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Generating Aptamers for Cancer Diagnosis and Therapy

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Abstract

Aptamers are single-stranded DNA or RNA oligonucleotides or peptides that are capable of binding to specific target proteins. Aptamers are generated in a process named SELEX or cell-SELEX. They resemble antigens in respect of their highly specific binding in the lower nanomolar and/or picomolar range. In contrast to antibodies, they can be generated *in vitro*, are more stable molecules and have less immunogenicity, better tissue penetration and faster clearance. Aptamers can be applied in many conditions, one of them being cancer medicine, because of their ability to detect cell-specific markers within complex systems that have no specified target molecules. Moreover, aptamers can be used as therapeutic agents, which are indirectly or directly conjugated with drugs, due to their toxic, inhibitory or activating effect on target cells.

Keywords: Aptamer; SELEX; Cell-SELEX; Cancer; Alimentary tract

The term aptamer is derived from the Latin word "aptus", meaning "to fit", since aptamers are specific three-dimensional *in vivo* structures that are capable of binding to specific target proteins. They are generated in a process named SELEX. End products of this method are single-stranded DNA or RNA oligonucleotides (12-30 bases) or peptides with high specificity and affinity to their target molecules, which are produced from libraries that contain 10^{13} to 10^{16} single stranded DNA (ssDNA) or RNA molecules [1,2].

The concept was first described in 1990 by three different research groups. Robertson et al. reported discovering an RNA enzyme (mutated tetrahymena ribozyme) that cleaves single-stranded DNA in a specific sequence [3]. Tuerk et al. described a procedure that depends on alternated cycles of ligand selection from pools with different nucleotide sequences and species-bound amplification. This is when the term "Systematic Evolution of Ligand by Exponential Enrichment"-SELEX was introduced [4]. The Ellington study group named the end products of the *in vitro* selection process "aptamer" [2]. Since 1990, numerous researches were carried out. To illustrate, a simple pubmed search for the term aptamer shows 2781 hits. Small molecules (dyes, metal ions, amino acids, small peptides) [5-10], proteins [11], viruses [12], biological cells [13,14] and bacteria [15] were studied as potential aptamer targets. Moreover, cancer related proteins were investigated, such as PDGF, VEGF, HER3, NFκB, tenascin-C and the prostate-specific membrane antigen [16-18]. In general, researches have proved that aptamers can be used in cancer medicine for detecting cancer cells, cancer biomarkers and enriching rare cancer cells with nanoparticle technology. Aptamers can be further implemented as target therapy for specific cancer. In this manner they can be toxic per se or increase the efficiency and sensitivity of specific chemotherapy [19].

Aptamers against Antibodies

Monoclonal antibodies that bind to tumor antigens are commonly used as therapeutic agents in different neoplasms. One such agent is a tyrosine kinase inhibitor (imatinib mesylate; industrial name Gleevec) that is used for treating chronic myeloid leukemia. Therapy with imatinib mesylate can lead to a regression of advanced tumors by inhibiting thyrosine kinase activity in overexpressed Brc-Abl oncoprotein [20,21].

Some other therapeutic antibodies include:

- bevacizumab (trade name Avastin), an angiogenesis (VEGF-A) inhibitor, used in case of a metastatic cancer disease, lung cancers, renal cancers and glioblastoma multiforme [22-25];
- cetuximab (trade name Erbitux), an epidermal growth factor inhibitor (EGFR) for treating head and neck cancer, as well as metastatic colorectal cancer [26,27];
- panitumumab (trade name Vectibix), an EGFR-, ErbB-1- and HER1-specific monoclonal antibody that has been approved for treating metastatic colorectal cancer [28];
- transtuzumab (trade name Herceptin), a HER2/neu receptor that is a specific antibody used for some types of breast cancer treatment [29] and many others.

Aptamers resemble antibodies in respect of their highly specific binding in the picomolar and/or nanomolar range. When comparing these two agents, we see that aptamers possess some advantages over antibodies. First of all, they are generated in an in vitro setting, which allows them screening against molecules with weak immunogenicity and toxicity [30,31]. Antibodies lack this property, because they are produced in an in vivo session. Aptamers are also stable molecules; they can be heated to 80°C, exposed to harsh environments and stored in different solvents without any irreversible conformation changes. Furthermore, compared to antibodies, they have little immunogenicity, as it was proven in a study on monkeys, which received doses that were 1000-fold larger than the therapeutic one, but little or no immunogenic effect was observed [32]. In addition, they can be chemically modified to improve stability in blood, biodistribution, tissue penetration and clearance [33]. The most often used chemical modifications of aptamers include 2′-amino pyrimidines, 2′-fluoro pyrimidines, 2′-O-methyl nucleotides, 2′-ribo nucleotides, 5-modofied pyrimidines and 4′-thio pyramidines [34]. Finally, the synthesis of aptamers is quite easy to perform.

However, some therapeutic aptamers were engineered to act against tumor-specific antigens like:

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- tenascine-C, an extracellular matrix glycoprotein that is overexpressed during tissue remodeling (tumor growth) [35];
- the prostate-specific membrane antigen (PSMA) that is up-regulated in prostate cancer [17]
- and the human epidermal growth factor receptor-3 (ErbB3/HER3) [36].

It has also been determined that the vascular endothelian growth factor (VEGF) is a potential target. It was tested in a mice model of renal cancer and was associated with tumor regression [37-39].

Different aptamer substances are at this time in various phases of clinical and preclinical trials. Studies especially focus on therapeutic use in conditions, such as age-related macula degeneration, acute myloid leukemia, autologous stem cell transplantation, type 2 diabetes mellitus, heart disease, coronary artery disease etc. In 2004, the first aptamer therapeutic was approved by the FDA. The agent (Pegaptanib, Macugen) has a binding domain on $VEGF_{165}$ and is used against age-related macula degeneration [40]. Some of the other aptamers that are in clinical trials are listed in Table 1.

SELEX Tecnology (Systematic Evolution of Ligand by Exponential Enrichment)

SELEX technology (Figure 1) is an evolutionary *in vitro* iterative panning procedure that is used for selecting aptamers, which specifically identify targets that range from small molecules to proteins [2,4]. However, cancer cells are characterized by cell surface molecules that overexpress or alter due to multiple oncogenic mutations. Different approaches have been discovered to target such surface molecules. The initial step of the SELEX procedure is the synthesis of a single-stranded nucleic acid (RNA/DNA) library of large sequence complexity (typical length is 20 to 100 nucleotides, flanked by two constant regions with primer sites for PCR amplification). The second is selecting oligonucleotides, which are capable of binding to target molecules with high affinity and specificity. DNA aptamers can be selected directly from a DNA library, whereas in case of RNA aptamers, an in vitro transcription of a DNA library into an RNA library has to be performed. A typical aptamer library with a variable region of 24 to 25 nucleotides has a predictive complexity of 4^{24} to 4^{25} different molecules.

The next steps of the SELEX procedures are: (a) nucleic acid pool folding in a specific buffer, in order to obtain specific conformational changes; (b) incubating the aptamer library with a target molecule, such as a recombinant protein within a binding buffer (smart aptamers with predefined equilibrium constant *Kd,* generated by the Equilibrium Capillary Electrophoresis of Equilbrium Mixtures (ECEEM), can be used [41]); (c) partitioning unbound nucleic acids from specifically bound ones to the target molecule; (d) dissociating the nucleic acid-protein complexes and (e) amplifying the nucleic acid pool that is enriched with sequences that allow binding to selected target molecules with PCR (DNA SELEX) or reverse transcriptase (RT)-PCR for RNA SELEX. The new generated nucleic acid pool serves as a starting library for a new SELEX cycle that consists of the same steps as the first round. The number of SELEX repetitions depends on the used library type and on specific enrichment achieved per selection cycle. After the last round of aptamer selection, PCR products are cloned and sequenced. Typically, about 50 aptamer clones are analyzed through sequencing [2,4,42]. After that, sequence alignments are performed, in order to assess the complexity of the selected aptamer pool and to group together aptamer clones with homologous sequences. Alignments are mostly performed with internet programs, such as CLUSTAL W (http://www.ebi.ac.uk/clustalw) [43]. Aptamer sequences are analyzed, in order to obtain relevant structures for binding. This step is conducted by the *mfold* program that is available online at http://www.bioinfo.rpi.edu/applications/mfold/DNAforml.cgi. The main purpose of this step is to identify possible configurations of single-stranded nucleic acids, such as stems, loops or bulges [44]. Binding studies follow, in order to determine specificity and affinity of selected aptamers. Besides, aptamer-target interactions are defined with footprinting [45-47]. The three-dimensional binding structure of the aptamer-target complex is visualized by using nuclear magnetic resonance (NMR) spectroscopy, X-ray structure analysis and crystallization [48,49].

Partitioning unbound nucleic acids from specifically bound ones represents one of the key steps in SELEX technology. That is why some new tools for isolation were implied, such as flow cytometry, surface plasmon resonance [50], capillary gel electrophoresis [41,51,52] and microfluid devices [53]. All this methods have simplified the procedure, making it more effective and faster.

Cell-SELEX Technology and Biopanning and Rapid Analysis of Selective Interactive Ligands

Cell-SELEX technology (Figure 2) allows targeting molecules on the surface of living cells, which has some advantages over classic SELEX technology. Cell-SELEX seems to be appropriate for identifying cancer cells and generating aptamers that bind specifically to altered surface molecules on cancer cells without the exact knowledge of changed sequences. The steps in cell-SELEX technology resemble the SELEX procedure, but have some differences. The DNA library, which consists of 30-40 bases that are flanked by primer sequences, is being incubated with target cells in specific conditions. The unbound DNA probes are washed out and the bound ones are collected with heat denaturing or phenol-chloroform and EtOH precipitation. The eluant is then amplified by PCR or used for counter selection. Counter selection represents a step in cell-SELEX, where aptamer probes are incubated with negative (control) cells. This time, the unbound probes are collected and amplified. This step is crucial for removing nucleic acids that bind to normally expressed membrane proteins. The final step consists of a double-stranded DNA (dsDNA) amplification that is treated with streptavidin beads, which results in antisens strands with biotin retaining on the beads and sens strand elution with sodium hydroxide solution. At the end, eluted ssDNA represents the enriched library for the next round of cell-SELEX. To prove that high affinity ligands are being enriched, flow cytometry or fluorescence microscopy is used to detect fluorescin isothiocyanate (FITC) labelled as a 5′ forward primer. The pool is usually composed of high affinity ligands after 15-25 cycles of the cell-SELEX procedure. In the last step, the pool is cloned and sequenced, in order to determine the sequence of enriched DNA pools. The identified sequences are then chemically synthesized and labelled with fluorophore to evaluate the binding affinity [40,54].

Another method, which allows targeting of cell-surface proteins in a cell population, is called biopanning and rapid analysis of selective interactive ligands (BRASIL). The method allows separation of phage-cell complexes from the remaining unbound phage. The separation is possible due to differential centrifugation, which leads cells from a hydrophilic environment to the hydrophobic-organic phase. This allows separation of unbound phage, which remains soluble in the upper hydrophilic phase, in just one centrifugation step. The above mentioned characteristics make BRASIL a simple and efficient cell panning method. Moreover, it can be used for targeting and isolating ligand-receptor pairs in cell populations, which are isolated from clinical samples as fine-needle aspirates [55].

Drug (Aptamer)	ClinicalTrials.gov Identifier:	Phase	Condition	Sponsor
ARC1905	NCT00950638	Phase I Active, not recruiting	Age-Related Macular Degeneration	Ophthotech Corporation
E10030	NCT01089517	Phase II Active, not recruiting	Age-Related Macular Degeneration	Ophthotech Corporation
E10030	NCT00569140	Phase I Completed	Age-Related Macular Degeneration	Ophthotech Corporation
ARC1905	NCT00709527	Phase I Completed	Age-Related Macular Degeneration	Ophthotech Corporation
EYE001 anti-VEGF aptamer	NCT00021736	Phase II Phase III completed	Macular Degeneration Choroidal Neovascularization	Eyetech Pharmaceuticals
REG1	NCT00113997	Phase I completed	Healthy, improve control of "blood thinning"	National Heart and Lung, and Blood Institute (NHLBI)
EYE001	NCT00056199	Phase I completed	Hippel-Lindau Disease	National Eye Institute (NEI)
pegaptanib sodium (Macugen)	NCT00215670	Phase II Phase III, completed	Age-Related Macular Degeneration	Eyetech Pharmaceuticals, Pfizer
Pegaptanib sodium	NCT00312351	Phase IV, terminated	Macular Degeneration	Eyetech Pharmaceuticals, Pfizer
Pegaptanib sodium	NCT00321997	Phase II Phase III, completed	Age-Related Macular Degeneration	Eyetech Pharmaceuticals, Pfizer
pegaptanib sodium (Macugen)	NCT00040313	Phase II, completed	Diabetic Macular Edema	Eyetech Pharmaceuticals, Pfizer
AS1411	NCT01034410	Phase II, terminated	Acute Myeloid Leukemia	Antisoma Research
NOX-E36	NCT00976729	Phase I, completed	Chronic Inflammatory Diseases Type 2 Diabetes Mellitus Systemic Lupus Erythematosus	Noxxon Pharma AG
NOX-A12	NCT01194934	Phase I, completed	Hematopoietic Stem Cell Transplantation	Noxxon Pharma AG
NOX-A12	NCT00976378	Phase I, completed	Autologous Stem Cell Transplantation	Noxxon Pharma AG
ARC1779	NCT00694785	Phase II, has been withdrawn prior to enrollment	Von Willebrand Disease	Archemix Corp.
ARC1779	NCT00632242	Phase II, completed	Purpura, Thrombotic Thrombocytopenic Von Willebrand Disease Type-2b	Archemix Corp.
ARC19499	NCT01191372	Phase I Phase II , no started	Hemophilia	Archemix Corp.
Macugen (Pegaptanib Sodium)	NCT01487044		Diabetic Macular Edema	Retina Institute of Hawaii
Drug: Macugen (Pegaptanib Sodium)	NCT01487070	Phase I, completed	PDR	Retina Institute of Hawaii

Table 1: Aptamers in clinical trials (www.ClinicalTrials.gov).

Nevertheless, in SELEX technology, aptamers, which act against complex targets, are selected in 8-10 repetitions of the cycle, while cell-SELEX requires more than 14 repetitions. BRASIL, on the other hand, requires a single centrifugation step. Despite of this, cell SELEX has one clear advantage over SELEX. The process can produce multiple ligands that have high affinity to target-positive cells, but not the control cells. This is the end product of counter-selection, which can be further used to discriminate two closely related cell populations as observed in malignant glioma cells, T-cell limphobastic leukemia against B-cell lymphoma, as well as small-cell and large-cell cancer [13,56,57]. Cell-SELEX technology makes it feasible to discriminate amongst closely related cell types or even within the same cell under different conditions [42].

When comparing RNA aptamers against DNA aptamers, some differences can be observed. The selection procedure for DNA aptamers is simpler and cheaper. Furthermore, reverse transcription is essential. DNA aptamers are generally more stable than RNA aptamers. On the other hand, RNA aptamers possess a higher level of structural diversity due to the frequent occurrence of modified nucleotides within their structure, their base paring properties and tendency to form three-dimensional structures [58].

Aptamers in Cancer Cell Diagnostics and Treatment

Cancer represents a group of diseases with a genetic compound, which can affect any part of the body. It is a leading cause of death worldwide, affecting around 13% of the population. Cancer is a major public health problem and an economic burden. The goal is to detect and treat the disease in its early stage, in order to achieve a better outcome. However, the main problem is the lack of sensitive and specific methods for discovering cancer in its early stages, due to the small amount of circulating cancer cells and low expression of biomarkers or specifically molecular markers. On the other hand, chemotherapy, as a main or supplementary therapy, is toxic for healthy cells. However, this is where aptamers can be applied, due to their ability to detect cell-specific markers within complex systems that are devoid of specified target molecules. Moreover, aptamers, which are highly specific for cancer cells, can be conjugated with therapeutic agents or small interfering RNA (siRNA). Aptamers can also be used directly, as medicinal substances, because of their toxic effect on certain target cells. They can be applied as regulators of the intrinsic intracellular pathway, due to their inhibitory effect on oncogens, and thus inhibit tumor growth and invasion.

As previously mentioned, aptamers represent one alternative

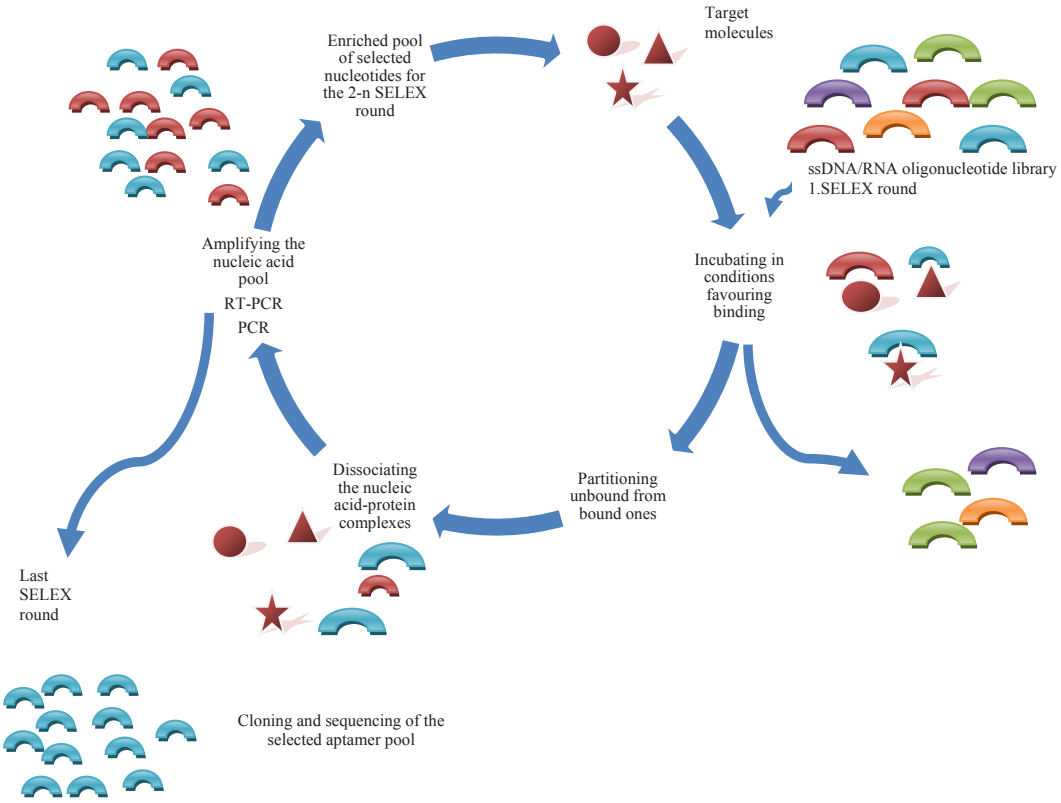

Figure 1: SELEX technology
The initial step of the SELEX procedure is the synthesis of a single-stranded nucleic acid (RNA/DNA) library of large sequence complexity (typical length is 20 to 100 nucleotides flanked by two constant primer regions with primer sites for PCR amplification) followed by a selection of oligonucleotides, which are capable of binding with high affinity and specificity to target molecules. The next steps of the SELEX procedures are: (a) incubating the aptamer library with a target molecule such as a recombinant protein in conditions favouring binding, (b) partitioning unbound nucleic acids from specifically bound ones to the target molecule, (c) dissociating the nucleic acid-protein complexes and (d) amplifying the nucleic acid pool enriched with sequences that allow binding to selected target molecules. The new generated nucleic acid pool serves as a starting library for a new SELEX cycle composed of identical steps as the first round. The number of SELEX repetition depends on the library type used and on specific enrichment achieved per a selection cycle. After the last round of aptamer selection, the PCR products are cloned and sequenced.

on how to capture and visualize cancer cells. Usually advanced biotechnology, nanotechnology or analytical methods are used in addition. So far, five promising approaches for cell detection seem to be: endogenous nucleic acid analysis [59]; flow cytometry analisis [60]; nanoparticle-based biosensing [61]; microfluid cell separation [62] and histological examination [63,64]. Also, non-invasive in vivo imaging techniques have been developed, in order to provide information on tumor progression and examination of cellular and biomolecular events.

Besides, aptamer-functionalized nanoparticles can be modified, so that they feature diagnostic and therapeutic properties, which were observed in a research by Bagalkot et al. that uses the anti-PSMA aptamer [65,66].

Therapeutic features of aptamers seem to be linked with their ability to recognize tumor markers or oncogens, the potential to couple drugs directly or to pack it into particles modified with aptamers. Regarding the literature, aptamers as medicinal substances can be general distinguished into: (a) aptamers as intracellular delivery vehicles – intramers [17,67-69]; (b) aptamer-directed drug conjugation [65,70]; (c) aptamer-liposome conjugates for target drug delivery [71,72]; (d) aptamer-micelle conjugates for target drug delivery [73]; (e) aptamer-protein conjugates [74]; (f) aptamer radionuclide conjugates [75] and (g) aptamer-nanostructure conjugates [76].

Aptamers Used in Alimentary Neoplasm

By reviewing recent research, we found some applications for aptamers in HCC (hepatocellular carcinoma), pancreatic adenocarcinoma and colorectal cancer. RNA and DNA aptamers were developed to target different cell surface proteins associated with tumor growth and biomarkers for certain types of cancer.

In hepatic neoplasm, osteopontin and the alfa-feto protein were observed as specific targets for aptamers. Osteopontin (OPN) is associated with HCC (hepatocellular carcinoma) growth and metastasis, and could therefore represent a unique therapeutic target. The study determined adhesion, migration/invasion and epithelial-mesenchymal transition (EMT) markers by using an aptamer or a mutant control aptamer (Mu-aptamer). The study found out that aptamer that targets OPN significantly decreases EMT and tumor growth of HCC [77]. An RNA aptamer that specifically binds alpha-fetoprotein was also developed by using the SELEX technology. The identified aptamer specifically and efficiently inhibited the AFP-mediated proliferation of hepatocarcinoma cells and down-regulated AFP-induced expression of oncogenes in the cells in a dose dependent manner [78]. Cell-SELEX was used to generate aptamers for liver cancer recognition. Two liver cell lines from Balb/cJ mice were used: a BNL 1ME A.7R.1 liver cancer cell line (MEAR) and a BNL CL.2 non-cancer cell line (BNL). In multiple rounds of selection and by using the BNL as

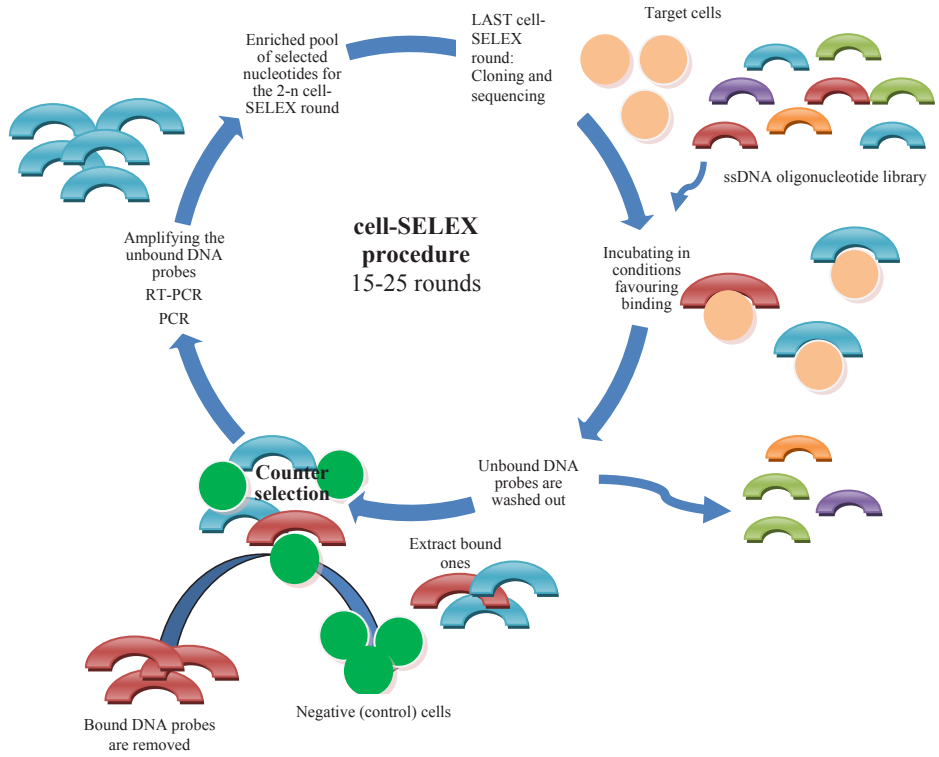

Figure 2: Cell-SELEX technology
The steps in cell-SELEX technology resemble SELEX procedure. The DNA library, which is composed of 30-40 bases flanked by primer sequences, is being incubated with target cells in specific conditions. The unbound DNA probes are washed out and the bound ones are collected with heat denaturing. The eluant is then amplified by PCR or used for counter selection. Counter selection represents a step in cell-SELEX, where apatamer probes are incubated with negative (control) cells. This time the unbound probes are collected and amplified. The final step is composed of dsDNA amplification treated with streptavidin beads resulting in antisens strands with biotin retaining on the beads and sens strand elution with sodium hydroxide solution. At the end, eluted ssDNA represents the enriched library for the next round of cell-SELEX. To prove that high affinity ligands are being enriched, flow cytometry or fluorescence microscopy is used. The pool is usually composed of high affinity ligands after 15-25 cycles of the cell-SELEX procedure. In the last step the pool is cloned and sequenced to determine the sequence of enriched DNA pools.

a control, we identified aptamers that specifically recognize the MEAR cancer cell line *in vivo and in vitro* [63]. In a research by Zhang et al., a mouse's MEAR-specific ssDNA aptamer TLS9a was used to fabricate a quantum dot-labelled aptamer bioprobe (QD-Apt). A bioprobe with optimal bioactivity was obtained by conjugating streptavidin-modified quantum dots (SA-QDs) with a biotin-derived aptamer in a ratio of 1:16. QD-Apt was able to specifically recognize MEAR cells, but not BNL cells and Hela cells, indicating that the probe was biocompatible and suitable for live cell imaging [79].

The P12FR2 RNA aptamer, which is specific for the pancreatic adenocarcinoma up-regulated factor (PAUF), was generated with the potential to be effective in treating human pancreatic cancer. The research group proved that the P12FR2 aptamer inhibits PAUF-induced migration of PANC-1 human pancreatic cancer cells and decreases tumor growth by about 60%, when injected intraperitoneally, without causing loss of weight in treated mice [80]. Along with this, the A1 high mobility group (HMGA1) proteins and their overexpression were investigated. These proteins are related to anchorage-independent growth and epithelial-mesenchymal transition and are therefore suggested as potential therapeutic targets. That is why bio-stable l-RNA oligonucleotides, the so-called Spiegelmers (NOX-A50 and NOX-f33), were generated, which specifically bind to HMGA1b. In a xenograft mouse study that used the PSN-1 pancreatic cancer cell line, subcutaneous administration of 2 mg/kg per day NOX-A50 formulated in polyplexes, showing an enhanced delivery of NOX-A50 to the tumor and a significant reduction of tumor volume [81]. Watanabe et al. proved in their research that aptamers can enhance chemotherapy efficiency in pancreatic cancer treatment. They used HMGA-targeted AT-rich phosphorothioate DNA (AT-sDNA) aptamers to suppress HMGA (high mobility group A), which is, when elevated, associated with resistance to the chemotherapy agent gemcitabine [82].

In colon cancer diagnostics and therapy, aptamers were used to identify specific biomarkers and to inhibit certain receptors involved in carcinogenesis. By using cell-SELEX technology and the DLD-1 and HCT 116 colorectal cancer cultured cell lines, a panel of target-specific aptamers was selected with high affinity and selectivity to colon cancer cells, but not to normal colon cells or other cancer cells [83]. In a study by Kwak et al., RNA aptamers, which are specific for peroxisome proliferator-activated receptor delta (PPAR-delta), were developed, which efficiently inhibited transcription and reduced mRNA levels of vascular endothelial cell growth factor-A and cyclooxygenase-2 resulting in a striking loss of tumorigenic potential, especially in HCT116 colon cancer cells with high-level expression of PPAR-delta-specific aptamers [84]. Moreover, the role of beta-catenin in transcription and splicing of oncogenic target genes was studied by using high-affinity RNA aptamers that connect to beta-catenin in vivo. The selected aptamer prevented alternative transcription and splicing induced by stabilized beta-catenin [85]. Furthermore, a DNA aptamer was created to target the CEA (human carcinoembryonic) antigen that is characteristic for gastrointestinal adenocarcinomas. The binding of

the DNA aptamer was monitored with flow cytometry in the MC-38 (CEA-) and MC (CEA+) mouse-colon adenocarcinoma lines. Results indicated high specific binding of the DNA aptamer in the CEA-positive MC-38 cell line, but not in the CEA-negative line. This way, aptamers could be used as intracellular delivery vehicles for aptamer-directed drug conjugates [86].

Conclusion

Cancer remains one of the leading causes of death worldwide. The main cancer types are lung (1 370 0000 deaths), stomach (736 000 deaths), liver (695 000 deaths), colorectal (608 000 deaths), breast (458 000 deaths) and cervical cancer (275,000 deaths) (76). The main challenge in cancer medicine is associated with early detection and specific treatment of cancer. The critical point is especially low expression of biomarkers in early stages and toxicity of chemotherapy. However, recent researches are investigating the therapeutic and diagnostic role of oligonucleotides. Oligonucleotides which are capable of binding to molecules other than nucleic acids are named aptamers. These are single stranded DNA or RNA oligonucleotides (12-30 bases), which represent a new way of targeting cancer cells and cancer biomarkers, as well enrich rare cancer cells with biotechnological, nanotechnological and analytical methods. Furthermore, they can be used as therapeutic agents. Aptamers are selected by using either the SELEX, the cell-SELEX or the brasil technology. Cell-SELEX seems to be especially appropriate for identifying cancer cells and generating aptamers that bind specifically to altered surface molecules on cancer cells without the exact knowledge on changed sequences. In the field of abdominal surgery, researches with aptamers were performed on pancreatic, colorectal and liver cancer. The selected aptamers could target certain biomolecules or inhibit receptors that are involved in carcinogenesis. This characteristic is of particular value in pancreatic cancer, due to late diagnosis and insufficient treatment opportunities resulting in poor prognosis. Further researches are required, in order to determine ,whether aptamers are capable of detecting biomarkers and inhibiting receptors or intrinsic pathways that are involved in carcinogenesis.

References

1. Gold L (1995) Oligonucleotides as research, diagnostic, and therapeutic agents. J Biol Chem 270: 13581-13584.
2. Ellington AD, Szostak JW (1990) In vitro selection of RNA molecules that bind specific ligands. Nature 346: 818-822.
3. Robertson DL, Joyce GF (1990) Selection in vitro of an RNA enzyme that specifically cleaves single-stranded DNA. Nature 344: 467-468.
4. Tuerk C, Gold L (1990) Systematic evolution of ligands by exponential enrichment: RNA ligands to bacteriophage T4 DNA polymerase. Science 249: 505-510.
5. Ciesiolka J, Yarus M (1996) Small RNA-divalent domains. RNA 2: 785-793.
6. Hofmann HP, Limmer S, Hornung V, Sprinzl M (1997) Ni2+-binding RNA motifs with an asymmetric purine-rich internal loop and a G-A base pair. RNA 3: 1289-1300.
7. Rajendran M, Ellington AD (2008) Selection of fluorescent aptamer beacons that light up in the presence of zinc. Anal Bioanal Chem 390: 1067-1075.
8. Geiger A, Burgstaller P, von der Eltz H, Roeder A, Famulok M (1996) RNA aptamers that bind L-arginine with sub-micromolar dissociation constants and high enantioselectivity. Nucleic Acids Res 24: 1029-1036.
9. Connell GJ, Illangesekare M, Yarus M (1993) Three small ribooligonucleotides with specific arginine sites. Biochemistry 32: 5497-5502.
10. Harada K, Frankel AD (1995) Identification of two novel arginine binding DNAs. EMBO J 14: 5798-5811.
11. Tuerk C, MacDougal S, Gold L (1992) RNA pseudoknots that inhibit human immunodeficiency virus type 1 reverse transcriptase. Proc Natl Acad Sci U S A 89: 6988-6992.
12. Pan W, Craven RC, Qiu Q, Wilson CB, Wills JW, et al. (1995) Isolation of virus-neutralizing RNAs from a large pool of random sequences. Proc Natl Acad Sci U S A 92: 11509-11513.
13. Chen HW, Medley CD, Sefah K, Shangguan D, Tang Z, et al. (2008) Molecular recognition of small-cell lung cancer cells using aptamers. ChemMedChem 3: 991-1001.
14. Sefah K, Tang ZW, Shangguan DH, Chen H, Lopez-Colon D, et al. (2009) Molecular recognition of acute myeloid leukemia using aptamers. Leukemia 23: 235-244.
15. Bruno JG, Kiel JL (1999) In vitro selection of DNA aptamers to anthrax spores with electrochemiluminescence detection. Biosens Bioelectron 14: 457-464.
16. Ireson CR, Kelland LR (2006) Discovery and development of anticancer aptamers. Mol Cancer Ther 5: 2957-2962.
17. Lupold SE, Hicke BJ, Lin Y, Coffey DS (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.
18. Daniels DA, Chen H, Hicke BJ, Swiderek KM, Gold L (2003) A tenascin-C aptamer identified by tumor cell SELEX: systematic evolution of ligands by exponential enrichment. Proc Natl Acad Sci U S A 100: 15416-15421.
19. Xiao Z, Shangguan D, Cao Z, Fang X, Tan W (2008) Cell-specific internalization study of an aptamer from whole cell selection. Chemistry 14: 1769-1775.
20. Huettner CS, Zhang P, Van Etten RA, Tenen DG (2000) Reversibility of acute B-cell leukaemia induced by BCR-ABL1. Nat Genet 24: 57-60.
21. Martinelli G, Soverini S, Rosti G, Baccarani M (2005) Dual tyrosine kinase inhibitors in chronic myeloid leukemia. Leukemia 19: 1872-1879.
22. Gaviani P, Silvani A, Corsini E, Erbetta A, Salmaggi A (2009) Neoplastic meningitis from breast carcinoma with complete response to liposomal cytarabine: case report. Neurol Sci 30: 251-254.
23. Koukourakis GV, Sotiropoulou-Lontou A (2011) Targeted therapy with bevacizumab (Avastin) for metastatic colorectal cancer. Clin Transl Oncol 13: 710-714.
24. Planchard D (2011) Bevacizumab in non-small-cell lung cancer: a review. Expert Rev Anticancer Ther 11: 1163-1179.
25. Albiges L, Salem M, Rini B, Escudier B (2011) Vascular endothelial growth factor-targeted therapies in advanced renal cell carcinoma. Hematol Oncol Clin North Am 25: 813-833.
26. Levy AR, Johnston KM, Sambrook J, Donato B, Penrod JR, et al. (2011) Indirect comparison of the efficacy of cetuximab and cisplatin in squamous cell carcinoma of the head and neck. Curr Med Res Opin 27: 2253-2259.
27. Garrett CR, Eng C (2011) Cetuximab in the treatment of patients with colorectal cancer. Expert Opin Biol Ther 11: 937-949.
28. Cacheux W, Le Tourneau C, Baranger B, Mignot L, Mariani P (2011) Targeted biotherapy in metastatic colorectal carcinoma: Current practice. J Visc Surg 148: 12-18.
29. Tsang RY, Finn RS (2012) Beyond trastuzumab: novel therapeutic strategies in HER2-positive metastatic breast cancer. Br J Cancer 106: 6-13.
30. Lee JH, Yigit MV, Mazumdar D, Lu Y (2010) Molecular diagnostic and drug delivery agents based on aptamer-nanomaterial conjugates. Adv Drug Deliv Rev 62: 592-605.
31. Tombelli S, Minunni M, Mascini M (2005) Analytical applications of aptamers. Biosens Bioelectron 20: 2424-2434.
32. Massarelli E, Onn A, Zinner R, Khuri FR, Kim ES, et al. (2002) New targets for the treatment of advanced non-small cell lung cancer. Cancer Chemother Biol Response Modif 20: 717-761.
33. Soontornworajit B, Wang Y (2011) Nucleic acid aptamers for clinical diagnosis: cell detection and molecular imaging. Anal Bioanal Chem 399: 1591-1599.
34. Keefe AD, Cload ST (2008) SELEX with modified nucleotides. Curr Opin Chem Biol 12: 448-456.
35. Hicke BJ, Marion C, Chang YF, Gould T, Lynott CK, et al. (2001) Tenascin-C aptamers are generated using tumor cells and purified protein. J Biol Chem 276: 48644-48654.

36. Chen CH, Chernis GA, Hoang VQ, Landgraf R (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 U S A 100: 9226-9231.

37. Ruckman J, Green LS, Beeson J, Waugh S, Gillette WL, et al. (1998) 2'-Fluoropyrimidine RNA-based aptamers to the 165-amino acid form of vascular endothelial growth factor (VEGF165). Inhibition of receptor binding and VEGF-induced vascular permeability through interactions requiring the exon 7-encoded domain. J Biol Chem 273: 20556-20567.

38. Huang J, Moore J, Soffer S, Kim E, Rowe D, et al. (2001) Highly specific antiangiogenic therapy is effective in suppressing growth of experimental Wilms tumors. J Pediatr Surg 36: 357-361.

39. Kim ES, Serur A, Huang J, Manley CA, McCrudden KW, et al. (2002) Potent VEGF blockade causes regression of coopted vessels in a model of neuroblastoma. Proc Natl Acad Sci U S A 99: 11399-11404.

40. Pu Y, Zhu Z, Liu H, Zhang J, Liu J, et al. (2010) Using aptamers to visualize and capture cancer cells. Anal Bioanal Chem 397: 3225-3233.

41. Drabovich AP, Berezovski M, Okhonin V, Krylov SN (2006) Selection of smart aptamers by methods of kinetic capillary electrophoresis. Anal Chem 78: 3171-3178.

42. Cerchia L, de Franciscis V (2010) Targeting cancer cells with nucleic acid aptamers. Trends Biotechnol 28: 517-525.

43. Chenna R, Sugawara H, Koike T, Lopez R, Gibson TJ, et al. (2003) Multiple sequence alignment with the Clustal series of programs. Nucleic Acids Res 31: 3497-3500.

44. Zuker M (2003) Mfold web server for nucleic acid folding and hybridization prediction. Nucleic Acids Res 31: 3406-3415.

45. Brown D, Brown J, Kang C, Gold L, Allen P (1997) Single-stranded RNA recognition by the bacteriophage T4 translational repressor, regA. J Biol Chem 272: 14969-14974.

46. Cho JS, Lee YJ, Shin KS, Jeong S, Park J, et al. (2004) In vitro selection of specific RNA aptamers for the NFAT DNA binding domain. Mol Cells 18: 17-23.

47. Tahiri-Alaoui A, Frigotto L, Manville N, Ibrahim J, Romby P, et al. (2002) High affinity nucleic acid aptamers for streptavidin incorporated into bi-specific capture ligands. Nucleic Acids Res 30: e45.

48. Förster C, Brauer AB, Brode S, Schmidt KS, Perbandt M, et al. (2006) Comparative crystallization and preliminary X-ray diffraction studies of locked nucleic acid and RNA stems of a tenascin C-binding aptamer. Acta Crystallogr Sect F Struct Biol Cryst Commun 62: 665-668.

49. Kelly JA, Feigon J, Yeates TO (1996) Reconciliation of the X-ray and NMR structures of the thrombin-binding aptamer d(GGTTGGTGTGGTTGG). J Mol Biol 256: 417-422.

50. Sayer N, Ibrahim J, Turner K, Tahiri-Alaoui A, 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.

51. Berezovski M, Musheev M, Drabovich A, Krylov SN (2006) Non-SELEX selection of aptamers. J Am Chem Soc 128: 1410-1411.

52. Mosing RK, Mendonsa SD, Bowser MT (2005) Capillary electrophoresis-SELEX selection of aptamers with affinity for HIV-1 reverse transcriptase. Anal Chem 77: 6107-6112.

53. Farokhzad OC, Khademhosseini A, Jon S, Hermmann A, Cheng J, et al. (2005) Microfluidic system for studying the interaction of nanoparticles and microparticles with cells. Anal Chem 77: 5453-5459.

54. Donovan MJ, Meng L, Chen T, Zhang Y, Sefah K, et al. (2011) Aptamer-drug conjugation for targeted tumor cell therapy. Methods Mol Biol 764: 141-152.

55. Giordano RJ, Cardó-Vila M, Lahdenranta J, Pasqualini R, Arap W (2001) Biopanning and rapid analysis of selective interactive ligands. Nat Med 7: 1249-1253.

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

57. Shangguan D, Li Y, Tang Z, Cao ZC, Chen HW, et al. (2006) Aptamers evolved from live cells as effective molecular probes for cancer study. Proc Natl Acad Sci U S A 103: 11838-11843.

58. Scott J (1989) Messenger RNA editing and modification. Curr Opin Cell Biol 1: 1141-1147.

59. Ring A, Smith IE, Dowsett M (2004) Circulating tumour cells in breast cancer. Lancet Oncol 5: 79-88.

60. Zhou J, Soontornworajit B, Wang Y (2010) A temperature-responsive antibody-like nanostructure. Biomacromolecules 11: 2087-2093.

61. Smith JE, Medley CD, Tang Z, Shangguan D, Lofton C, et al. (2007) Aptamer-conjugated nanoparticles for the collection and detection of multiple cancer cells. Anal Chem 79: 3075-3082.

62. Phillips JA, Xu Y, Xia Z, Fan ZH, Tan W (2009) Enrichment of cancer cells using aptamers immobilized on a microfluidic channel. Anal Chem 81: 1033-1039.

63. Shangguan D, Meng L, Cao ZC, Xiao Z, Fang X, et al. (2008) Identification of liver cancer-specific aptamers using whole live cells. Anal Chem 80: 721-728.

64. Li S, Xu H, Ding H, Huang Y, Cao X, et al. (2009) Identification of an aptamer targeting hnRNP A1 by tissue slide-based SELEX. J Pathol 218: 327-336.

65. Bagalkot V, Zhang L, Levy-Nissenbaum E, Jon S, Kantoff PW, et al. (2007) Quantum dot-aptamer conjugates for synchronous cancer imaging, therapy, and sensing of drug delivery based on bi-fluorescence resonance energy transfer. Nano Lett 7: 3065-3070.

66. Wang AZ, Bagalkot V, Vasilliou CC, Gu F, Alexis F, et al. (2008) Superparamagnetic iron oxide nanoparticle-aptamer bioconjugates for combined prostate cancer imaging and therapy. ChemMedChem 3: 1311-1315.

67. Chang SS, Reuter VE, Heston WD, Bander NH, Grauer LS, et al. (1999) Five different anti-prostate-specific membrane antigen (PSMA) antibodies confirm PSMA expression in tumor-associated neovasculature. Cancer Res 59: 3192-3198.

68. Ross JS, Sheehan CE, Fisher HA, Kaufman RP Jr, Kaur P, et al. (2003) Correlation of primary tumor prostate-specific membrane antigen expression with disease recurrence in prostate cancer. Clin Cancer Res 9: 6357-6362.

69. McNamara JO 2nd, Andrechek ER, Wang Y, Viles KD, Rempel RE, et al. (2006) Cell type-specific delivery of siRNAs with aptamer-siRNA chimeras. Nat Biotechnol 24: 1005-1015.

70. Huang YF, Shangguan D, Liu H, Phillips JA, Zhang X, et al. (2009) Molecular assembly of an aptamer-drug conjugate for targeted drug delivery to tumor cells. Chembiochem 10: 862-868.

71. Cao Z, Tong R, Mishra A, Xu W, Wong GC, et al. (2009) Reversible cell-specific drug delivery with aptamer-functionalized liposomes. Angew Chem Int Ed Engl 48: 6494-6498.

72. Kang H, O'Donoghue MB, Liu H, Tan W (2010) A liposome-based nanostructure for aptamer directed delivery. Chem Commun (Camb) 46: 249-251.

73. Wu Y, Sefah K, Liu H, Wang R, Tan W (2010) DNA aptamer-micelle as an efficient detection/delivery vehicle toward cancer cells. Proc Natl Acad Sci U S A 107: 5-10.

74. Chu TC, Marks JW 3rd, Lavery LA, Faulkner S, Rosenblum MG, et al. (2006) Aptamer:toxin conjugates that specifically target prostate tumor cells. Cancer Res 66: 5989-5992.

75. Hicke BJ, Stephens AW, Gould T, Chang YF, Lynott CK, et al. (2006) Tumor targeting by an aptamer. J Nucl Med 47: 668-678.

76. Orava EW, Cicmil N, Gariépy J (2010) Delivering cargoes into cancer cells using DNA aptamers targeting internalized surface portals. Biochim Biophys Acta 1798: 2190-2200.

77. Bhattacharya SD, Mi Z, Kim VM, Guo H, Talbot LJ, et al. (2012) Osteopontin regulates epithelial mesenchymal transition-associated growth of hepatocellular cancer in a mouse xenograft model. Ann Surg 255: 319-325.

78. Lee YJ, Lee SW (2012) Regression of hepatocarcinoma cells using RNA aptamer specific to alpha-fetoprotein. Biochem Biophys Res Commun 417: 521-527.

79. Zhang J, Jia X, Lv XJ, Deng YL, Xie HY (2010) Fluorescent quantum dot-labeled aptamer bioprobes specifically targeting mouse liver cancer cells. Talanta 81: 505-509.

80. Kim YH, Sung HJ, Kim S, Kim EO, Lee JW, et al. (2011) An RNA aptamer that specifically binds pancreatic adenocarcinoma up-regulated factor inhibits migration and growth of pancreatic cancer cells. Cancer Lett 313: 76-83.

81. Maasch C, Vater A, Buchner K, Purschke WG, Eulberg D, et al. (2010) Polyetheylenimine-polyplexes of Spiegelmer NOX-A50 directed against intracellular high mobility group protein A1 (HMGA1) reduce tumor growth in vivo. J Biol Chem 285: 40012-40018.

82. Watanabe M, Sheriff S, Lewis KB, Tinch SL, Cho J, et al. (2012) HMGA-targeted phosphorothioate DNA aptamers increase sensitivity to gemcitabine chemotherapy in human pancreatic cancer cell lines. Cancer Lett 315: 18-27.

83. Sefah K, Meng L, Lopez-Colon D, Jimenez E, Liu C, et al. (2010) DNA aptamers as molecular probes for colorectal cancer study. PLoS One 5: e14269.

84. Kwak H, Hwang I, Kim JH, Kim MY, Yang JS, et al. (2009) Modulation of transcription by the peroxisome proliferator-activated receptor delta--binding RNA aptamer in colon cancer cells. Mol Cancer Ther 8: 2664-2673.

85. Lee HK, Choi YS, Park YA, Jeong S (2006) Modulation of oncogenic transcription and alternative splicing by beta-catenin and an RNA aptamer in colon cancer cells. Cancer Res 66: 10560-10566.

86. Robbins PF, Kantor JA, Salgaller M, Hand PH, Fernsten PD, et al. (1991) Transduction and expression of the human carcinoembryonic antigen gene in a murine colon carcinoma cell line. Cancer Res 51: 3657-3662.