

Nucleic acid aptamers for targeting of shRNA-based cancer therapeutics

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Abstract: Aptamers are nucleic acid ligands which have been validated to bind to epitopes with a specificity similar to that of monoclonal antibodies. Aptamers have been primarily investigated for their direct function in terms of inhibition of protein targets; however, recent evidence gives reason to actively explore aptamers as targeting moieties for delivery of anticancer therapeutics. Many aptamers have been developed to bind to extracellular membrane domains of proteins overexpressed on cancer cells and have the potential to be modified for use in targeting cancer therapeutics. The use of DNA vector-based short hairpin RNA (shRNA) for RNA interference (RNAi) is a precise means for the disruption of target gene expression but its clinical usage in cancer is limited by obstacles related to delivery into cancer cells. Nucleic acid aptamers are attractive candidates for targeting of shRNA therapies. Their small size, ease of production and modification, and high specificity are valued attributes in comparison to other targeting moieties currently being tested. Here we review the development of aptamers directed to PSMA, Nucleolin, HER-3, RET, TN-C, and MUC1 and focus on their potential for use in targeting of shRNA-based cancer therapeutics.

Keywords: aptamer, tumor targeting, oligonucleotides therapeutics, cancer, shRNA, gene therapy

Introduction

Delivery vehicles optimal for shRNA delivery ideally are targeted in order to maximize biodistribution to the cancer cell population, thereby increasing their effectiveness in the clinical setting and minimizing potentially adverse off target (ie, nonmalignant) cell effects. Tumor cellular uptake at the target site(s) can be enhanced by conferring to the delivery vehicle the ability to recognize surface features of the target cells. This can be accomplished most readily by recognizing surface antigens or promoting receptor mediated endocytosis. Delivery vehicles can be complexed with monoclonal antibodies, peptides, small molecule ligands, and aptamers to recognize cell surface markers (Lu and Low 2002; Dyba et al 2004; Stefanidakis and Koivunen 2004; Hughes and Rao 2005).

Aptamers are noncoding nucleic acid oligomers made from either RNA or DNA. Much like tRNA, they fold through intramolecular bonds into varied tertiary structures. The folded structure of aptamers creates binding pockets that allow the molecules to interact with the three dimensional structure of specific domains on a protein's surface. Aptamers bind to epitopes with affinity comparable to that of monoclonal antibodies. Their dissociation constants are often in the picomolar to low nanomolar range. Because of their high surface area, the area in contact with the target is large, minimizing the chances of off target effects by increasing binding specificity. In some cases aptamers can inhibit the function of a target protein by binding to its active site, or inducing a conformational change, or inhibiting dimerization or DNA binding (Lupold et al 2002; Umehara et al 2004; Shi et al 2007).

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The idea of developing nucleic acid ligands for therapeutic purposes was born out of the study of activation of viral gene expression during HIV infection. There exists a nucleic acid ligand that binds specifically to a transactivator of human immunodeficiency virus type 1 (HIV-1) gene called Tat. The Tat protein normally binds to an RNA stem-loop structure called a trans-acting response element (TAR) which occurs within the first 60 nucleotides of all HIV-1 transcripts, thus activating expression of viral genes. A series of studies showed that overexpression of TAR sequence decoys conferred HIV-1 resistance to cells, presumably because the TAR oligonucleotides bound specifically to the Tat protein, thus inhibiting it from activating viral gene expression (Sullenger et al 1990; Sullenger et al 1991; Held et al 2006).

Since this initial discovery, a wide range of potential therapeutic applications for aptamers have been proposed and pursued (Bunka and Stockley 2006). Aptamers have been successfully developed against extracellular protein ligands, such as transforming growth factor receptors, platelet-derived growth factor, basic fibroblast growth factor, and vascular endothelial growth factor (VEGF) as well as intracellular proteins such as NF κ B (Jellinek et al 1995; Golden et al 2000; Cassidy and Maher 2001; Pietras et al 2001; Ohuchi et al 2005, 2006; McCauley et al 2006). Pegaptanib, an aptamer that binds to and inhibits VEGF has been approved by the FDA for treatment of age related macular generation (Ng et al 2006). Aptamers that bind to blood coagulation factors VIIa and IXa are being investigated as anticoagulants (Rusconi et al 2002). A subset of aptamers that have been developed for therapeutic purposes have the potential to be used as targeting moieties for shRNA.

Aptamers represent an attractive alternative to traditional methods of targeting cancer gene therapy by circumventing many of the stumbling blocks that inhibit the effectiveness of these methods. Aptamers are smaller than monoclonal antibodies. With a molecular weight of 10–15 kDa they are one order of magnitude lighter than antibodies used for targeting, which have an average molecular weight of 150 kDa (White et al 2000). Aptamers are also less immunogenic than antibodies (Drolet et al 2000; Pestourie et al 2005; Apte et al 2007), making them more suited to systemic administration and long term therapy.

Aptamers can facilitate cell entry of an shRNA delivery vehicle in similar ways as traditional targeting moieties. Aptamers to the extracellular domains of transmembrane receptor proteins can penetrate the cell membrane by receptor mediated endocytosis (Hughes and Rao 2005). When aptamers bind to cell surface proteins they increase

the proximity of the delivery vehicle and the cell membrane. This facilitates charge interactions between a positively charged delivery vehicle and the negatively charged membrane that result in internalization of the payload. Here we will briefly review the process of aptamer development and then give examples of the current development of aptamers directed to good candidate proteins for tumor targeting.

Here we review the development of aptamers directed to PSMA, Nucleolin, HER-3, RET, TN-C, and MUC1 and focus on their potential for use in targeting of shRNA-based cancer therapeutics. Though most of these aptamers have not yet been used as shRNA targeting moieties *in vivo* this selection of aptamers represents a group that is well suited to the targeting shRNA-based cancer therapies thereby enhancing their efficiency and safety.

Aptamer production

RNA and DNA aptamers with specific binding properties can now be amplified *in vitro* from libraries through a streamlined, automated iterative selection process. Because of their small, relatively simple structure, the chemical modification of aptamers to increase serum stability or bioavailability can be accomplished readily. The production and modification of aptamers is very uncomplicated when compared to the production of monoclonal antibodies, which require prokaryotic or eukaryotic expression systems. The chemical methods involved are well defined and easily conformable to good manufacturing process requirements, which has eased the transition of aptamers into the clinical setting.

Aptamers that bind to a specific target are isolated from a group of candidate oligonucleotides, either RNA or DNA, through ‘systematic evolution of ligands by exponential enrichment’ (SELEX) (Tuerk and Gold 1990). SELEX is an *in vitro* process that can be automated to allow the rapid production of aptamers (Cox et al 2002; Ellington et al 2005).

The SELEX process begins with a degenerate sequence library of $\geq 10^{15}$ molecules. With a library of this size it is likely that there is a molecule that will bind to almost any target (Ellington and Szostak 1990). The library is then treated with the target molecule at physiological pH and temperature. After some aptamers bind the aptamer–target complexes are partitioned from the nonbinding aptamers and bound species are regenerated and amplified by PCR in the case of DNA aptamers or RT-PCR in the case of RNA aptamers. The amplified molecules are then subjected to

another round of the selection process. Over this repetitive process of binding and amplification the pool is enriched for aptamers that bind most efficiently to the target (Gopinath 2007). SELEX can also be tailored by ligation and cleavage of small 10nt fixed primer sites before and after amplification. These smaller primer sequences allow the isolation of shorter aptamer sequences which can later be more readily synthesized chemically (Vater et al 2003).

Whole-cell SELEX is a procedure that has been developed for the selection of aptamers to extracellular matrix proteins and transmembrane proteins. It involves the introduction of cells overexpressing the protein target of interest to oligomer libraries. This procedure offers the advantage of selecting molecules more suited to recognizing their target in its natural glycosylation state under physiological conditions. Because of the great variety of proteins of the cell surface, a second set of traditional SELEX with the purified protein is sometimes necessary to obtain a purified specific aptamer product (Hicke et al 2001; Cerchia et al 2005; Ohuchi et al 2005). This method has been used to isolate aptamers that bind to human transforming growth factor-beta type III receptor displayed on cell surface (Ohuchi et al 2005, 2006).

Naked nucleotide oligomers are in general very unstable in the blood and are unsuitable for therapeutic or targeting applications. One study showed that an unmodified aptamer against thrombin had an *in vivo* serum half-life of only 108 seconds (Griffin et al 1993). A variety of chemical modifications can be applied to nucleic acid aptamers during and after the SELEX process in order to increase their suitability for systemic tumor targeting (Table 1). Natural nucleotides within aptamers can be substituted with 2' fluoro, O-methyl, or amino modified or 4' thio modified nucleotides that are poor substrates for nuclease degradation (Beigelman et al 1995; Gold et al 1995; Burmeister et al 2005; Kato et al 2005) 3' end caps can also be modified to confer nuclease resistance (Burmeister et al 2005). Aptamers can be bound to bulky lipids or polymers to reduce their renal clearance and increase their serum half-life (Boomer et al 2005). Aptamer intramolecular bonds can be altered to increase structural stability (Schmidt et al 2004). Beyond chemical modifications to increase stability, aptamers can also be modified with small functional groups to facilitate their attachment as targeting moieties to polymeric or lipid shRNA delivery vehicles. Amino groups have been added to aptamers in order to facilitate their conjugation to a nanoparticle composed of poly(lactic acid)-block-polyethylene glycol copolymer with a terminal carboxylic acid functional group (Farokhzad et al 2004).

Likely candidates for aptamer mediated cancer targeting

The following cell surface proteins are good candidates for tumor targeting. Aptamers are actively being developed for all of them, whether for therapeutic or for targeting purposes (Table 2).

Prostate-specific membrane antigen (PSMA)

The most extensively studied aptamer for cancer targeting binds to the prostate-specific membrane antigen (PSMA). PSMA is a well described protein that is expressed in prostate cancer epithelial cells (Israeli et al 1994; Murphy et al 1998). Its use in targeting of many different types of cancer therapeutics is a constantly developing field. In normal prostate epithelial cells, it is primarily expressed as the intracellular protein referred to as PSM' but in prostate carcinoma the splice variant termed PSMA is overexpressed. PSMA, which is more pertinent for targeting purposes, is a type 2 integral membrane glycoprotein folate carboxypeptidase expressed exclusively in prostate acinar epithelium (Su et al 1995). Increased PSMA expression has been documented in prostatic intraepithelial neoplasia, prostatic adenocarcinoma, and in tumor associated neovasculature. Its expression generally increases with disease progression and metastasis (Bostwick et al 1998).

PSMA was originally described as the target for mAb 7E11 in the androgen-dependent LNCaP human prostatic adenocarcinoma cell line (Horoszewicz et al 1987). Antibodies that bind to the extracellular domain of the PSMA are used clinically for imaging and staging of prostate cancer (Freeman et al 2002) and are in clinical trials to determine therapeutic potential (Bander et al 2005). Monoclonal antibodies to PSMA have also been used to target delivery of gene therapy *in vivo* (Moffatt et al 2006).

The practicality of PSMA as a target for various kinds of anticancer therapies has placed it in center stage of aptamer-based cancer targeting research. The first reported RNA aptamers selected to bind a tumor-associated membrane antigen were two 2'-fluoro-pyrimidine RNA oligomers that bind to the extracellular domain of the PSMA. These two aptamers, called xPSM-A9 and xPSM-A10, which share no consensus sequences, bind to the PSMA at different extracellular epitopes. Aptamer xPSM-A9 was shown to inhibit the PSMA noncompetitively with an average $K(i)$ of 2.1 nM, while aptamer xPSM-A10 inhibits the PSMA competitively with an average $K(i)$ of 11.9 nM (Lupold et al 2002).

Table 1 Common chemical modifications of aptamers for tumor targeting

Modification	Effect	Reference
surround with lipoproteins	Reduced renal clearance	Willis 1998
PEGylation	Reduced renal clearance	Boomer 2005
binding to cholesterol	Reduced renal clearance	Rusconi 2004 de Smidt 1991
biotin-streptavidin	Reduced renal clearance	Dougan 2000
2'-fluoro pyrimidine substitution	Nuclease resistance	Burmeister 2005
2'-O-methyl nucleotide substitution	Nuclease resistance	Chelliserrykattil 2004 Beigelman 1995 Burmeister 2005
3' end cap modification	Nuclease resistance	Burmeister 2005
2'-amino nucleotide substitution	Nuclease resistance	Burmeister 2005
use of locked nucleic acid modification	Improve structural stability	Schmidt 2004
4'thio nucleotide substitution	Nuclease resistance	Kato 2005
3'-amino nucleotide substitution	Facilitation of conjugation to delivery vehicle	Lupold 2002

de Smidt PC, Doan TL, de Falco S and van Berkel TJC. 1991. Association of antisense oligonucleotides with lipoproteins prolongs the plasma half-life and modifies the tissue distribution. *Nucleic Acids Res*, 19, 4695–700.

Dougan H. et al. 2000. Extending the lifetime of anticoagulant oligodeoxynucleotide aptamers in blood. *Nucl Med Bio*, 27, 289–97.

Boomer RM. et al. 2005. Conjugation to polyethylene glycol polymer promotes aptamer biodistribution to healthy and inflamed tissues. *Oligonucleotides*, 15, 183–95.

Willis MC. et al. 1998. Liposome anchored vascular endothelial growth factor aptamers. *Bioconjug Chem*, 9, 573–82.

Beigelman L, McSwiggen JA, Draper KG, Gonzalez C, Jensen K, Karpeisky AM, Modak AS, Matulic-Adamic J, DiRenzo AB, Haeberli P, et al. 1995. Chemical modification of hammerhead ribozymes. Catalytic activity and nuclease resistance. *J Biol Chem*. Oct 27;270(43):25702-8. PMID: 7592749.

Burmeister PE, Lewis SD, Silva RF, Preiss JR, Horwitz LR, Pendergrast PS, McCauley TG, Kurz JC, Epstein DM, Wilson C, Keefe AD. 2005. Direct in vitro selection of a 2'-O-methyl aptamer to VEGF. *Chem Biol*, 12:25–33.

Rusconi CP, Roberts JD, Pitoc GA, Nimjee SM, White RR, Quick G Jr, Scardino E, Fay WPR, Sullenger BA. 2004. Antidote-mediated control of an anticoagulant aptamer in vivo. *Nat Biotechnol*, 22:1373–84.

Kato, Y. et al. 2005. New NTP analogs: the synthesis of 4'-thioUTP and 4'-thioCTP and their utility for SELEX. *Nucleic Acids Res*, 33, 2942–51.

The nuclease resistant xPSMA-A10 aptamer has been exploited to target the delivery of various nanoparticulate drug delivery systems. It has been particularly well studied as a targeting moiety for biodegradable nanoparticles conjugated with chemotherapeutics. In one study, the xPSMA-A10 aptamer was bound to a poly(lactic acid)-block-polyethylene glycol (PEG) copolymer with a terminal carboxylic acid functional group (PLA-PEG-COOH) and used to deliver rhodamine-labeled dextran as a model drug in vitro. This

study showed that the targeted nanoparticles preferentially bind to and are taken up in vitro by LNCaP prostate epithelial cells which express PSMA, but not by PC3 prostate epithelial cells, which do not express detectable levels of the PSMA (Farokhzad et al 2004).

In a more recent study, 5'-NH₂ modified xPSMA-A10 aptamers were conjugated to poly(D,L-lactic-co-glycolic acid)-*block*-poly(ethylene glycol) nanoparticles with terminal carboxylic acid groups (PLGA-PEG-COOH).

Table 2 Nucleotide aptamers in development with potential for use in targeting of shRNA therapy

Aptamer	Target	Testing		Clinical
		in vitro	in vivo	
AS1411 (Agro100)	Nucleolin	antiproliferation, growth inhibition	Antitumor activity in nude mouse xenograft models, synergy with chemotherapeutic agents	Phase 1 and 2 trials for advanced solid malignancies
TTA1	TN-C	Binding	Successful aptamer penetration in various murine xenograft tumor models	not tested
various	MUC1	Binding	Successful aptamer penetration in a murine xenograft tumor model	not tested
xPSM-A10	PSMA	Inhibition of PSMA via competitive binding	Successful targeting of a chemotherapy nanoparticle in a murine xenograft model	not tested
xPSM-A9	PSMA	Inhibition of PSMA via noncompetitive binding	not tested	not tested
A30	HER3	Inhibitor of HER-3 signaling via noncompetitive binding	not tested	not tested
D4	RET ^{C634Y}	Inhibitor of RET ^{C634Y} dimer formation	not tested	not tested

These nanoparticles were then encapsulated with the chemotherapeutic docetaxel and tested *in vitro* and *in vivo*. The docetaxel-encapsulated nanoparticle-aptamer (Dtxl-NP-Apt) bioconjugates enhanced *in vitro* cellular toxicity with LNCaP prostate epithelial cells as compared with nanoparticles that lack the PSMA aptamer. After a single intratumoral injection of Dtxl-NP-Apt bioconjugates using a murine LNCaP xenograft model of prostate cancer, complete tumor reduction was noted in five of seven nude mice. 100% of the mice that received the Dtxl-NP-Apt bioconjugate survived the 109-day study. In contrast, only two of seven mice that received the non-targeted Dtxl-NP had complete tumor reduction and only 57% of the mice in this group survived the 109-day study. Only 14% of mice treated with Dtxl alone survived the 109-day study (Farokhzad et al 2006). A similar study tested PLGA-PEG-COOH-xPSMA-A10 conjugates encapsulating docetaxel and ¹⁴C labeled paclitaxel. It was found that the surface association of nanoparticles with targeting aptamer significantly enhanced delivery to tumors when compared to equivalent but nontargeted nanoparticles in a murine LNCaP xenograft model (Cheng et al 2007).

PSMA aptamers are particularly attractive as targeting moieties for RNAi-based cancer therapeutics because the PSMA itself participates in membrane recycling, becoming internalized through ligand induced endocytosis, thus facilitating entry of the therapeutic payload into the cytoplasm (Ghosh and Heston 2004). This property of the PSMA has been exploited in the targeting of therapeutic siRNAs to prostate cancers using aptamer-siRNA chimeras and conjugates. In one study, a 27mer anti-Lamin siRNA was noncovalently bound to xPSMA-A9 aptamers using a modular streptavidin bridge and tested *in vitro* to transfect PC3 cells and LNCaP cells that naturally expressed the PSMA. The conjugates were successfully transfected with this method and inhibition of lamin expression was achieved. The conjugates displayed similar transfection efficiency to oligofectamine controls but better specificity, as they were only taken up by PSMA expressing LNCaP cells and not PSMA-negative PC3 cells (Chu et al 2006).

In another study, aptamer-siRNA chimeras were used to target prostate cancer cells and knock out expression of survival genes. Chimeras of the xPSMA-A10 aptamer and polo-like kinase 1 (PLK1) and BCL2 siRNAs were generated via *in vitro* transcription from DNA templates. The chimeras were shown to be internalized and promote apoptosis preferentially in PSMA expressing LNCaP cells and not PC3 control cells. This specificity was retained in murine LNCaP xenograft tumor models where chimeras bearing PLK1

siRNAs mediated pronounced tumor regression while having no statistically significant effect on non-PSMA expressing PC3 xenografts (McNamara et al 2006).

Nucleolin

An aptamer (AS1411) that binds to the external domain of the membrane protein nucleolin has been well characterized because of its therapeutic potential. Due to the properties of the aptamer and the properties of nucleolin itself AS1411 is an excellent candidate for aptamer mediated targeting of gene therapy.

Human nucleolin is an abundant multifunctional 76 kDa membrane associated phosphoprotein that has 707 amino acids in its sequence (Derenzini et al 1995; Sarcevic et al 1997). It is composed of three main domains, an acidic histone-like NH₂ terminus, a central domain containing four RNA-binding sub-domains, and a carboxy terminal domain that contains its four sites of phosphorylation (Lapeyre et al 1987; Ginisty et al 1999). Though it was originally characterized as a nucleolar protein, nucleolin is also present on the external cell membrane where it functions as a signaling receptor of poorly defined function (Hovanessian et al 2000; Storck et al 2007).

Though its function on the surface of cancer cells has not been clearly defined, Nucleolin has been extensively investigated in the context of cancer. It has been shown to be associated with survival, growth and proliferation of cells, nuclear transport, transcription, packing and transport of rRNA, and replication and recombination of DNA (Srivastava and Pollard 1999). In addition, overexpression of nucleolin is related to poor clinical prognosis for some cancer types (Derenzini et al 1995; Derenzini 2000).

One pathophysiologic function of cell surface nucleolin that makes it ideal for targeting anticancer therapies is its involvement in internalization of specific ligands. Nucleolin has been shown to bind to and internalize the heparin-binding growth factor midkine (Said et al 2002), the iron-binding glycoprotein lactoferrin (Legrand et al 2004), acharan sulfate (Joo et al 2005), as well as the tumor-homing 34-amino acid peptide (F3) (Christian et al 2003).

The aptamer known as AS1411 was first developed as AGRO100 by Aptamera (Louisville, KY). It is an unmodified guanosine rich 26-mer, which binds to nucleolin. AS1411 was originally investigated because of observations that guanosine-rich oligonucleotides (GRO) had nonantisense-based *in vitro* antiproliferative properties against cancer cells (Bates et al 1999). These oligonucleotides were further studied and it was found that the unmodified DNA phosphodiester

GRO analog GRO29A-OH, which retains the G quartet structure essential for nucleolin binding (Dapic et al 2003), displayed resistance against serum nuclease degradation (Dapic et al 2002). AS1411 is simply GRO29A-OH with its three 5' thymidines cleaved.

A nontraditional aptamer because of its G quartet-based binding to its target, AS1411 has been shown to bind to cell surface nucleolin and inhibit growth of many cancer cell lines *in vitro* such as the prostate cancer cell line DU145, the breast cancer cell lines MDA-MB-231 and MCF-7, the lung cancer cell line A549, as well as the cervical cancer cell line HeLa (Bates et al 1999; Dapic et al 2002).

In anticipation of its use as a therapeutic, biodistribution studies were realized to characterize the *in vivo* pharmacodynamics, distribution, and metabolism of the aptamer following IV administration. AS1411 metabolism was studied in mice bearing lung and renal human tumor xenografts derived from A549 and A498 cells using IV administration of AS1411 radiolabeled with tritiated thymidine. Following 1, 10, and 25 mg/kg IV boluses of [3H]AS1411, 63% of the radiolabeled aptamer was excreted in urine within 5 hours while less than 1% of the injected dose was detected upon analysis of bile, thus suggesting that the main mode of metabolism is renal. This same study also showed that AS1411 displayed dose related pharmacokinetics with an elimination half life of 2 days in plasma and whole blood. Regardless of the dose administered, tumor/blood ratios were 4:6 for the renal carcinoma xenograft and 2:4 and for the lung carcinoma xenograft, showing that the aptamer effectively accumulated in tumor tissue (Ireson and Kelland 2006).

In vivo xenograft efficacy studies have collectively shown that AS1411 results in cytostasis rather than cytotoxicity. After treatment with AS1411 cells are blocked at S-phase. Because of this observation, there has been interest in combining AS1411 with nucleoside analogs such as gemcitabine, which act on cells during S-phase. Early *in vitro* and *in vivo* studies of AS1411 in combination with gemcitabine against PANC-1 pancreatic cancer cells have shown enhanced anti-tumor activity in comparison to either agent alone (Ireson and Kelland 2006).

Preclinical toxicology of AS1411 has been evaluated in rats and dogs with IV bolus dosages of up to 100 mg/kg in rats and 96-hour continuous IV infusions at doses up to 10 mg/kg/d. No significant toxicity was observed in either species, as determined by clinical observations, clinical pathology evaluation, and gross examination of tissues at necropsy (Ireson and Kelland 2006).

AS1411 was the first nucleic acid-based aptamer approved for Phase I clinical testing for the treatment of cancer in humans. Results have been published on the progress of 17 patients with advanced solid tumors receiving AS1411 at the Brown Cancer Center in Louisville, KY (Laber et al 2005). This report describes a continuous IV infusion dose escalation study starting from 1 mg/kg/d and thus far reported up to 10 mg/kg/d. Fifteen patients received a 4 day infusion while 2 patients received a 7 day infusion. No serious toxicity related to AS1411 administration was observed. A patient with renal cancer achieved a sustained partial response at 16 months post-treatment and 41% of patients were reported to have stable disease at 2 months post-treatment. From these initial results the Phase I study has continued with a focus on renal and non-small cell lung cancers (Ireson and Kelland 2006; Laber et al 2006).

The extensive preclinical and burgeoning clinical data amassed about AS1411 makes it an ideal candidate for targeting of cancer gene therapy in humans.

Human epidermal growth factor receptor 3 (HER-3)

Human epidermal growth factor receptor 3 (HER-3) is a receptor tyrosine kinase that is overexpressed on the surface of many different cancer cells (Yokota et al 1988; Schneider et al 1989; Slamon et al 1989; Berchuck et al 1990; Dougall et al 1993). The extra-cellular domains of receptor tyrosine kinases are rational and accessible targets for anticancer therapies. The most prominent example of a therapy targeted to the extra-cellular domain of a receptor tyrosine kinase is Herceptin, a humanized monoclonal antibody that targets the extra-cellular domain of HER-2. This molecule has proved effective in the treatment of HER2-overexpressing breast cancers (Pegram et al 1999).

An aptamer to the extracellular domain of HER-3, called A30, has been isolated using SELEX. A30 was demonstrated to have high binding specificity to HER-3, despite the sequence similarities shared by the extracellular domains of HER-2 and HER-3. *In vitro*, A30 inhibits the growth stimulatory effects of HER-3's natural ligand, heregulin, though it does not compete for its binding site. A30 also inhibits HER-3 from binding to HER-2 which is a necessary step in HER-mediated growth induction (Chen et al 2003).

Though A30 is still in the early stages of development and would likely need to undergo modifications to enhance its serum stability prior to its introduction in the clinical setting, it represents a possible future targeting moiety for shRNA delivery in the therapy of cancer.

RET

Another receptor tyrosine kinase for which an aptamer has been developed is the RET oncoprotein. Mutated forms of the RET Receptor tyrosine kinase are implicated as the cause of the dominant inherited cancer syndrome MEN2 (Hansford and Mulligan 2000). In MEN2, germline to 'missense mutations of the RET gene result in the conversion of cysteine 634 to tyrosine (RET^{C634Y}), which causes cancers of the neuroendocrine organs. These mutations affect the cysteine rich extracellular domain of RET causing the loss of an intramolecular disulfide bond. The loss of this bond allows the formation dimers of mutated RET monomers via an intermolecular disulfide bond. Dimerization results in a constitutively activated receptor (Iwashita et al 1999).

Using 16 cycles of whole-cell SELEX several RNA aptamers that bind to the extracellular domain of RET^{C634Y} have been isolated. PC12 cells which expressed the mutant receptor were used for the selection procedure. The aptamer with the best signaling inhibition and the most nuclease resistance, termed D4, was selected for further development (Cerchia et al 2005).

An aptamer that binds specifically to RET could be useful for targeting of specific anticancer gene therapies and it also is another example of the use of whole-cell SELEX to effectively develop aptamers that bind to transmembrane proteins in their natural glycosylation states. This method could be employed to develop aptamers to other potential targets.

Tenascin-C (TN-C)

Tenascin-C is a hexameric extracellular matrix protein that is highly expressed during active tissue development processes including wound healing, angiogenesis, embryonic development, and tumor growth (Mackie et al 1988; Erickson and Bourdon 1989; Koukoulis et al 1991; Zagzag et al 1995). TN-C is an ideal candidate for targeting of anticancer shRNA therapy because it is highly expressed in tumor and in general its expression is limited in normal tissues (Borsi et al 1992). High TN-C expression has been noted in osteosarcomas, lymphomas, glioblastomas, melanomas as well as carcinomas of breast, colon lung, and prostate (Chiquet-Ehrismann 2004; Orend 2005; Orend and Chiquet-Ehrismann 2006).

Using SELEX with TN-C expressing U251 glioblastoma cells RNA aptamers that bind to the extracellular fibrinogen-like domain of TN-C with high affinity were isolated. Several modifications were made to the aptamer in order to increase its usefulness as a targeting moiety. Its size was reduced in order to increase tissue penetration and efficiency of synthesis. It was 2'-OCH₃ substituted, and 3' capped in order to increase nuclease stability. A 5' amine was also added to

serve as a conjugation site. After these modifications the resultant aptamer, termed TTA1, had an increased equilibrium K_d but the final value of 5×10^{-9} M still represents a very high affinity (Hicke et al 2001).

In a later experiment the potential of TTA1 to be used for *in vivo* drug delivery was evaluated. Uptake and tumor distribution of rhodamine red-X-labeled TTA1 was studied using fluorescence microscopy in a U251 human glioblastoma cell line xenograft nude mouse model. Within 10 minutes after IV injection of the fluorescently labeled aptamer, bright perivascular fluorescence was noted in the xenografts. The fluorescence then diffused throughout the tumor stroma over the following 3 hours (Hicke et al 2006).

Biodistribution and radioimaging studies were performed using (99m)Tc labeled TTA1 and glioblastoma (U251) and breast cancer (MDA-MB-435) tumor xenografts in nude mice. IV administered (99m)Tc labeled TTA1 showed rapid blood clearance with a serum half-life of less than 2 min, and rapid tumor penetration. TTA1 was cleared much more rapidly from the blood than from the tumor. Both renal and hepatobiliary clearance pathways were observed, but it is estimated that due to rapid nuclease degradation of the aptamer the clearance patterns observed were those of the aptamer metabolites. In these studies it was found that TTA1 affectively targeted a wide variety of TN-C expressing tumor types including colon (SW620), breast (MDA-MB-468, MDA-MB-435), glioblastoma (U251), rhabdomyosarcoma (A673). As a control TTA1 was also tested with a squamous cell carcinoma of the head and neck (KB) xenograft that did not express TN-C. As expected, KB xenografts did not display appreciable aptamer uptake (Hicke et al 2006).

The results of these studies are promising in terms of TTA1s potential use in tumor targeting. Before its use in targeting of shRNA-based cancer therapies it would probably need further modifications to increase its serum half life. While short half lives and rapid elimination are beneficial for minimizing potential toxicity, such a short serum half life may inhibit aptamer penetration in areas of the target tumor that are poorly perfused (Hicke et al 2006).

MUC1

MUC1 is a large, highly glycosylated transmembrane epithelial cell surface protein. Mucin proteins are expressed on the apical membrane of various epithelial cell types. They have many roles which facilitate the function of mucosal cells such as lubrication of epithelial cell surfaces, prevention of tissue dehydration, protection of cells from proteolytic degradation and provision of a barrier against infection

(von Mensdorff-Pouilly et al 2000). Mucins also function as signal transduction receptors involved in triggering cellular responses such as proliferation, differentiation, and apoptosis (Taylor-Papadimitriou et al 1999; Hollingsworth and Swanson 2004). MUC1 overexpression has been well documented in many human adenocarcinomas including breast, pancreatic, ovarian, colorectal, lung, prostate, esophageal (Maeshima et al 1997; Aoki et al 1998; Zhang et al 1998; Hough et al 2000; Ginestier et al 2002; Kohlgraf et al 2003; Burjonrappa et al 2007). Its expression has also been documented in other types of tumors including astrocytoma, melanoma, neuroblastoma, as well as hematological malignancies (Oosterkamp et al 1997; Brossart et al 2001).

The cell surface expression pattern of MUC1 across human malignancies makes it an excellent and versatile target for cancer targeting with an aptamer. Using 10 rounds of in vitro SELEX, a group of DNA aptamers has been isolated that binds selectively to synthetic MUC1 peptides. This pool of aptamers, when fluorescently labeled has been verified to bind specifically to MUC1 expressing cancer cell lines in vitro (Ferreira et al 2006). In a later study this group of aptamers was demonstrated by radio-imaging to have good tumor penetration in a murine xenograft (Perkins and Missailidis 2007). These aptamers are in the early stages of development and before their transition into the clinic they would need further modification to minimize size and maximize nuclease resistance.

Conclusions

Aptamer development could ameliorate many of the traditional problems with the introduction of exogenous genetic material for therapeutic purposes. There is a wide range of experimentation occurring, from the laboratory to the preclinical setting, which has the potential to revolutionize the targeting of gene-based therapy. The aptamers reviewed here represent the most promising areas of current development pertinent to aptamer-based targeting of shRNA cancer therapeutics and will likely be among the first to make transition to targeting in the clinic.

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References

Aoki R, Tanaka S, et al. 1998. MUC-1 expression as a predictor of the curative endoscopic treatment of submucosally invasive colorectal carcinoma. *Dis Colon Rectum*, 41(10):1262–72.

Apte R, Modi M, Masonson H, et al.; for the Macugen AMD Study Group. 2007. Pegaptanib 1-year systemic safety results from a safety-pharmacokinetic trial in patients with neovascular age-related macular degeneration. *Ophthalmology*, 114(9):1702–12.

Bander NH, Milowsky MI, Nanus DM, et al. 2005. Phase I trial of 177lutetium-labeled J591, a monoclonal antibody to prostate-specific membrane antigen, in patients with androgen-independent prostate cancer. *J Clin Oncol*, 23(21):4591–601.

Bates PJ, Kahlon JB, Thomas SD, et al. 1999. Antiproliferative activity of G-rich oligonucleotides correlates with protein binding. *J Biol Chem*, 274(37):26369–77.

Beigelman L, McSwiggen JA, Draper KG, et al. 1995. Chemical modification of hammerhead ribozymes. Catalytic activity and nuclease resistance. *J Biol Chem*, 270(43):25702–8.

Berchuck A, Kamel A, Whitaker R, et al. 1990. Overexpression of HER-2/neu is associated with poor survival in advanced epithelial ovarian cancer. *Cancer Res*, 50(13):4087–91.

Boomer RM, Lewis SD, Healy JM, et al. 2005. Conjugation to polyethylene glycol polymer promotes aptamer biodistribution to healthy and inflamed tissues. *Oligonucleotides*, 15(3):183–95.

Borsi L, Carnemolla B, Nicolo G, et al. 1992. Expression of different tenascin isoforms in normal, hyperplastic and neoplastic human breast tissues. *Int J Cancer*, 52(5): 688–92.

Bostwick DG, Pacelli A, Blute M, et al. 1998. Prostate specific membrane antigen expression in prostatic intraepithelial neoplasia and adenocarcinoma: a study of 184 cases. *Cancer*, 82(11):2256–61.

Brossart P, Schneider A, Dill P, et al. 2001. The epithelial tumor antigen MUC1 is expressed in hematological malignancies and is recognized by MUC1-specific cytotoxic T-lymphocytes. *Cancer Res*, 61(18):6846–50.

Bunka DH and Stockley PG. 2006. Aptamers come of age – at last. *Nat Rev Microbiol*, 4(8):588–96.

Burjonrappa SC, Reddimasu S, Nawaz Z, et al. 2007. Mucin expression profile in Barrett's, dysplasia, adenocarcinoma sequence in the esophagus. *Indian J Cancer*, 44(1):1–5.

Burmeister PE, Lewis SD, Silva RF, et al. 2005. Direct in vitro selection of a 2'-O-methyl aptamer to VEGF. *Chem Biol*, 12(1):25–33.

Cassiday LA and Maher LJ 3rd. 2001. In vivo recognition of an RNA aptamer by its transcription factor target. *Biochemistry*, 40(8):2433–8.

Cerchia L, Ducongé F, Pestourie C, et al. 2005. Neutralizing aptamers from whole-cell SELEX inhibit the RET receptor tyrosine kinase. *PLoS Biol*, 3(4):e123.

Chen CH, Chernis GA, Hoang VQ, et al. 2003. Inhibition of heregulin signaling by an aptamer that preferentially binds to the oligomeric form of human epidermal growth factor receptor-3. *Proc Natl Acad Sci USA*, 100(16):9226–31.

Cheng J, Tepley BA, Sherifi I, et al. 2007. Formulation of functionalized PLGA-PEG nanoparticles for in vivo targeted drug delivery. *Biomaterials*, 28(5):869–76.

Chiquet-Ehrismann, R. 2004. Tenascins. *Int J Biochem Cell Biol*, 36(6):986–90.

Christian S, Pilch J, Akerman ME, et al. 2003. Nucleolin expressed at the cell surface is a marker of endothelial cells in angiogenic blood vessels. *J Cell Biol*, 163(4):871–8.

Chu TC, Twu KY, Ellington AD, et al. 2006. Aptamer mediated siRNA delivery. *Nucleic Acids Res*, 34(10):e73.

Cox JC, Hayhurst A, Hesselberth J, et al. 2002. Automated selection of aptamers against protein targets translated in vitro: from gene to aptamer. *Nucleic Acids Res*, 30(20):e108.

Dapic V, Abdomerovic V, Marrington R, et al. 2003. Biophysical and biological properties of quadruplex oligodeoxyribonucleotides. *Nucleic Acids Res*, 31(8):2097–107.

Dapic V, Bates PJ, Trent JO, et al. 2002. Antiproliferative activity of G-quartet-forming oligonucleotides with backbone and sugar modifications. *Biochemistry*, 41(11):3676–85.

Derenzini M. 2000. The AgNORs. *Micron*, 31(2):117–20.

Derenzini M, Sirri V, Trere D, et al. 1995. The quantity of nucleolar proteins nucleolin and protein B23 is related to cell doubling time in human cancer cells. *Lab Invest*, 73(4):497–502.

de Smidt PC, Le Doan T, de Falco S, et al. 1991. Association of antisense oligonucleotides with lipoproteins prolongs the plasma half-life and modifies the tissue distribution. *Nucleic Acids Res*, 19(17):4695–700.

- Dougall WC, Qian X, Greene MI. 1993. Interaction of the neu/p185 and EGF receptor tyrosine kinases: implications for cellular transformation and tumor therapy. *J Cell Biochem*, 53(1):61–73.
- Dougan H, Lyster DM, Vo CV, et al. 2000. Extending the lifetime of anticoagulant oligodeoxynucleotide aptamers in blood. *Nucl Med Biol*, 27(3):289–97.
- Drolet DW, Nelson J, Tucker CE, et al. 2000. Pharmacokinetics and safety of an anti-vascular endothelial growth factor aptamer (NX1838) following injection into the vitreous humor of rhesus monkeys. *Pharm Res*, 17(12):1503–10.
- Dyba M, Tarasova NI, Michejda CJ. 2004. Small molecule toxins targeting tumor receptors. *Curr Pharm Des*, 10(19):2311–34.
- Ellington AD, Cox JC, Hayhurst A, et al. 2005. Automated in vitro selection and microarray applications for functional RNA sequences. *The RNA World, 3rd Ed.*, Cold Spring Harbor, New York, Cold Spring Harbor Laboratory Press: 683–719.
- Ellington AD and Szostak JW. 1990. In vitro selection of RNA molecules that bind specific ligands. *Nature*, 346(6287):818–22.
- Erickson HP and Bourdon MA. 1989. Tenascin: an extracellular matrix protein prominent in specialized embryonic tissues and tumors. *Annu Rev Cell Biol*, 5:71–92.
- Farokhzad OC, Cheng J, Teply BA, et al. 2006. Targeted nanoparticle-aptamer bioconjugates for cancer chemotherapy in vivo. *Proc Natl Acad Sci USA*, 103(16):6315–20.
- Farokhzad OC, Jon S, Khademhosseini A, et al. 2004. Nanoparticle-aptamer bioconjugates: a new approach for targeting prostate cancer cells. *Cancer Res*, 64(21):7668–72.
- Ferreira CS, Matthews CS, Missailidis S. 2006. DNA aptamers that bind to MUC1 tumour marker: design and characterization of MUC1-binding single-stranded DNA aptamers. *Tumour Biol*, 27(6):289–301.
- Freeman LM, Krynyckiy BR, Li Y, et al. 2002. The role of (111)In Capromab Pentetide (Prosta-ScintR) immunoscintigraphy in the management of prostate cancer. *Q J Nucl Med*, 46(2):131–7.
- Ghosh A and Heston WD. 2004. Tumor target prostate specific membrane antigen (PSMA) and its regulation in prostate cancer. *J Cell Biochem*, 91(3):528–39.
- Ginestier C, Charafe-Jauffret E, Bertucci F, et al. 2002. Distinct and complementary information provided by use of tissue and DNA microarrays in the study of breast tumor markers. *Am J Pathol*, 161(4):1223–33.
- Ginisty H, Sicard H, Roger B, et al. 1999. Structure and functions of nucleolin. *J Cell Sci*, 112(Pt 6):761–72.
- Gold L, Polisky B, Uhlenbeck O, et al. 1995. Diversity of oligonucleotide functions. *Annu Rev Biochem*, 64:763–97.
- Golden MC, Collins BD, Willis MC, et al. 2000. Diagnostic potential of PhotoSELEX-evolved ssDNA aptamers. *J Biotechnol*, 81(2-3):167–78.
- Gopinath SC. 2007. Methods developed for SELEX. *Anal Bioanal Chem*, 387(1):171–82.
- Griffin LC, Tidmarsh GF, Bock LC, et al. 1993. In vivo anticoagulant properties of a novel nucleotide-based thrombin inhibitor and demonstration of regional anticoagulation in extracorporeal circuits. *Blood*, 81(12):3271–6.
- Hansford JR and Mulligan LM. 2000. Multiple endocrine neoplasia type 2 and RET: from neoplasia to neurogenesis. *J Med Genet*, 37(11):817–27.
- Held DM, Kissel JD, Patterson JT, et al. 2006. HIV-1 inactivation by nucleic acid aptamers. *Front Biosci*, 11:89–112.
- Hicke BJ, Marion C, Chang YF, et al. 2001. Tenascin-C aptamers are generated using tumor cells and purified protein. *J Biol Chem*, 276(52):48644–54.
- Hicke BJ, Stephens AW, Gould T, et al. 2006. Tumor targeting by an aptamer. *J Nucl Med*, 47(4):668–78.
- Hollingsworth MA and Swanson BJ. 2004. Mucins in cancer: protection and control of the cell surface. *Nat Rev Cancer*, 4(1):45–60.
- Horoszewicz JS, Kawinski E, Murphy GP. 1987. Monoclonal antibodies to a new antigenic marker in epithelial prostatic cells and serum of prostatic cancer patients. *Anticancer Res*, 7(5B):927–35.
- Hough CD, Sherman-Baust CA, Pizer ES, et al. 2000. Large-scale serial analysis of gene expression reveals genes differentially expressed in ovarian cancer. *Cancer Res*, 60(22):6281–7.
- Hovanessian AG, Puvion-Dutilleul F, Nisole S, et al. 2000. The cell-surface-expressed nucleolin is associated with the actin cytoskeleton. *Exp Cell Res*, 261(2):312–28.
- Hughes JA and Rao GA. 2005. Targeted polymers for gene delivery. *Expert Opin Drug Deliv*, 2(1):145–57.
- Ireson CR and Kelland LR. 2006. Discovery and development of anticancer aptamers. *Mol Cancer Ther*, 5(12):2957–62.
- Israeli RS, Powell CT, Corr JG, et al. 1994. Expression of the prostate-specific membrane antigen. *Cancer Res*, 54(7):1807–11.
- Iwashita T, Kato M, Murakami H, et al. 1999. Biological and biochemical properties of Ret with kinase domain mutations identified in multiple endocrine neoplasia type 2B and familial medullary thyroid carcinoma. *Oncogene*, 18(26):3919–22.
- Jellinek D, Green LS, Bell C, et al. 1995. Potent 2'-amino-2'-deoxypyrimidine RNA inhibitors of basic fibroblast growth factor. *Biochemistry*, 34(36):11363–72.
- Joo EJ, ten Dam GB, van Kuppevelt TH, et al. 2005. Nucleolin: a chondroitin sulfate-binding protein on the surface of cancer cells. *Glycobiology*, 15(1):1–9.
- Kato Y, Minakawa N, Komatsu Y, et al. 2005. New NTP analogs: the synthesis of 4'-thioUTP and 4'-thioCTP and their utility for SELEX. *Nucleic Acids Res*, 33(9):2942–51.
- Kohlgraf KG, Gawron AJ, Higashi M, et al. 2003. Contribution of the MUC1 tandem repeat and cytoplasmic tail to invasive and metastatic properties of a pancreatic cancer cell line. *Cancer Res*, 63(16):5011–20.
- Koukoulis GK, Gould VE, Bhattacharyya A, et al. 1991. Tenascin in normal, reactive, hyperplastic, and neoplastic tissues: biologic and pathologic implications. *Hum Pathol*, 22(7):636–43.
- Laber DA, Sharma VR, Bhupalam L, et al. 2005. Update on the first phase I study of AGRO100 in advanced cancer. *J Clin Oncol (Meet Abstr)*, 23:3064.
- Laber DA, Taft BS, Kloecker GH, et al. 2006. Extended phase I study of AS1411 in renal and non-small cell lung cancers. *J Clin Oncol*, 24[18S(June 20 Supplement)]:13098.
- Lapeyre B, Bourbon H, Amalric F. 1987. Nucleolin, the major nucleolar protein of growing eukaryotic cells: an unusual protein structure revealed by the nucleotide sequence. *Proc Natl Acad Sci USA*, 84(6):1472–6.
- Legrand D, Vigié K, Said EA, et al. 2004. Surface nucleolin participates in both the binding and endocytosis of lactoferrin in target cells. *Eur J Biochem*, 271(2):303–17.
- Lu Y and Low PS. 2002. Folate-mediated delivery of macromolecular anticancer therapeutic agents. *Adv Drug Deliv Rev*, 54(5):675–93.
- Lupold SE, Hicke BJ, Lin Y, et al. 2002. Identification and characterization of nuclease-stabilized RNA molecules that bind human prostate cancer cells via the prostate-specific membrane antigen. *Cancer Res*, 62(14):4029–33.
- Mackie EJ, Halfter W, Liverani D. 1988. Induction of tenascin in healing wounds. *J Cell Biol*, 107(6 Pt 2):2757–67.
- Maeshima A, Miyagi A, Hirai T, et al. 1997. Mucin-producing adenocarcinoma of the lung, with special reference to goblet cell type adenocarcinoma: immunohistochemical observation and Ki-ras gene mutation. *Pathol Int*, 47(7):454–60.
- McCauley TG, Kurz JC, Merlino PG, et al. 2006. Pharmacologic and pharmacokinetic assessment of anti-TGFbeta2 aptamers in rabbit plasma and aqueous humor. *Pharm Res*, 23(2):303–11.
- McNamara JO 2nd, Andrechek ER, Wang Y, et al. 2006. Cell type-specific delivery of siRNAs with aptamer-siRNA chimeras. *Nat Biotechnol*, 24(8):1005–15.
- Moffatt S, Papasakelariou C, Wiehle S, et al. 2006. Successful in vivo tumor targeting of prostate-specific membrane antigen with a highly efficient J591/PEI/DNA molecular conjugate. *Gene Ther*, 13(9):761–72.
- Murphy GP, Elgamil AA, Su SL, et al. 1998. Current evaluation of the tissue localization and diagnostic utility of prostate specific membrane antigen. *Cancer*, 83(11):2259–69.

- Ng EW, Shima DT, Calias P, et al. 2006. Pegaptanib, a targeted anti-VEGF aptamer for ocular vascular disease. *Nat Rev Drug Discov*, 5(2):123–32.
- Ohuchi SP, Ohtsu T, Nakamura Y. 2005. A novel method to generate aptamers against recombinant targets displayed on the cell surface. *Nucleic Acids Symp Ser (Oxf)*, (49):351–2.
- Ohuchi SP, Ohtsu T, Nakamura Y. 2006. Selection of RNA aptamers against recombinant transforming growth factor-beta type III receptor displayed on cell surface. *Biochimie*, 88(7):897–904.
- Oosterkamp HM, Scheiner L, Stefanova MC, et al. 1997. Comparison of MUC-1 mucin expression in epithelial and non-epithelial cancer cell lines and demonstration of a new short variant form (MUC-1/Z). *Int J Cancer*, 72(1):87–94.
- Orend G. 2005. Potential oncogenic action of tenascin-C in tumorigenesis. *Int J Biochem Cell Biol*, 37(5):1066–83.
- Orend G and Chiquet-Ehrismann R. 2006. Tenascin-C induced signaling in cancer. *Cancer Lett*, 244(2):143–63.
- Pegram M, Hsu S, Lewis G, et al. 1999. Inhibitory effects of combinations of HER-2/neu antibody and chemotherapeutic agents used for treatment of human breast cancers. *Oncogene*, 18(13):2241–51.
- Perkins AC and Missailidis S. 2007. Radiolabelled aptamers for tumour imaging and therapy. *Q J Nucl Med Mol Imaging*.
- Pestourie C, Tavitian B, Duconge F. 2005. Aptamers against extracellular targets for in vivo applications. *Biochimie*, 87(9-10):921–30.
- Pietras K, Ostman A, Sjoquist M, et al. 2001. Inhibition of platelet-derived growth factor receptors reduces interstitial hypertension and increases transcapillary transport in tumors. *Cancer Res*, 61(7):2929–34.
- Rusconi CP, Scardino E, Layzer J, et al. 2002. RNA aptamers as reversible antagonists of coagulation factor IXa. *Nature*, 419(6902):90–4.
- Rusconi CP, Roberts JD, Pitoc GA, et al. 2004. Antidote-mediated control of an anticoagulant aptamer in vivo. *Nat Biotechnol*, 22(11):1423–8.
- Said EA, Krust B, Nisole S, et al. 2002. The anti-HIV cytokine midkine binds the cell surface-expressed nucleolin as a low affinity receptor. *J Biol Chem*, 277(40):37492–502.
- Sarcevic B, Lilischkis R, Sutherland RL. 1997. Differential phosphorylation of T-47D human breast cancer cell substrates by D1-, D3-, E-, and A-type cyclin-CDK complexes. *J Biol Chem*, 272(52):33327–37.
- Schmidt KS, Borkowski S, Kurreck J, et al. 2004. Application of locked nucleic acids to improve aptamer in vivo stability and targeting function. *Nucleic Acids Res*, 32(19):5757–65.
- Schneider PM, Hung MC, Chiocca SM, et al. 1989. Differential expression of the c-erbB-2 gene in human small cell and non-small cell lung cancer. *Cancer Res*, 49(18):4968–71.
- Shi H, Fan X, Sevilimedu A, et al. 2007. RNA aptamers directed to discrete functional sites on a single protein structural domain. *Proc Natl Acad Sci USA*, 104(10):3742–6.
- Slamon DJ, Godolphin W, Jones LA, et al. 1989. Studies of the HER-2/neu proto-oncogene in human breast and ovarian cancer. *Science*, 244(4905):707–12.
- Srivastava M and Pollard HB. 1999. Molecular dissection of nucleolin's role in growth and cell proliferation: new insights. *Faseb J*, 13(14):1911–22.
- Stefanidakis M and Koivunen E. 2004. Peptide-mediated delivery of therapeutic and imaging agents into mammalian cells. *Curr Pharm Des*, 10(24):3033–44.
- Storck S, Shukla M, Dimitrov S, et al. 2007. Functions of the histone chaperone nucleolin in diseases. *Subcell Biochem*, 41:125–44.
- Su SL, Huang IP, Fair WR, et al. 1995. Alternatively spliced variants of prostate-specific membrane antigen RNA: ratio of expression as a potential measurement of progression. *Cancer Res*, 55(7):1441–3.
- Sullenger BA, Gallardo HF, Ungers GE, et al. 1990. Overexpression of TAR sequences renders cells resistant to human immunodeficiency virus replication. *Cell*, 63(3):601–8.
- Sullenger BA, Gallardo HF, Ungers GE, et al. 1991. Analysis of trans-acting response decoy RNA-mediated inhibition of human immunodeficiency virus type 1 transactivation. *J Virol*, 65(12):6811–6.
- Taylor-Papadimitriou J, Burchell J, Miles DW, et al. 1999. MUC1 and cancer. *Biochim Biophys Acta*, 1455(2-3):301–13.
- Tuerk C and Gold L. 1990. Systematic evolution of ligands by exponential enrichment: RNA ligands to bacteriophage T4 DNA polymerase. *Science*, 249(4968):505–10.
- Umehara T, Fukuda K, Nishikawa F, et al. 2004. Designing and analysis of a potent bi-functional aptamers that inhibit protease and helicase activities of HCV NS3. *Nucleic Acids Symp Ser (Oxf)*, (48):195–6.
- Vater A, Jarosch F, Buchner K, et al. 2003. Short bioactive Spiegelmers to migraine-associated calcitonin gene-related peptide rapidly identified by a novel approach: tailored-SELEX. *Nucleic Acids Res*, 31(21):e130.
- von Mensdorff-Pouilly S, Snijdwint FG, Verstraeten AA, et al. 2000. Human MUC1 mucin: a multifaceted glycoprotein. *Int J Biol Markers*, 15(4):343–56.
- White RR, Sullenger BA, Rusconi CP. 2000. Developing aptamers into therapeutics. *J Clin Invest*, 106(8):929–34.
- Willis MC, Collins BD, Zhang T, et al. 1998. Liposome-anchored vascular endothelial growth factor aptamers. *Bioconjug Chem*, 9(5):573–82.
- Yokota J, Yamamoto T, Miyajima N, et al. 1988. Genetic alterations of the c-erbB-2 oncogene occur frequently in tubular adenocarcinoma of the stomach and are often accompanied by amplification of the v-erbA homologue. *Oncogene*, 2(3):283–7.
- Zagzag D, Friedlander DR, Miller DC, et al. 1995. Tenascin expression in astrocytomas correlates with angiogenesis. *Cancer Res*, 55(4):907–14.
- Zhang S, Zhang HS, Reuter VE, et al. 1998. Expression of potential target antigens for immunotherapy on primary and metastatic prostate cancers. *Clin Cancer Res*, 4(2):295–302.