PostDoc Journal
Vol. 1, No. 8, August 2013

Journal of Postdoctoral Research
www.postdocjournal.com

Current Progress and Future Prospects of aptamer-based cancer therapy

Silvia Catuogno[1] and Carla L. Esposito[1,2]

[1]Istituto di Endocrinologia ed Oncologia Sperimentale-CNR, Naples, Italy

[2]E-Mail: c.esposito@ieos.cnr.it

Abstract

Aptamers are short, structured, single-stranded RNA or DNA ligands that bind with high affinity to target molecules. They have a number of important advantages over proteins as therapeutic reagents. Most importantly they are rapidly isolated *in vitro,* show high specificity, low toxicity and non immunogenicity and can be easily stabilized by chemical modifications to improve their application *in vivo*.

To date an increasing number of aptamers have been generated with a great potential as cancer therapeutics. These first include inhibitory aptamers which bind to their target proteins directly hampering their function. In addition more recently, aptamers against cell surface molecules have been developed to specifically deliver different secondary reagents, including iRNAs, chemotherapeutic agents, toxins and nanoparticles to a cancer cells or tissues, thus increasing the efficacy of a given therapy as well as attenuating the overall toxicity.

In this article, we review recent advances in the use of aptamers for cancer therapy, particularly focusing on aptamers targeting cell surface. The future perspectives of aptamers as cancer therapeutics will be discussed as well.

Introduction

Cancer pathogenesis results from the accumulation of multiple genetic alterations that affect the activity and/or the expression of proteins involved in different cellular signalling pathways, thus conferring the proliferative and invasive characteristics to tumour cells [1, 2].

Despite great advances in surgical techniques, radiotherapy and chemotherapy, the identification of new therapies able to specifically target cancer cells remain a primary challenge in the field of oncology.

To this purpose, aptamers represent very promising molecules. They are single stranded nucleic acid ligands able to bind with high affinity and specificity protein or non-protein targets by folding into complex tertiary structures. They have a number of important advantages over proteins as therapeutic agents [3, 4]. Most importantly, they are readily chemically synthesized by a technique named Systematic Evolution of Ligands by EXponential enrichment (SELEX), thus avoiding the use of animal cells. Therefore they are cost-effective and easier to characterize than antibodies that are produced by recombinant means. Aptamers show binding affinities in the low nanomolar-picomolar range, comparable to those of antibodies, but different from them, they are sufficiently stable, since aptamers can be heated to 80°C, exposed to harsh environments and stored in different solvents without any irreversible conformation changes. Moreover, aptamers can be readily chemically modified to further enhance their stability in blood, bioavailability and pharmacokinetics [5-7] thus improving their effectiveness for clinic application.

Since oligonucleotides, aptamers are not affected by proteases, but, especially RNAs are rapidly degraded by nucleases in whole

organisms resulting in a very short half-life in the blood, a variety of approaches have been developed to improve their stability. The most effective modifications to circumvent this limitation are the substitutions at the 2′-ribose of the pyrimidines that are mainly affected by serum nucleases degradation. Even if pyrimidine-modified aptamers show considerable increase in serum stability in the absence of other modifications, introduction of 2′-O-Metyl purines [8] and changes in the internucleotide linkages (such as the use of phosphorothioate) and in the nucleobases (for example, the substitution of uridine at position 5) as well as the capping at the oligonucleotide 3′-terminus, have been reported [5]. Further, the use of locked nucleic acids (LNA), containing a methylene bridge to connect the 2′-O to the 4′-C, increases the stability of base pairing, stabilizing the duplex and enhancing the resistance to nucleases. For example the introduction of LNA-modifications in the anti-tenascin C (TN-C) aptamer significantly improves its plasma stability of approximately 25% and enhances the tumor uptake [6]. Even if the low molecular weight of aptamers allows cost-effective chemical synthesis and good target accessibility, it renders them susceptible to a rapid clearance by renal filtration. To overcome this problem, the most used strategy is to increase the size of the aptamers by conjugation with polyethylene glycol (PEG) [7]. As an example, 40 kDa PEG conjugation to anti-vascular endothelial growth factor (VEGF) aptamer (known as Macugen or pegaptanib) enhances its half-life from 2 hours to 1 day in rodents [8] whereas the half-life was 10 days when administered to humans (see prescribing information for Macugen).

Another important propriety of aptamers is that their toxicity and immunogenicity has never been reported [9]. This is based on the fact that antibodies against synthetic oligonucleotides are not generally produced and, in addition, the Toll like receptors mediated innate immune response against non-self RNAs is abrogated by 2′-modified nucleotides containing aptamers [10].

The potential clinical importance of this class of molecules aptamers is underlined by the fact that different aptamers are currently in clinical and preclinical trials and the aptamer therapeutic (Macugen or pegaptanib) directed against the VEGF has been approved by US Food and Drug Administration for the treatment of age-related macular degeneration (AMD) [11].

To date, an increasing numbers of aptamers provide innovative tools for cancer therapy. They can be used directly as therapeutics, because of their direct inhibitory properties or, thanks to their excellent targeting can be implemented as delivery tools for secondary therapeutic agents.

Here we will review the aptamer potentiality in cancer medicine focusing on the most promising recent examples and on their future prospective.

Selection of tumor-targeting aptamers

SELEX technology (Figure 1) is an evolutionary *in vitro* iterative procedure used to select aptamers specifically directed against a wide variety of potential targets, ranging from small molecules to proteins [12, 13]. The initial step of the SELEX procedure is the synthesis of a single-stranded nucleic acid (DNA, RNA or modified RNA) library of a high complexity. Randomisation of a sequence stretch from 20 up to 100 nucleotides in length is used to create an enormous diversity of possible sequences, which in consequence generate a great number of different conformations with different binding properties, from which ligands that may stably interact with specific target molecules are isolated. Random sequences are flanked by two constant regions with primer sites for PCR amplification.

The SELEX method (**Figure 1**) includes different steps: (1) counter-selection step, incubating the library with non target molecule; (2) positive selection, incubating aptamers unbound in the previous step with the target molecule (a protein or a cell line for protein- or cell-SELEX respectively); (3) partitioning unbound nucleic acids from those bound specifically to the selector molecule; (4) dissociating the nucleic acid-protein complexes; and (5) amplifying the nucleic acids pool enriched for specific ligands.

After the final round of the process, the PCR products are cloned into a vector and sequenced to allow for the identification of the best binding sequences.

Cancer cells are characterized by cell surface molecules that are over-expressed or altered due to multiple oncogenic mutations. Different approaches have been developed to target such surface molecules with aptamers.

In many cases soluble, purified cell surface proteins have been used as targets for aptamers selection in vitro (protein-SELEX). This approach is easy to control but the selection is performed in a non-physiological context, leading to the possibility that the selected aptamer might not recognize the same target in its native conformation.

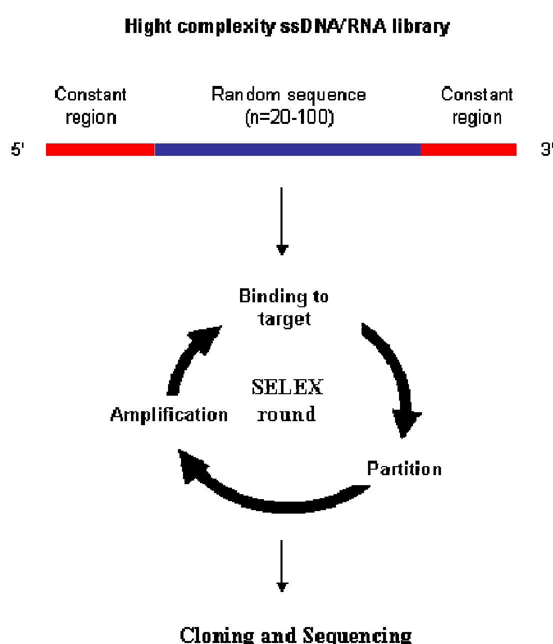

Figure 1. **Scheme of SELEX technology**. The RNA/DNA aptamer library contains a variable region flanked by two constant regions (primer sites for PCR amplification and T7-RNA polymerase promoter in the case of an RNA library). The library is used for the selection process (see text for details).

The application of the SELEX technology to complex target including whole living cells (cell-SELEX) allow to overcame this problem. By such an approach, it is possible to select aptamers against a desired protein, by using as target of selection a cell line over-expressing the protein. For example, this strategy has been successfully adopted to generate RNA aptamers specific for the transmembrane RTK, Ret [14, 15] or against the transforming growth factor (TGF)-β type III receptor [16].

It is important to notice that in cancer, receptor mutations are one of the major sources of proliferation deregulation and tumor initiation. Given the high specificity of aptamer-target recognition, aptamer selected against wild type proteins may not recognise the mutant form, thus a specific protocol of selection may be necessary (i.e. cell line that over-express the mutant protein).

More interesting in oncology, is the application of cell-SELEX to target a specific cancer cell phenotype (differential cell-SELEX) without a prior knowledge of the target protein. Indeed, a major challenge in cancer therapy is to distinguish with high accuracy, between closely related cell populations. In this respect, different selection strategies have been developed to isolate aptamers that can specifically recognize and discriminate between distinct cancer cell types. For example, aptamers that discriminate cells from distant tumor types, like T-cell acute lymphocytic leukemia (ALL) versus B-cell lymphoma [17] and small lung cancer cells versus large cell lung cancer [18], colon cancer cells versus other cancer cells [19] have been generated. The approach has been further developed to discriminate even in the same cancer cell type different properties (such as malignancy, therapeutic response, metastatic potential, proliferation or apoptotic rate). In this regard, in our laboratory, we isolated by a differential cell-SELEX approach, a panel of aptamers that bind human malignant glioblastoma cells, discriminating them from non-tumorigenic glioblastoma cells [20].

In addition, the great advances in cell-SELEX offer also the opportunity to develop innovative approaches to identify and isolate cancer stem cells, that are an emerging important target to develop more effective cancer therapy [21, 22]. Aptamers that specifically bind to brain tumor intiating cells with excellent Kds or that target the cancer

stem cells subpopulation of prostate cancer cells have been recently described [23, 24]. Further, an RNA aptamer that specifically recognise the cancer stem cell surface marker CD133 has been isolated and characterized [25].
In addition to cell-SELEX, even a tumor implanted in mice (*in vivo*-SELEX) [26] have been used to select aptamers.

Aptamers as cancer therapeutics

The utility of aptamers in cancer therapy has first employed those molecules able to bind and consequently inhibit the activity of their target proteins.
Proliferation and/or survival of tumor cells is promoted by the deregulation of specific cell-surface signaling receptors due to their over-expression or constitutive activation. Thus, the inhibition of these receptors using neutralizing aptamers represents an innovative approach to interfere with the growth of many human malignancies.
Among the aptamers in clinical trial, the AS1411 DNA aptamer (AGRO100) is very interesting for cancer therapy [27]. This aptamer is a 26-mer guanine-rich oligonucleotide (GRO), which folds into quadruplex structures [28] and is directed against nucleolin, a ubiquitous intranuclear and cytoplasmic phosphoprotein implicated in cellular proliferation and often over-expressed on the surface of tumor cells. Once bound to nucleolin, the AS1411 aptamer is taken into the cancer cell, where it causes cellular death by apoptosis through inhibiting nuclear factor-κB (NF-κB) [29] and Bcl-2 [30] pathways. It shows its effectiveness as an anticancer therapy for different solid human malignancies as well as for acute myeloid leukemia (AML) and is currently in phase IIb clinical trial to evaluate its effectiveness in combination with high-dose cytarabine in patients with relapsed and refractory AML.
Apart AS1411, in the last years an increasing number of aptamers has been generated to target specific cell-surface signaling receptors of cancer cells. For example, a key target for cancer treatment is the EGFR that is highly expressed in different types of cancer [31]. Recently, different 2′-Fluoro-Pyrimidine (2′-F-Py) RNA aptamers have been selected against a purified Fc-EGFR fusion protein. One of these aptamers, named E07, bound to the purified receptor and to EGFR expressing A431 cells and inhibit EGF-dependent receptor activation *in vitro* [32]. In addition, we generated by cell-SELEX, a 2′-F-Py RNA aptamer named CL4, that binds to EGFR with a high affinity (binding constant of 10 nM). CL4 aptamer inhibits EGFR-mediated signal pathways inducing apoptosis even in cells that are resistant to the most frequently used EGFR-inhibitors, such as gefitinib and cetuximab. Interestingly, CL4 has an antitumuor activity in mouse xenograft models of human cancer, and the combined treatment with cetuximab shows clear synergy in inducing apoptosis and inhibiting tumor growth *in vivo* [33].
Activated deletion variant EGFRvIII is the most common EGFR mutation found in cancer. RNA aptamers have been recently generated against the histidine-tagged EGFRvIII ectodomain using an *Escherichia coli* expression system. These aptamers can not bind the protein expressed by eukaryotic cells likely because of receptor glycosylation [34]. Hence, transfecting EGFRvIII aptamers into cells, they bind to the newly synthesized EGFRvIII and inhibit its glycosylation thus reducing the amount of mature EGFRvIII on the cell surface and increasing the percentage of cells undergoing apoptosis.
Other examples of aptamers that target and inhibit important cancer cell surface receptors include the oligonucleotides against epidermal growth factor receptor 2 and 3 (Her2/ErbB2 and Her3/ErbB3) [35-37], transforming growth factor β type III receptor (TGF-β type III) [16], tyrosine kinase Axl and Ret receptors [14, 15, 38].
Neutralizing aptamers able to inhibit tumor growth have been isolated also against cell-surface receptor ligands. One recent example is the 2′-F-Py modified RNA aptamer P12FR2, that is specific for the pancreatic adenocarcinoma up-regulated factor (PAUF). PAUF is a secretory factor highly expressed in pancreatic cancer that contributes to the oncogenesis of pancreatic cells [39]. P12FR2 aptamer inhibits migration of PANC-1 human pancreatic cancer

cells and decreases tumor growth in vivo when injected intraperitoneally [40].
A great challenge in cancer therapy is to prevent or inhibit angiogenesis which is associated with the increased vascularity necessary for tumor progression and metastasis. In this regard, different aptamers able to interfere with this process have been isolated. One notable early example is the anti - VEGF RNA aptamer. This aptamer binds and antagonizes the action of VEGF-165 and is the only aptamer commercially approved (Macugen or pegaptanib) for the treatment of exudative AMD [41, 42]. Inhibiting neovascularization, Macugen is also a potential candidate for the treatment of highly vascularized tumors, even if the effectiveness of a systemic administration is still unclear .
More recently, it has been characterized an RNA aptamer that specifically blocks Ang 2 inhibiting Tie2 receptor that is important for tumor neovascularization [43]. The *in vivo* administration of this aptamer modified with PEG inhibits tumor angiogenesis and growth thus providing a new anti-neoplastic strategy.
In order to develop anti-agiogenesis molecules, DNA aptamer have been selected against primary cultured mouse tumor endothelial cells (mTECs). One of the selected aptamer (AraHH001) binds specifically to mTECs and to cultured human tumor endothelial cells (hTECs), isolated from a clinical patient with a renal carcinoma. AraHH001 showed significant anti-angiogenesis activity in vitro inhibiting tube formation by mTECs on matrigel [44].

Aptamers have been applied also to cancer immunotherapy. A recent example is an RNA aptamer able to block the murine or human IL-4 receptor-α (IL4Rα) that is critical for myeloid-derived suppressor cells (MDSC) suppression function. MDSC promote tumor progression and metastasis by enhancing angiogenesis and invasion, but also inhibit antitumor T-cell functions, limiting immunotherapeutic interventions effectiveness. The selected anti–IL4Rα aptamer promoted MDSC elimination, increasing the number of tumor-infiltrating T cells and a reducing tumor growth in vivo [45].

Moreover, different aptamers against tumor metastasis associated factors have been developed. For example, An RNA aptamer that binds to carcinoembryonic antigen (CEA), inhibits migration and invasion of colon cancer cells *in vitro* and hepatic metastasis of colon cancer cells *in vivo* [46]. More recently, a DNA aptamer able to recognize epithelial cell adhesion molecule (EpCAM), overexpressed in most solid cancers, has been dscribed and represents a promising tool for targeted cancer therapy [47].

In addition to cell-surface proteins, intracellular proteins of cancer medical interest can be targeted with aptamers (so-called intramers) as well by means of strategies allowing their entry into intracellular compartments [48-.50].

Aptamers for *in vivo* cancer delivery of therapeutics

Apart that such inhibitory molecules, thanks to their high binding specificity, aptamers against cell surface molecules can be developed to deliver any kind of secondary reagents to a given cancer cell or tissue (**Figure 2**). In this way, only targeted cells will be exposed to the secondary reagent thus increasing the efficacy of a given therapy as well as attenuating the overall toxicity of the drug [51, 52]. At this regard, currently an increasing number of aptamers targeting cancer cell surface epitopes have been successfully used for the specific delivery of active drug substances both in vitro and in vivo, including nanoparticles [24, 53], anti-cancer therapeutics [54], toxins [55], enzymes [56], radionuclides [57], viruses [58], small interfering RNAs (siRNAs) [59-71] and more recently microRNAs [72-74].

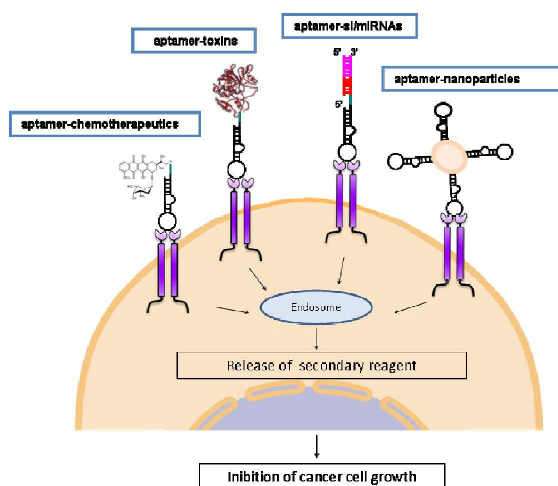

Figure 2. Aptamers as delivery tools. Aptamers against cell surface molecules has been developed to deliver different secondary reagents including cancer therapeutics, toxins, siRNAs/microRNAs and nanoparticles to a given cancer cell.

The best-characterized aptamers for targeted delivery are the RNAs directed against Prostate specific menbrane antigen (PSMA) [75] that were successfully developed to drive to prostate cancer cells nanoparticles [76], quantum dots [77], toxins [55], or siRNAs [61, 65, 66].

PSMA–aptamer has been linked to siRNAs by different approaches including non-covalent conjugation of siRNA with aptamer via a streptavidin connector [60] or generation of aptamer–siRNA chimeras by extending the 3' end of the aptamer with a nucleotide sequence complementary to the antisense strand of the siRNA [61, 65]. In addition to siRNAs, PSMA–aptamer has been further use to deliver to prostate cancer cells toxins [55] or chemotherapeutic agents encapsulated within nanoparticles or directly intercaleted into the aptamer [76, 78-81].

In addition to PSMA–aptamer, in a recent study, a DNA aptamer targeting human hepatocellular carcinoma LH86 cells, was used to delivery Doxorubicin to liver cancer. The aptamer was modified with a long GC tail to achieve greater intercalation efficiency of Doxorubicin, showing a specific killing efficiency for target cancer cells [82].

Currently, the number of cancer cell surface epitopes targeting aptamers that have been developed as delivery tools is growing rapidly and includes those against the extracellular proteins involved in cancer protein tyrosine kinase 7 (PTK7) [83], nucleolin [84], mucin 1 (MUC1) [85, 86], epidermal growth factor receptor (EGFR) [87] and transferrin receptor [88].

Furthermore, recently it has been explored the use of aptamer to deliver microRNAs as cancer therapeutics. Second generation PSMA-targeting aptamer (A10-3.2) was conjugated to a polyamidoamine (PAMAM)-based microRNA (miR-15a and miR-16-1) via bifunctional PEG to deliver microRNAs to prostate cancer cells. The construct selective induced in vitro cell death of PSMA positive prostate cancer cells by loading miR-15a and miR-16-1 [72]. In addition, a chimera that combines MUC1 aptamer and let-7i microRNA was able to sensitized OVCAR-3 ovarian cancer cells to paclitaxel [73]. MUC1 aptamer was also combined to miR-29b that inhibits DNA methyltransferases expression, inducing PTEN gene. The resulting chimeric molecule (Chi-29b chimera) effectively induced apoptosis through specific delivery of miR-29b into OVCAR-3 cells [74]. These new compounds are provide innovative cancer therapeutic strategies even if their in vivo effectiveness has not yet been proven.

Conclusions and Future prospects

According to World Health Organization, cancer is a leading cause of death worldwide, accounting around 13% of all deaths and this percentage is projected to continue to increase in the next year. Thus, cancer is a major public health problem and have a great economical and social impact.
The identification of new therapies is essential and a crucial challenge is to specifically target cancer cells. Indeed, an important drawback in cancer treatment is that conventional therapies are toxic for healthy cells.

As discussed above, a very highly innovative approach is the development of aptemer-microRNAs chimeras. Even if the aptamer-based microRNAs delivery has not yet been validated *in vivo*, it represents an important prospective with a secure future development. Indeed, microRNAs are one of the most promising new tools for the study of tumors, but their development have a compelling need to be confirmed at functional and therapeutic level.

In addition, current evidence suggests that combinations of specific inhibitors result in improved anti-tumor response and decreased toxicity. Thus, the possibility to use combinations of aptamers to multiple targets or inhibitory aptamers with current chemotherapies, represent a great future prospective.

References

1. María Apellániz-Ruiz and Zafira Castaño. Adapt or Dye: Tumor Microenvironment, A Powerful Regulator of Cancer Progression. PDJ 2013, 1 (5): 1-8.
2. Hanahan, D., Weinberg, R.A. Hallmarks of cancer: the next generation. Cell. 2011, 144(5):646-74.
3. Cerchia, L., de Franciscis V. Targeting cancer cells with nucleic acid aptamers. Trends Biotechnol. 2010, 28(10):517-25.
4. Missailidis, S., Hardy, A. Aptamers as inhibitors of target proteins. Expert Opin Ther Pat. 2009, 19(8):1073-82.
5. Keefe, A.D., Cload, S.T. SELEX with modified nucleotides. Curr Opin Chem Biol. 2008, 12(4):448-56.
6. Schmidt, K.S. et al. Application of locked nucleic acids to improve aptamer in vivo stability and targeting function. Nucleic Acids Res. 2004, 27;32(19):5757-65.
7. Healy, J.M. et al. Pharmacokinetics and biodistribution of novel aptamer compositions. Pharm Res. 2004, 21(12):2234-2246.
8. Burmeister, P.E. et al. Direct in vitro selection of a 2′-O-methyl aptamer to VEGF. Chem Biol. 2005, 12(1):25-33.
9. Foy, J.W.D. et al. Local tolerance and systemic safety of pegaptanib sodium in the dog and rabbit. J Ocul Pharmacol Ther. 2007, 23(5):452-466.
10. Yu, D. et al. Modifications incorporated in CpG motifs of oligodeoxynucleotides lead to antagonist activity of toll-like receptors 7 and 9. J Med Chem. 2009, 52(16):5108-5114.
11. Esposito, C.L. et al. New insight into clinical development of nucleic acid aptamers. *Discov Med*. 2011 11(61): 487-96.
12. Ellington, A.D., Szostak, J.W. In vitro selection of RNA molecules that bind specific ligands. Nature. 1990, 346: 818-822.
13. Tuerk, C., Gold, L. Systematic evolution of ligands by exponential enrichment: RNA ligands to bacteriophage T4 DNA polymerase. Science 1990, 249: 505-510.
14. Cerchia, L. et al. Neutralizing aptamers from whole-cell SELEX inhibit the RET receptor tyrosine kinase. PLoS Biol. 2005, 3(4):e123.
15. Vento, M.T. et al. Distribution and bioactivity of the Ret-specific D4 aptamer in three-dimensional collagen gel cultures. Mol Cancer Ther. 2008, 7(10):3381-8.
16. Ohuchi, S.P., Ohtsu, T., Nakamura, Y. Selection of RNA aptamers against recombinant transforming growth factor-beta type III receptor displayed on cell surface. Biochimie. 2006,88(7):897-904.
17. Shangguan, D. et al. Aptamers evolved from live cells as effective molecular probes for cancer study. Proc Natl Acad Sci U S A. 2006, 103(32):11838-43.
18. Chen, H.W. et al. Molecular recognition of small-cell lung cancer cells using aptamers. ChemMedChem. 2008,3(6):991-1001.
19. Sefah, K. et al. DNA Aptamers as Molecular Probes for Colorectal Cancer Study. PLoS One 2010, 5(12): e14269.
20. Cerchia, L. et al. Differential SELEX in human glioma cell lines. PLoS One. 2009, 24;4(11):e7971.
21. Guo, KT. Et al. Aptamer-based strategies for stem cell research. Mini Rev Med Chem 2007, 7(7):701-705.
22. Hoffmann, J. et al. Immobilized DNA aptamers used as potent attractors for porcine endothelial precursor cells. J Biomed Mater Res A. 2008, 84(3):614-21.

23. Kim, Y. et al. Aptamer Identification of Brain Tumor Initiating Cells. Cancer Res. 2013 [Epub ahead of print]
24. Wang, J. et al. Aptamer-Conjugated Nanorods for Targeted Photothermal Therapy of Prostate Cancer Stem Cells. Chem Asian J. 2013 [Epub ahead of print]
25. Shigdar, S. RNA aptamers targeting cancer stem cell marker CD133. Cancer Lett. 2013, 330(1):84-95.
26. Mi, J. et al. In vivo selection of tumor-targeting RNA motifs. Nat Chem Biol 2010, 6(1):22-24.
27. Reyes-Reyes, E.M., Teng, Y., Bates, P.J. A new paradigm for aptamer therapeutic AS1411 action: uptake by macropinocytosis and its stimulation by a nucleolin-dependent mechanism. Cancer Res. 2010, 70(21): 8617-29.
28. Dapic, V. et al. Biophysical and biological properties of quadruplex oligodeoxyribonucleotides. Nucleic Acids Res 2003, 31(8): 2097-2107.
29. Girvan, A.C. et al. AGRO100 inhibits activation of nuclear factor-kappaB (NF-kappaB) by forming a complex with NF-kappaB essential modulator (NEMO) and nucleolin. Mol Cancer Ther 2006, 5(7):1790-1799.
30. Soundararajan, S. et al. The nucleolin targeting aptamer AS1411 destabilizes Bcl-2 messenger RNA in human breast cancer cells. Cancer Res 2008, 68(7): 2358-2365.
31. Hynes, N.E., Lane, H.A. ERBB receptors and cancer: the complexity of targeted inhibitors. Nature Reviews Cancer. 2005, 5(5):341–354.
32. Li, N. et al. Inhibition of cell proliferation by an anti-egfr aptamer. PLoS One 2011, 6(6): e20299.
33. Esposito, C.L. et al. A neutralizing RNA aptamer against EGFR causes selective apoptotic cell death. PLoS One 2011, 6(9): e24071.
34. Liu, Y. et al. Aptamers selected against the unglycosylated EGFRvIII ectodomain and delivered intracellularly reduce membrane-bound EGFRvIII and induce apoptosis. Biological Chemistry 2009, 390(2):137–144.
35. Kim, M.Y., Jeong S. In Vitro Selection of RNA Aptamer and Specific Targeting of ErbB2 in Breast Cancer Cells. Nucleic Acid Ther. 2011, 21(3): 173–178.
36. Chen, C.H. et al. 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. U. S. A. 2003, 100:9226–9231.
37. Park, E., Baron, R., Landgraf, R. Higher-order association states of cellular ERBB3 probed with photo-cross-linkable aptamers. Biochemistry 2008, 18:11992–12005.
38. Cerchia, L. et al. Targeting Axl with an high-affinity inhibitory aptamer. Mol Ther. 2012, 20(12):2291-303.
39. Cho, I.R. et al. Pancreatic adenocarcinoma up-regulated factor (PAUF) enhances the expression of β-catenin, leading to a rapid proliferation of pancreatic cells. Exp Mol Med. 2011, 43(2):82-90.
40. Kim, Y.H. et al. An RNA aptamer that specifically binds pancreatic adenocarcinoma up-regulated factor inhibits migration and growth of pancreatic cancer cells. Cancer Lett 2011, 313: 76-83.
41. Ng, E.W. et al. Pegaptanib, a targeted anti-VEGF aptamer for ocular vascular disease. Nat Rev Drug Discov 2006, 5(2):123-132.
42. Chakravarthy, U. et al. Year 2 efficacy results of 2 randomized controlled clinical trials of pegaptanib for neovascular age-related macular degeneration. Ophthalmology 2006, 113(9):1508.e1-25.
43. Sarraf-Yazdi, S.J. et al. Inhibition of in vivo tumor angiogenesis and growth via systemic delivery of an angiopoietin 2-specific RNA aptamer. Surg Res. 2008, 146(1):16-23.
44. Ara M.N. et al. Development of a novel DNA aptamer ligand targeting to primary cultured tumor endothelial cells by a cell-based SELEX method. PLoS One. 2012,7(12):e50174.
45. Roth, F. Et al. Aptamer-mediated blockade of IL4Rα triggers apoptosis of MDSCs and limits tumor progression. Cancer Res. 2012, 72(6):1373-83.
46. Lee, Y.J. An RNA aptamer that binds carcinoembryonic antigen inhibits hepatic metastasis of colon cancer cells in mice. Gastroenterology. 2012 143(1):155-65.

47. Song, Y. Selection of DNA aptamers against epithelial cell adhesion molecule for cancer cell imaging and circulating tumor cell capture. Anal Chem. 2013, 85(8):4141-9.
48. Lee, H.K. et al. β-Catenin regulates multiple steps of RNA metabolism as revealed by the RNA aptamer in colon cancer cells. Cancer Res 2007, 67(19):9315-9321.
49. Mi, J. et al. RNA aptamer-targeted inhibition of NF-kappa B suppresses non-small cell lung cancer resistance to doxorubicin. Mol Ther 2007, 16(1):66-73.
50. Watanabe, M. et al. HMGA-targeted phosphorothioate DNA aptamers increase sensitivity to gemcitabine chemotherapy in human pancreatic cancer cell lines. Cancer Lett. 2012, 315(1):18-27.
51. Cerchia L, de Franciscis V. Targeting cancer cells with nucleic acid aptamers. Trends Biotechnol 2010, 28(10):517-525.
52. Zhou, J., Rossi, J. Aptamer-targeted cell-specific RNA interference. Silence 2010, 1(1):4.
53. Farokhzad, O.C. et al. Targeted nanoparticle-aptamer bioconjugates for cancer chemotherapy in vivo. Proc. Natl.Acad.Sci. U. S.A. 2006, 103:6315–20.
54. Bagalkot, V. et al. Anaptamer-doxorubicin physical conjugateas a novel targeted drug-delivery platform. Angew. Chem. Int. Ed. Engl. 2006, 45: 8149–52.
55. Chu, T.C. et al. Aptamer:toxin conjugates that specifically target prostate tumor cells. Cancer Res. 2006, 66: 5989–92.
56. Chen, C.H. et al. Aptamer-based endocytosis of a lysosomal enzyme. Proc. Natl. Acad. Sci. U.S.A. 2008, 105: 15908-13.
57. Hicke, B.J. et al. Tumor targeting by an aptamer. J. Nucl. Med. 2006, 47: 668–78.
58. Tong, G.J. et al. Viral capsid DNA aptamer conjugates as multivalent cell-targeting vehicles. J. Am. Chem. Soc. 2009, 131: 11174– 78.
59. Thiel K.W., Giangrande, P.H. Intracellular delivery of RNA-based therapeutics using aptamers. Ther Deliv. 2010, 1(6):849-61.
60. Chu, T.C. et al. Aptamer mediated siRNA delivery. Nucleic Acids Res. 2006, 34: e73.
61. McNamara, J.O. 2nd et al. Cell type-specific delivery of siRNAs with aptamer-siRNA chimeras. Nat Biotechnol. 2006, 24(8):1005-15.
62. Zhou, J. et al. Novel dual inhibitory function aptamer-siRNA delivery system for HIV-1 therapy. Mol Ther. 2008, 16(8), 1481-9.
63. Wullner, U. et al. Cell-specific induction of apoptosis by rationally designed bivalent aptamer-siRNA transcripts silencing eukaryotic elongation factor 2. Curr Cancer Drug Targets 2008, 8(7):554-65.
64. Zhou, J. et al. 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. 2009, 37(9): 3094-109.
65. Dassie, J.P. et al. Systemic administration of optimized aptamer-siRNA chimeras promotes regression of PSMA-expressing tumors. Nat Biotechnol. 2009, 27(9): 839-49.
66. Ni, X. et al. Prostate-targeted radiosensitization via aptamer-shRNA chimeras in human tumor xenografts. J Clin Invest. 2011, 121(6):2383-90.
67. Wheeler, L.A. et al. Inhibition of HIV transmission in human cervicovaginal explants and humanized mice using CD4 aptamer-siRNA chimeras. J Clin Invest. 2011, 121(6): 2401-12.
68. Neff, C.P. et al. An aptamer-siRNA chimera suppresses HIV-1 viral loads and protects from helper CD4(+) T cell decline in humanized mice. Sci Transl Med. 2011, 3(66): 66ra6.
69. Zhu, Q., Shibata, T., Kabashima, T., Kai, M. Inhibition of HIV-1 protease expression in T cells owing to DNA aptamer-mediated specific delivery of siRNA. Eur J Med Chem. 2012, 56: 396-9.
70. Thiel, K.W. et al. Delivery of chemo-sensitizing siRNAs to HER2+-breast cancer cells using RNA aptamers. Nucleic Acids Res. 2012, 40(13): 6319-37.
71. Zhou, J. et al. Functional in vivo delivery of multiplexed anti-HIV-1 siRNAs via a chemically synthesized aptamer with a sticky bridge. Mol Ther. 2013, 21(1):192-200.
72. Wu, X. et al. Second-generation aptamer-conjugated PSMA-targeted delivery

system for prostate cancer therapy. Int J Nanomedicine. 2011, 6: 1747-56.

73. Liu, N., Zhou, C., Zhao, J., Chen, Y. Reversal of paclitaxel resistance in epithelial ovarian carcinoma cells by a MUC1 aptamer-let-7i chimera. Cancer Invest. 2012, 30(8): 577-82.

74. Dai, F. et al. Anticancer role of MUC1 aptamer-miR-29b chimera in epithelial ovarian carcinoma cells through regulation of PTEN methylation. Target Oncol. 2012, 7(4): 217-25.

75. Lupold, S.E. et al. Identification and characterization of nuclease-stabilized RNA molecules that bind human prostate cancer cells via the prostate-specific membrane antigen. Cancer Res 2002, 62(14):4029-33.

76. Farokhzad, O.C. et al. Nanoparticle-aptamer bioconjugates: a new approach for targeting prostate cancer cells. Cancer Res 2004, 64(21):7668-72.

77. Chu, T.C. et al. Labeling tumor cells with fluorescent nanocrystal- aptamer bioconjugates. Biosens Bioelectron 2006b, 21(10):1859-66.

78. Bagalkot, V. et al. An aptamer-doxorubicin physical conjugate as a novel targeted drug-delivery platform. Angew Chem Int Ed Engl. 2006, 45(48):8149-52.

79. Dhar, S. et al. Targeted delivery of cisplatin to prostate cancer cells by aptamer functionalized Pt(IV) prodrug-PLGA-PEG nanoparticles. Proc. Natl. Acad. Sci. U.S.A. 2008, 105: 17356–61.

80. Wang, A.Z. et al. Super paramagnetic iron oxide nanoparticle–aptamer bioconjugates for combined prostate cancer imaging and therapy. Chem. Med. Chem. 2008, 3: 1311–15

81. Zhang, L. et al. Co-delivery of hydrophobic and hydrophilic drugs from nanoparticle-aptamer bioconjugates. Chem. Med. Chem. 2007, 2: 1268- 71

82. Meng, L. et al. Targeted Delivery of Chemotherapy Agents Using a Liver Cancer-Specific Aptamer . PLoS ONE 2012, 7(4): e33434.

83. Huang, Y.F. et al. Molecular assembly of an aptamer-drug conjugate for targeted drug delivery to tumor cells. ChemBioChem 2009, 10(5):862-68.

84. Ko, M.H. et al. In vitro derby imaging of cancer biomarkers using quantum dots. Small 2009, 5(10):1207-12.

85. Cheng, A.K. et al. Aptamer-based detection of epithelial tumor marker mucin 1 with quantum dot-based fluorescence readout. Anal Chem 2009, 81(15):6130-39.

86. Ferreira, C.S. et al. Phototoxic aptamers selectively enter and kill epithelial cancer cells. Nucleic Acids Res 2009, 37(3):866-76.

87. Li, N. et al. Directed evolution of gold nanoparticle delivery to cells. Chem Commun 2010, 46(3):392-94.

88. Wilner, S.E. et al. An RNA alternative to human transferrin: a new tool for targeting human cells. Mol Ther Nucleic Acids 2012, 1: e21.