-EGFR aptamer J18 with a K_d of about 7 nM was noncovalently installed on the surface of gold nanoparticles (GNPs) *via* a facile hybridization approach in which GNPs were coated with capture DNA oligonucleotides. These were next hybridized with the corresponding complementary oligonucleotides that were appended to 5'-terminal of aptamers. Their results demonstrated that the anti-EGFR aptamer can elicit the specific internalization of GNPs to EGFR-expressing cells through receptor-mediated endocytosis, as well as reduce nonspecific absorption of GNPs.

Anti-protein tyrosine kinase 7 aptamers

As mentioned before, the objective of a cell-based selection is to generate aptamers that can specifically recognize a particular target cell population. An example using a cell-based SELEX procedure was reported by Tan and his colleagues in 2006 (Shangguan et al., 2006). A panel of cell-specific DNA aptamers was evolved with binding affinity to CCRF-CEM cells (a T-cell acute lymphoblastic leukemia cell line) without prior information about the molecular targets. These selected aptamers were able to distinguish molecular differences on

cancer cells in patient samples (Shangguan et al., 2007). Further target validation revealed that the human protein tyrosine kinase 7 (PTK7), a transmembrane receptor highly expressed on CCRF-CEM cells, is the molecular target of one of the selected aptamers (sgc8 sequence). Moreover, the sgc8 DNA aptamer was demonstrated to be specifically internalized into the target cells *via* a receptor-mediated endocytosis, suggesting that it is a promising targeting ligand-directed lymphoblastic leukemia T-cells (Xiao et al., 2008).

Following this line the Tan group covalently linked the anti-PTK7 aptamer (sgc8) with chemotherapeutic agents for cell-specific delivery (Fig. 1F) (Huang et al., 2009). The aptamer 5'-SH group was chemically cross-linked with doxorubicin (Dox) *via* an acid-labile linkage that could allow cleavage and release the Dox inside the acidic endosomal environment. The aptamer-Dox conjugates not only maintained specific binding and high affinity to target cancer cells, but also showed selectivity for killing targeted cancer cells. The aforementioned approach also was employed to noncovalently assemble an sgc8 aptamer with daunorubicin, which was able to intercalate within the helical strand of the sgc8 aptamer for targeted delivery to T-cell acute lymphoblastic leukemia cells (Taghdisi et al., 2009).

Most recent work from the Tan lab has provided an aptamer-modified liposome system for exploiting an anti-PTK7 aptamer as a promising drug delivery vehicle (Kang et al., 2010). In this example, the sgc8 aptamer was chemically linked to the liposome by a PEG spacer, resulting in enhanced stability compared with plain liposomes. Their results demonstrated that this aptamer-modified liposome Dextran delivery system has ~250 aptamers per liposome, thus resulting in multiple aptamer-receptor interactions and facilitated binding with the target cells. As a consequence, high specificity and efficient release of the loaded model drug was achieved using this delivery system. In another example, the multivalent aptamer-conjugated nanostructure was designed that combined the specific recognition ability of aptamers with the high absorption efficiency of Au-Ag nanorods, serving as a selective therapeutic agent for targeted cancer cell photothermal destruction (Huang et al., 2008). When these aptamer-nanorod conjugates were applied to both suspension cells and artificial solid tumor samples, selective and efficient photothermal killing of target tumor cells, and excellent hyperthermia efficiency and selectivity were displayed.

An anti-PTK7 aptamer (sgc8c) also was attached to the surface of a genome-free viral capsid carrier through an efficient oxidative coupling strategy (Tong et al., 2009). The resulting multivalent aptamer-capsid-targeted delivery system showed significant levels of binding to the cells relative to those of control samples.

Despite these successful reports, in a recent study published by Ellington (Li et al., 2009) the authors instead claim that their work indicates that this aptamer does not specifically bind to PTK7 but instead exhibits a propensity to associate with adhering cells. The considerable argument surrounding the binding and specificity of PTK7 aptamers therefore needs further investigation.

Anti-transferrin receptor aptamers

It was previously known that transferrin can serve as a targeting ligand for receptor-mediated endocytosis *via* the

transferrin receptor (TfR). In 2008, Neufeld and colleagues first isolated several RNA and DNA aptamers against the extracellular domain of the mouse TfR, which is also subject to rapid internalization by endocytosis (Chen et al., 2008). Therefore, the investigators took advantage of the TfR binding properties of an anti-TfR aptamer to explore the potential of using a DNA aptamer for delivery of the enzyme α -L-iduronidase into enzyme deficient mouse fibroblasts. In this method, the terminal glycerol of anti-TfR DNA aptamer was chemically oxidized by periodate. The resulting aldehyde functional group was subsequently reacted with amino groups on the protein (lysosomal enzyme). This study was the first to show the rescue of an enzymatic cellular activity through aptamer-mediated enzyme delivery, suggesting a potential therapeutic approach for the treatment of lysosomal storage diseases.

Anti-MUC1 aptamer

Membrane-associated glycoforms are an important class of tumor surface biomarkers that are uniquely and abundantly expressed on a broad range of epithelial cancer cells. These are also rapidly recycled through intracellular compartments (endosomes, Golgi) and internalized, therefore representing an excellent entry conduit for importing aptamers (Ceriani et al., 1992; Altschuler et al., 2000; Gendler, 2001). Mucins are a group of glycoproteins found in the secretions of mucous membranes. Overexpression of the mucin proteins, especially MUC1, is associated with many types of cancer. Ferreira et al. recently performed an *in vitro* selection process with an engineered mimic of known mucin MUC1 determinants and selected several DNA aptamers (Ferreira et al., 2009). The aptamers with the highest affinity were efficiently internalized by epithelial cancer cells, and specifically delivered a photodynamic therapy agent (chlorine e_6) into epithelial cancer cells with a remarkable enhancement of efficiency (>500-fold increase) compared with the drug alone. No cytotoxicity was observed in the cells lacking the target O-glycan-peptide markers. These data suggested the anti-MUC1 aptamers can function as drug carriers to specific epithelial cancer cells.

Anti-nucleolin aptamer

In addition to the aptamers selected by SELEX, AS1411 (formerly named AGRO100) is a 26-mer guanine-rich oligonucleotide that was not evolved by SELEX. It, nevertheless, also functions as an aptamer and is now being used as an anticancer agent in phase II clinical trials (Bates et al., 2009). AS1411 is known to bind with high affinity to nucleolin (NCL), a bcl-2 mRNA binding protein involved in cell proliferation (Bates et al., 1999). However, the biological consequences of this interaction are not well understood. Although NCL is found within the nucleus of all cells, it is uniquely expressed on the surface of tumor cells. NCL overexpression on the plasma membrane has been linked to various human diseases such as breast cancer; therefore, it provides a potential target for cancer-cell-specific drug delivery. Moreover, the binding of AS1411 can result in internalization of the aptamer-NCL complex and a consequent antiproliferative activity in several cancer cell lines, including breast cancer cells, thus making AS1411 a promising approach for targeting breast cancer. In 2009, Cao et al. developed a reversible

AS1411 aptamer-liposome bioconjugate that can effectively deliver cisplatin in a cancer-cell-specific manner (Cao et al., 2009). The 3'-terminal cholesterol tag of the aptamer ensures immobilization of aptamers on the liposome surface by insertion into the hydrophobic lipid membrane. The formulation of the multifunctional aptamer-liposome-cisplatin conjugate enhanced receptor-mediated cell uptake and improved the therapeutic efficacy. Because the essential feature of aptamer affinity and specificity are the unique 3-dimensional structures, aptamer active structure or binding affinities can be disrupted by their complementary DNAs. Thus, when needed (such as the alleviation of overdose or drug-induced allergic reactions) a nucleic acid-based antidote can be employed to deactivate NCL-aptamers on the liposome surface and inhibit cell uptake of the drug-encapsulated liposome.

Conclusion and Perspectives

Nucleic acid-based aptamers evolved *in vitro* have been shown to play significant roles in biosensing, medical diagnostics, molecular imaging, targeted delivery, and therapy. In particular, during the last 6 years, an ever-increasing number of studies concerning development of cell-specific aptamers have been emphasizing their potential as either targeting ligands or guided carriers for drug delivery. With the rapid progresses in cell-based SELEX technology and versatile chemical modification methods, it has become clear that synthetic, cell-specific aptamers can be readily adapted for drug delivery.

Despite the advances described above, continued efforts are definitely required to improve targeting efficiency, drug loading capacity, circulation time, and binding kinetics. Multimerization of aptamers with drugs, decoration of multivalent aptamers on nano-carriers, and development of facile conjugation strategies are also remaining challenges. Nevertheless, nucleic acid-based aptamers are amenable to rapid changes in design and structure that can address these challenges.

Acknowledgments

This work was supported by grants from the National Institutes of Health AI29329, AI42552, and HL07470 awarded to J.J.R.

Authors' Contributions

J.Z. drafted the article. J.R. revised it and gave final approval of the version to be published. All authors read and approved the final article.

Author Disclosure Statement

The authors declare that they have no competing financial interests.

References

- ALTSCHULER, Y., KINLOUGH, C.L., POLAND, P.A., BRUNS, J.B., APODACA, G., WEISZ, O.A., and HUGHEY, R.P. (2000). Clathrin-mediated endocytosis of MUC1 is modulated by its glycosylation state. *Mol. Biol. Cell* **11**, 819–831.
- BAGALKOT, V., FAROKHZAD, O.C., LANGER, R., and JON, S. (2006). An aptamer-doxorubicin physical conjugate as a

- novel targeted drug-delivery platform. *Angew. Chem. Int. Ed. Engl.* **45**, 8149–8152.
- BATES, P.J., KAHN, J.B., THOMAS, S.D., TRENT, J.O., and MILLER, D.M. (1999). Antiproliferative activity of G-rich oligonucleotides correlates with protein binding. *J. Biol. Chem.* **274**, 26369–26377.
- BATES, P.J., LABER, D.A., MILLER, D.M., THOMAS, S.D., and TRENT, J.O. (2009). Discovery and development of the G-rich oligonucleotide AS1411 as a novel treatment for cancer. *Exp. Mol. Pathol.* **86**, 151–164.
- BEREZOVSKI, M., DRABOVICH, A., KRYLOVA, S.M., MUSHEEV, M., OKHONIN, V., PETROV, A., and KRYLOV, S.N. (2005). Nonequilibrium capillary electrophoresis of equilibrium mixtures: a universal tool for development of aptamers. *J. Am. Chem. Soc.* **127**, 3165–3171.
- BEREZOVSKI, M., MUSHEEV, M., DRABOVICH, A., and KRYLOV, S.N. (2006). Non-SELEX selection of aptamers. *J. Am. Chem. Soc.* **128**, 1410–1411.
- BLANK, M., WEINSCHENK, T., PRIEMER, M., and SCHLUESENER, H. (2001). Systematic evolution of a DNA aptamer binding to rat brain tumor microvessels. selective targeting of endothelial regulatory protein pigpen. *J. Biol. Chem.* **276**, 16464–16468.
- CAO, Z., TONG, R., MISHRA, A., XU, W., WONG, G.C., CHENG, J., and LU, Y. (2009). Reversible cell-specific drug delivery with aptamer-functionalized liposomes. *Angew. Chem. Int. Ed. Engl.* **48**, 6494–6498.
- CERIANI, R.L., CHAN, C.M., BARATTA, F.S., OZZELLO, L., DEROSA, C.M., and HABIF, D.V. (1992). Levels of expression of breast epithelial mucin detected by monoclonal antibody BrE-3 in breast-cancer prognosis. *Int. J. Cancer* **51**, 343–354.
- CHEN, C.H., DELLAMAGGIORE, K.R., OUELLETTE, C.P., SEDANO, C.D., LIZADJOHRY, M., CHERNIS, G.A., GONZALES, M., BALTASAR, F.E., FAN, A.L., MYEROWITZ, R., and NEUFELD, E.F. (2008). Aptamer-based endocytosis of a lysosomal enzyme. *Proc. Natl. Acad. Sci. U. S. A.* **105**, 15908–15913.
- CHENG, J., TEPLY, B.A., SHERIFI, I., SUNG, J., LUTHER, G., GU, F.X., LEVY-NISSENBAUM, E., RADOVIC-MORENO, A.F., LANGER, R., and FAROKHZAD, O.C. (2007). Formulation of functionalized PLGA-PEG nanoparticles for *in vivo* targeted drug delivery. *Biomaterials* **28**, 869–876.
- CHU, T.C., MARKS, J.W., 3RD, LAVERY, L.A., FAULKNER, S., ROSENBLUM, M.G., ELLINGTON, A.D., and LEVY, M. (2006a). Aptamer:toxin conjugates that specifically target prostate tumor cells. *Cancer Res.* **66**, 5989–5992.
- CHU, T.C., TWU, K.Y., ELLINGTON, A.D., and LEVY, M. (2006b). Aptamer mediated siRNA delivery. *Nucleic Acids Res.* **34**, e73.
- DASSIE, J.P., LIU, X.Y., THOMAS, G.S., WHITAKER, R.M., THIEL, K.W., STOCKDALE, K.R., MEYERHOLZ, D.K., MCCAFFREY, A.P., MCNAMARA, J.O., 2ND, and GIANGRANDE, P.H. (2009). Systemic administration of optimized aptamer-siRNA chimeras promotes regression of PSMA-expressing tumors. *Nat. Biotechnol.* **27**, 839–849.
- DEY, A.K., KHATI, M., TANG, M., WYATT, R., LEA, S.M., and JAMES, W. (2005). An aptamer that neutralizes R5 strains of human immunodeficiency virus type 1 blocks gp120-CCR5 interaction. *J. Virol.* **79**, 13806–13810.
- DHAR, S., GU, F.X., LANGER, R., FAROKHZAD, O.C., and LIPPARD, S.J. (2008). Targeted delivery of cisplatin to prostate cancer cells by aptamer functionalized Pt(IV) prodrug-PLGA-PEG nanoparticles. *Proc. Natl. Acad. Sci. U. S. A.* **105**, 17356–17361.
- DRABOVICH, A.P., BERZOVSKI, M., OKHONIN, V., and KRYLOV, S.N. (2006). Selection of smart aptamers by methods of kinetic capillary electrophoresis. *Anal. Chem.* **78**, 3171–3178.
- ELLINGTON, A.D., and SZOSTAK, J.W. (1990). *In vitro* selection of RNA molecules that bind specific ligands. *Nature* **346**, 818–822.
- ERICKSON, H.P., and BOURDON, M.A. (1989). Tenascin: an extracellular matrix protein prominent in specialized embryonic tissues and tumors. *Annu. Rev. Cell Biol.* **5**, 71–92.
- FAMULOK, M., HARTIG, J.S., and MAYER, G. (2007). Functional aptamers and aptazymes in biotechnology, diagnostics, and therapy. *Chem. Rev.* **107**, 3715–3743.
- FANG, X., and TAN, W. (2009). Aptamers generated from cell-SELEX for molecular medicine: a chemical biology approach. *Acc. Chem. Res.* **43**, 48–57.
- FAROKHZAD, O.C., CHENG, J., TEPLY, B.A., SHERIFI, I., JON, S., KANTOFF, P.W., RICHIE, J.P., and LANGER, R. (2006). Targeted nanoparticle-aptamer bioconjugates for cancer chemotherapy *in vivo*. *Proc. Natl. Acad. Sci. U. S. A.* **103**, 6315–6320.
- FAROKHZAD, O.C., JON, S., KHADEMHOSEINI, A., TRAN, T.N., LAVAN, D.A., and LANGER, R. (2004). Nanoparticle-aptamer bioconjugates: a new approach for targeting prostate cancer cells. *Cancer Res.* **64**, 7668–7672.
- FAROKHZAD, O.C., KHADEMHOSEINI, A., JON, S., HERMMANN, A., CHENG, J., CHIN, C., KISELYUK, A., TEPLY, B., ENG, G., and LANGER, R. (2005). Microfluidic system for studying the interaction of nanoparticles and microparticles with cells. *Anal. Chem.* **77**, 5453–5459.
- FERREIRA, C.S., CHEUNG, M.C., MISSAILIDIS, S., BISLAND, S., and GARIEPY, J. (2009). Phototoxic aptamers selectively enter and kill epithelial cancer cells. *Nucleic Acids Res.* **37**, 866–876.
- GENDLER, S.J. (2001). MUC1, the renaissance molecule. *J. Mammary Gland Biol. Neoplasia* **6**, 339–353.
- GU, F., ZHANG, L., TEPLY, B.A., MANN, N., WANG, A., RADOVIC-MORENO, A.F., LANGER, R., and FAROKHZAD, O.C. (2008). Precise engineering of targeted nanoparticles by using self-assembled biointegrated block copolymers. *Proc. Natl. Acad. Sci. U. S. A.* **105**, 2586–2591.
- GUO, P. (2005). RNA nanotechnology: engineering, assembly and applications in detection, gene delivery and therapy. *J. Nanosci. Nanotechnol.* **5**, 1964–1982.
- GUO, S., TSCHAMMER, N., MOHAMMED, S., and GUO, P. (2005). Specific delivery of therapeutic RNAs to cancer cells via the dimerization mechanism of phi29 motor pRNA. *Hum. Gene Ther.* **16**, 1097–1109.
- HESSELBERTH, J., ROBERTSON, M.P., JHAVERI, S., and ELLINGTON, A.D. (2000). *In vitro* selection of nucleic acids for diagnostic applications. *J. Biotechnol.* **74**, 15–25.
- HICKE, B.J., and STEPHENS, A.W. (2000). Escort aptamers: a delivery service for diagnosis and therapy. *J. Clin. Invest.* **106**, 923–928.
- HICKE, B.J., MARION, C., CHANG, Y.F., GOULD, T., LYNOTT, C.K., PARMA, D., SCHMIDT, P.G., and WARREN, S. (2001). Tenascin-C aptamers are generated using tumor cells and purified protein. *J. Biol. Chem.* **276**, 48644–48654.
- HICKE, B.J., STEPHENS, A.W., GOULD, T., CHANG, Y.F., LYNOTT, C.K., HEIL, J., BORKOWSKI, S., HILGER, C.S., COOK, G., WARREN, S., and SCHMIDT, P.G. (2006). Tumor targeting by an aptamer. *J. Nucl. Med.* **47**, 668–678.
- HUANG, Y.F., CHANG, H.T., and TAN, W. (2008). Cancer cell targeting using multiple aptamers conjugated on nanorods. *Anal. Chem.* **80**, 567–572.

- HUANG, Y.F., SHANGGUAN, D., LIU, H., PHILLIPS, J.A., ZHANG, X., CHEN, Y., and TAN, W. (2009). Molecular assembly of an aptamer-drug conjugate for targeted drug delivery to tumor cells. *Chembiochem* **10**, 862–868.
- JAVIER, D.J., NITIN, N., LEVY, M., ELLINGTON, A., and RICHARDS-KORTUM, R. (2008). Aptamer-targeted gold nanoparticles as molecular-specific contrast agents for reflectance imaging. *Bioconjug. Chem.* **19**, 1309–1312.
- KANG, H., O'DONOGHUE, M.B., LIU, H., and TAN, W. (2010). A liposome-based nanostructure for aptamer directed delivery. *Chem. Commun. (Camb.)* **46**, 249–251.
- KEEFE, A.D., and CLOAD, S.T. (2008). SELEX with modified nucleotides. *Curr. Opin. Chem. Biol.* **12**, 448–456.
- KHALED, A., GUO, S., LI, F., and GUO, P. (2005). Controllable self-assembly of nanoparticles for specific delivery of multiple therapeutic molecules to cancer cells using RNA nanotechnology. *Nano Lett.* **5**, 1797–1808.
- KHATI, M., SCHUMAN, M., IBRAHIM, J., SATTENTAU, Q., GORDON, S., and JAMES, W. (2003). Neutralization of infectivity of diverse R5 clinical isolates of human immunodeficiency virus type 1 by gp120-binding 2'F-RNA aptamers. *J. Virol.* **77**, 12692–12698.
- KRAUS, E., JAMES, W., and BARCLAY, A.N. (1998). Cutting edge: novel RNA ligands able to bind CD4 antigen and inhibit CD4+ T lymphocyte function. *J. Immunol.* **160**, 5209–5212.
- LEE, J.F., STOVALL, G.M., and ELLINGTON, A.D. (2006). Aptamer therapeutics advance. *Curr. Opin. Chem. Biol.* **10**, 282–289.
- LEVY-NISSENBAUM, E., RADOVIC-MORENO, A.F., WANG, A.Z., LANGER, R., and FAROKHZAD, O.C. (2008). Nanotechnology and aptamers: applications in drug delivery. *Trends Biotechnol.* **26**, 442–449.
- LI, N., EBRIGHT, J.N., STOVALL, G.M., CHEN, X., NGUYEN, H.H., SINGH, A., SYRETT, A., and ELLINGTON, A.D. (2009). Technical and biological issues relevant to cell typing with aptamers. *J. Proteome Res.* **8**, 2438–2448.
- LI, N., LARSON, T., NGUYEN, H.H., SOKOLOV, K.V., and ELLINGTON, A.D. (2010). Directed evolution of gold nanoparticle delivery to cells. *Chem. Commun. (Camb.)* **46**, 392–394.
- LIU, H., RAJASEKARAN, A.K., MOY, P., XIA, Y., KIM, S., NAVARRO, V., RAHMATI, R., and BANDER, N.H. (1998). Constitutive and antibody-induced internalization of prostate-specific membrane antigen. *Cancer Res.* **58**, 4055–4060.
- LORGER, M., ENGSTLER, M., HOMANN, M., and GORINGER, H.U. (2003). Targeting the variable surface of African trypanosomes with variant surface glycoprotein-specific, serum-stable RNA aptamers. *Eukaryot. Cell* **2**, 84–94.
- LUPOLD, S.E., HICKE, B.J., LIN, Y., and COFFEY, D.S. (2002). Identification and characterization of nuclease-stabilized RNA molecules that bind human prostate cancer cells via the prostate-specific membrane antigen. *Cancer Res.* **62**, 4029–4033.
- MALLIKARATCHY, P., STAHELIN, R.V., CAO, Z., CHO, W., and TAN, W. (2006). Selection of DNA ligands for protein kinase C- δ . *Chem. Commun. (Camb.)* **14**, 3229–3231.
- MAYER, G. (2009). The chemical biology of aptamers. *Angew. Chem. Int. Ed. Engl.* **48**, 2672–2689.
- MCMANARA, J.O., 2ND, ANDRECHEK, E.R., WANG, Y., VILES, K.D., REMPEL, R.E., GILBOA, E., SULLENGER, B.A., and GIANGRANDE, P.H. (2006). Cell type-specific delivery of siRNAs with aptamer-siRNA chimeras. *Nat. Biotechnol.* **24**, 1005–1015.
- MOSING, R.K., MENDONSA, S.D., and BOWSER, M.T. (2005). Capillary electrophoresis-SELEX selection of aptamers with affinity for HIV-1 reverse transcriptase. *Anal. Chem.* **77**, 6107–6112.
- NIMJEE, S.M., RUSCONI, C.P., and SULLENGER, B.A. (2005). Aptamers: an emerging class of therapeutics. *Annu. Rev. Med.* **56**, 555–583.
- PHILLIPS, J.A., LOPEZ-COLON, D., ZHU, Z., XU, Y., and TAN, W. (2008). Applications of aptamers in cancer cell biology. *Anal. Chim. Acta.* **621**, 101–108.
- SASSANFAR, M., and SZOSTAK, J.W. (1993). An RNA motif that binds ATP. *Nature* **364**, 550–553.
- SAYER, N., IBRAHIM, J., TURNER, K., TAHIRI-ALAOU, A., and JAMES, W. (2002). Structural characterization of a 2'F-RNA aptamer that binds a HIV-1 SU glycoprotein, gp120. *Biochem. Biophys. Res. Commun.* **293**, 924–931.
- SHANGGUAN, D., CAO, Z.C., LI, Y., and TAN, W. (2007). Aptamers evolved from cultured cancer cells reveal molecular differences of cancer cells in patient samples. *Clin. Chem.* **53**, 1153–1155.
- SHANGGUAN, D., LI, Y., TANG, Z., CAO, Z.C., CHEN, H.W., MALLIKARATCHY, P., SEFAH, K., YANG, C.J., and TAN, W. (2006). Aptamers evolved from live cells as effective molecular probes for cancer study. *Proc. Natl. Acad. Sci. U. S. A.* **103**, 11838–11843.
- SHAW, B.R., MOUSSA, L., SHARAF, M., CHEEK, M., and DOBRIKOV, M. (2008). Boranophosphate siRNA-aptamer chimeras for tumor-specific downregulation of cancer receptors and modulators. *Nucleic Acids Symp. Ser. (Oxf.)* **52**, 655–656.
- SINGH, A.B., and HARRIS, R.C. (2005). Autocrine, paracrine and juxtacrine signaling by EGFR ligands. *Cell Signal.* **17**, 1183–1193.
- TAGHDISI, S.M., ABNOUS, K., MOSAFFA, F., and BEHRANVAN, J. (2009). Targeted delivery of daunorubicin to T-cell acute lymphoblastic leukemia by aptamer. *J. Drug Target* **18**, 277–281.
- TANG, Z., PAREKH, P., TURNER, P., MOYER, R.W., and TAN, W. (2009). Generating aptamers for recognition of virus-infected cells. *Clin. Chem.* **55**, 813–822.
- TASCH, J., GONG, M., SADELAIN, M., and HESTON, W.D. (2001). A unique folate hydrolase, prostate-specific membrane antigen (PSMA): a target for immunotherapy? *Crit. Rev. Immunol.* **21**, 249–261.
- THIEL, K.W., and GIANGRANDE, P.H. (2009). Therapeutic applications of DNA and RNA aptamers. *Oligonucleotides* **19**, 209–222.
- TONG, G.J., HSIAO, S.C., CARRICO, Z.M., and FRANCIS, M.B. (2009). Viral capsid DNA aptamer conjugates as multivalent cell-targeting vehicles. *J. Am. Chem. Soc.* **131**, 11174–11178.
- TUERK, C., and GOLD, L. (1990). Systematic evolution of ligands by exponential enrichment: RNA ligands to bacteriophage T4 DNA polymerase. *Science* **249**, 505–510.
- VEEDU, R.N., VESTER, B., and WENGEL, J. (2009). Efficient enzymatic synthesis of LNA-modified DNA duplexes using KOD DNA polymerase. *Org. Biomol. Chem.* **7**, 1404–1409.
- WANG, A.Z., BAGALKOT, V., VASILIOU, C.C., GU, F., ALEXIS, F., ZHANG, L., SHAIKH, M., YUET, K., CIMA, M.J., LANGER, R., KANTOFF, P.W., BANDER, N.H., JON, S., and FAROKHZAD, O.C. (2008). Superparamagnetic iron oxide nanoparticle-aptamer bioconjugates for combined prostate cancer imaging and therapy. *Chem. Med. Chem.* **3**, 1311–1315.
- WULLNER, U., NEEF, I., ELLER, A., KLEINES, M., TUR, M.K., and BARTH, S. (2008). Cell-specific induction of apoptosis

- by rationally designed bivalent aptamer-siRNA transcripts silencing eukaryotic elongation factor 2. *Curr. Cancer Drug Targets* **8**, 554–565.
- XIAO, Z., SHANGGUAN, D., CAO, Z., FANG, X., and TAN, W. (2008). Cell-specific internalization study of an aptamer from whole cell selection. *Chemistry* **14**, 1769–1775.
- XU, Y., PHILLIPS, J.A., YAN, J., LI, Q., FAN, Z.H., and TAN, W. (2009). Aptamer-based microfluidic device for enrichment, sorting, and detection of multiple cancer cells. *Anal. Chem.* **81**, 7436–7442.
- YAN, A.C., and LEVY, M. (2009). Aptamers and aptamer targeted delivery. *RNA Biol.* **6**, 316–320.
- ZHANG, L., RADOVIC-MORENO, A.F., ALEXIS, F., GU, F.X., BASTO, P.A., BAGALKOT, V., JON, S., LANGER, R.S., and FAROKHZAD, O.C. (2007). Co-delivery of hydrophobic and hydrophilic drugs from nanoparticle-aptamer bioconjugates. *Chem. Med. Chem.* **2**, 1268–1271.
- ZHOU, J., and ROSSI, J.J. (2009). The therapeutic potential of cell-internalizing aptamers. *Curr. Top. Med. Chem.* **9**, 1144–1157.
- ZHOU, J., LI, H., LI, S., ZAIA, J., and ROSSI, J.J. (2008). Novel dual inhibitory function aptamer-siRNA delivery system for HIV-1 therapy. *Mol. Ther.* **16**, 1481–1489.
- ZHOU, J., SWIDERSKI, P., LI, H., ZHANG, J., NEFF, C.P., AKKINA, R., and ROSSI, J.J. (2009). Selection, characterization and application of new RNA HIV gp 120 aptamers for facile delivery of Dicer substrate siRNAs into HIV infected cells. *Nucleic Acids Res.* **37**, 3094–3109.

Address correspondence to:

Dr. John J. Rossi
Fox North Room 2002, City of Hope
1500 East Duarte Road
Duarte, CA 91010

E-mail: jrossi@coh.org

Received for publication October 30, 2010; accepted after revision November 16, 2010.