Appl Microbiol Biotechnol (2016) 100:6955–6969
DOI 10.1007/s00253-016-7686-2

MINI-REVIEW

Cell-targeting aptamers act as intracellular delivery vehicles

Subash C. B. Gopinath[1,2] · **Thangavel Lakshmipriya**[1] · **Yeng Chen**[3,4] · **M. K. Md Arshad**[1] · **Jesinda P. Kerishnan**[3] · **A. R. Ruslinda**[1] · **Yarub Al-Douri**[1,5] · **C. H. Voon**[1] · **Uda Hashim**[1]

Received: 23 April 2016 / Revised: 12 June 2016 / Accepted: 13 June 2016 / Published online: 28 June 2016

Abstract Aptamers are single-stranded nucleic acids or peptides identified from a randomized combinatorial library through specific interaction with the target of interest. Targets can be of any size, from small molecules to whole cells, attesting to the versatility of aptamers for binding a wide range of targets. Aptamers show drug properties that are analogous to antibodies, with high specificity and affinity to their target molecules. Aptamers can penetrate disease-causing microbial and mammalian cells. Generated aptamers that target surface biomarkers act as cell-targeting agents and intracellular delivery vehicles. Within this context, the "cell-internalizing aptamers" are widely investigated via the process of cell uptake with selective binding during in vivo systematic evolution of ligands by exponential enrichment (SELEX) or by cell-internalization SELEX, which targets cell surface antigens to be receptors. These internalizing aptamers are highly preferable for the localization and functional analyses of multiple targets. In this overview, we discuss the ways by which internalizing aptamers are generated and their successful applications. Furthermore, theranostic approaches featuring cell-internalized aptamers are discussed with the purpose of analyzing and diagnosing disease-causing pathogens.

Keywords Aptamer · Internalization · SELEX · Receptor · Surface antigen

✉ Subash C. B. Gopinath
subash@unimap.edu.my

[1] Institute of Nano Electronic Engineering, Universiti Malaysia Perlis, 01000 Kangar, Perlis, Malaysia

[2] School of Bioprocess Engineering, Universiti Malaysia Perlis, 02600 Arau, Perlis, Malaysia

[3] Department of Oral Biology and Biomedical Sciences, Faculty of Dentistry, University of Malaya, 50603 Kuala Lumpur, Malaysia

[4] Oral Cancer Research and Coordinating Center, Faculty of Dentistry, University of Malaya, 50603 Kuala Lumpur, Malaysia

[5] Physics Department, Faculty of Science, University of Sidi-Bel-Abbes, Sidi-Bel-Abbes 22000, Algeria

Introduction

Aptamers are comprised of nucleic acids or peptides that have been proven to mimic functions of antibodies. Nucleic acid aptamers are high-affinity molecules retrieved from the assembled pool of oligonucleotide base combinations. This selection process, called systematic evolution of ligands by exponential enrichment or "SELEX," was formulated in 1990 (Tuerk and Gold 1990; Gopinath 2007a; Gopinath 2011). Similar to nucleic acid aptamers, peptide aptamers can be generated on protein scaffolds, most commonly using a yeast two-hybrid system (Gopinath 2011). The target can range from a small molecule to a whole cell (cell-SELEX). An excellent advantage of aptamer generation is that no prior knowledge is required about the targets. Aptamers are currently widely believed to be good substitutes for antibodies due to their advantages of stability, extended lifespan, and uniform activity irrespective of batch-to-batch variations. In the past, aptamers were attractive alternatives to antibodies because of the production of higher numbers of antipathogenic aptamers with wide applications in developing novel platforms for diagnosis (Gopinath et al. 2006a). In several instances, aptamers have shown to function better than antibodies, and these two molecules complement each other (Gopinath et al. 2006a; Kim et al. 2010a).

Using the basic SELEX method developed by three groups in 1990, different separation methods incorporated into SELEX strategies (Gopinath 2007b) have yielded aptamers in several fields. The basic SELEX involves the use of a library of approximately 10^{14} molecules, which are allowed to bind the target of interest under appropriate buffering conditions in which they retain their activities. Upon complex formation, the bound oligonucleotides are partitioned from the unbound oligonucleotides, and the bound molecules are enzymatically amplified for the iterative rounds of selection. Through the progression of selection cycles, the ratio between target and separated molecules is manipulated to increase the stringency in selection, along with the right competitor. The successful ligand can be selected by repeated processes of partition and amplification after it has complexed with the target. The progression of selection cycles with the enriched molecules can be monitored by convenient assay(s); usually, radio isotope-based labeling is recommended. After revealing the binding, the successfully bound molecules in the last selection cycle will be cloned for sequencing. The efficiencies of the selected nucleic acid aptamers (DNA and RNA) are varied due to the pattern of stem-loop structures, and RNA allows more chances of binding to the target than does DNA. DNA molecules, however, are more stable than RNA molecules, so RNA needs to undergo chemical modification to enhance its half-life. In receptor binding studies, RNA aptamers succeed more often than do DNA aptamers, due to the availability of higher structural variants.

Cell-targeted aptamer generation—SELEX methods

Aptamers are wonderful candidates because they are capable of attacking complex targets, and they are versatile molecules for intracellular delivery that can be expressed constantly and stably in target cells. Aptamers also play vital roles in preventing cell–cell adhesion, cell–pathogen adhesion, and the entry of pathogens into the cell. In the field of oncology, aptamers are valued for their roles in both functional and localization aspects.

With the basic developments in SELEX, different strategies have been demonstrated for generating cell-internalizing aptamers, such as whole-cell SELEX (also termed cell-internalization SELEX or cell-SELEX) and in vivo SELEX (Mi et al. 2010; Thiel et al. 2012; Zhou et al. 2012; Cheng et al. 2013; Gourronc et al. 2013; Camorani et al. 2014; Yan 2014). These SELEX processes target membrane/surface-bound antigens, leading to the production of internalizing aptamers. Distinct surface "aptatopes" are involved in the SELEX process, and the selected aptamer will be internalized upon binding to the surface targets on the cell. It should be noted that with cell-internalization SELEX, there is a possibility of generating aptamers against multiple targets on the single cell (Thiel et al., 2012).

Cell-SELEX

Cell-SELEX is a straightforward way to generate internalizing aptamers because it involves direct interaction with whole cells and the selection of aptamer exclusively depends on the surface antigens. These surface antigens vary from organism to organism and genus to genus, but within the related species, they are limited. Often, there are only minute differences among species because of their similar origin, and in some cases, mutations make changes to surface antigens based on variations in the environment (Gopinath et al. 2006a). Cell-SELEX can be carried out either with collected whole cells or by solid-phase methodology. The steps involved in cell-SELEX include (1) complex formation of the whole-cell and nucleic acid (DNA/RNA) library; (2) separation of bound molecules from molecules not to the cells; (3) amplification of selected molecules for the next round of SELEX; and (4) cloning and sequencing of selected molecules after the final round of SELEX (Fig. 1; Gopinath et al. 2006a). Another important step usually involved in cell-SELEX is performing counter-SELEX with negative cells (non-pathogenic cells in the case of selection against pathogenic cells). Cell-SELEX is referred to as "blind cell-SELEX" when performed against an unknown target, especially in the case of selection with pathogenic cells. Usually, cell-SELEX is difficult to perform compared to routine-SELEX against purified protein, as cell-SELEX may require additional selection cycles. Furthermore, due to the possibility of multiple targets on the cell surface, additional characterization is necessary to find an appropriate target. Selection should be carried out against live cells, as dead cells have higher chances of losing membrane integrity (Shum et al. 2013). Cell-SELEX currently plays a major role in the field of oncology, where its major advantage is that there is no need to purify the surface antigen because aptamers can be generated via native selection. This is a clear advantage over recombinant proteins, which may lose native conformation. Even native purified proteins may sometimes not fold properly. A disadvantage of cell-SELEX, however, is that it is difficult to select the aptamer against the embedded surface antigen to the membrane. A specific aptamer targeting the ectodomain of a human platelet-derived growth factor receptor has been generated to inhibit receptor signaling, thereby inhibiting glioblastoma cancer growth (Camorani et al. 2014). Thiel et al. (2012) have introduced bioinformatics-based cell-internalization SELEX, using vascular smooth muscle cell as the model.

Using cell-SELEX strategy, several aptamers have been generated to target acute lymphocytic leukemia, T-cell leukemia (Taghdisi et al. 2010), liver cancer (Shangguan et al. 2008), acute myeloid leukemia (Sefah et al. 2009), lung cancer (Chen et al. 2008; Zhao et al. 2009), ovarian cancer (Van Simaeys et al. 2010), B cell lymphoma (Mallikaratchy et al. 2007),

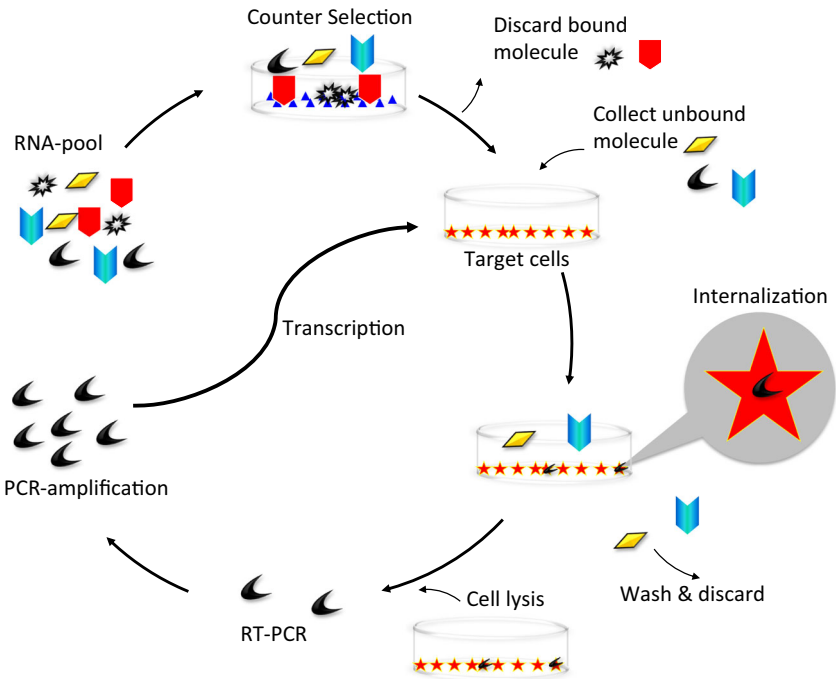

Fig. 1 Illustration for steps involved in the cell-specific SELEX process. The whole cell would be the target molecule. Involves the following three steps: complex formation, separation, and amplification. These steps are usually repeated for 14 cycles

colorectal cancer (Sefah et al. 2010), and breast cancer (Zhang et al. 2012). One DNA aptamer, Sgc8, has been generated to target leukemia cells. Leukemia begins in the bone marrow when abnormal white blood cells are produced in higher numbers. The Sgc8 aptamer has a high potential for binding, with a dissociation constant of 800 pM. This aptamer also has a higher specificity because it cannot bind to normal CD3+ T (Xiao et al. 2008). Homann and Göringer (1999) used cell-SELEX to select aptamers that targeted African trypanosomes, aiding in the development of drugs to fight African trypanosomiasis.

Tissue-specific SELEX

Although internalizing aptamers can be generated by whole cell-SELEX, as stated above, they can also be generated via tissue penetration using the SELEX strategies demonstrated by Mi et al. (2010) and Cheng et al. (2013). Mi et al. (2010) generated an aptamer to target tumors based on in vivo SELEX. Their study used a nuclease-resistant (2′ fluoropyrimidine-modified) RNA library to select an aptamer to target hepatic colon cancer metastases. They intravenously injected the RNA library into a mouse bearing a previously implanted hepatic tumor, and then the RNA molecules bound to the tumor tissue were extracted and enriched for the next round of SELEX, followed by 14 repeated rounds (Fig. 2). The authors were able to reveal the progression in SELEX based on an aptamer's specificity to the extracted tumor proteins. The selected aptamer had an affinity of threefold higher than the initial library, and its binding was proven by both in vitro and in vivo assays. Furthermore, to find the right target, affinity purification was performed using a selected aptamer modified with biotin. The selected aptamer was found to specifically bind to p68, an RNA helicase involved in upregulating colorectal cancer.

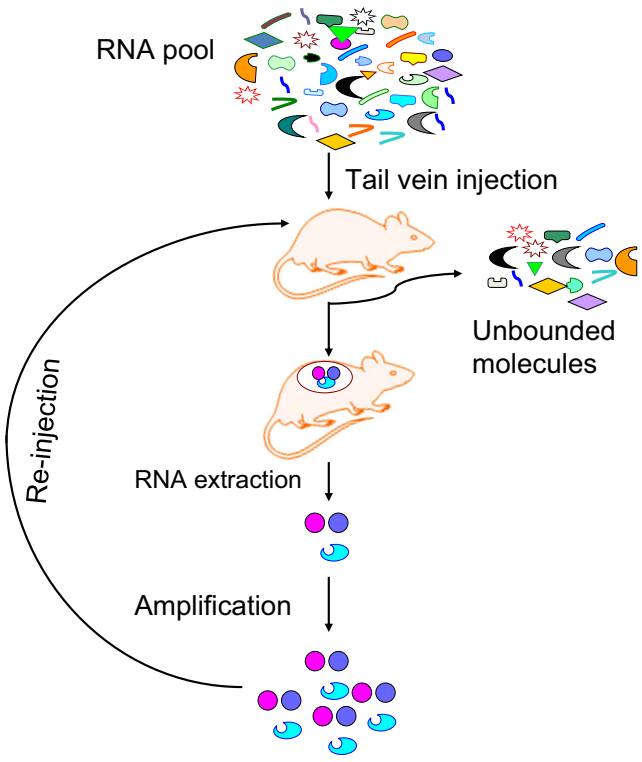

Fig. 2 Illustration for steps involved in the in vivo-SELEX process. Oligonucleotide library is injected into the tail of the mouse. The targeted tissue will be dissected for isolation of bound molecules. Three mandatory steps are complex formation, partition, and amplification

Similarly, Cheng et al. (2013) used a 2′fluoropyrimidine-modified RNA library and made injections into the tail vein of a mouse, after which molecules bound to the brain tissue were extracted and amplified to proceed with the next round of SELEX. Overall, 90 % of the sequences had three motifs that were found after 22 rounds of SELEX. The authors evaluated the aptamer enrichment from kidney, liver, and brain and found enrichment only in the brain tissue. They determined that molecules had an affinity to brain capillary endothelia and entered the parenchyma. The authors evaluated the internalization ability of the selected aptamer and found that it possessed a 25 % higher level of binding to endothelial cells when compared to scrambled sequences. Tissue-specific aptamer generation based on in vivo SELEX is more specific because the experiments are performed under real conditions. This method can be performed only with a stable aptamer library, however, because unmodified molecules are susceptible and can undergo degradation with nucleases present in the animal's system.

Fluorescent-SELEX

Modifications to the above SELEX methods can be incorporated into the exact position of aptamer decided by the user in order to obtain stable aptamers, or they can be introduced during the selection. Similarly, for localization purposes, an aptamer library can be modified with fluorescent molecules. This facilitates tracing the bound molecules via image analyses, which is suitable for theranostics. This kind of SELEX is considered to be an alternate strategy for post-SELEX modifications (Jhaveri et al. 2000; Rajendran and Ellington 2003). Jhaveri et al. (2000) performed fluorescent-SELEX by incorporating F-12-UTP during the transcription of DNA molecules, thereby generating an aptamer that targeted ATP. After 11 iterative rounds of SELEX, the researchers found higher affinity molecules, with indications of fluorescence increments in ATP. The selected aptamer has been found to have two uridine residues, found at the 52nd and 61st positions. Comparative analyses between U52 and U61 indicated that the U61 residue displayed ATP-dependent changes to the level of about 56 % fluorescence.

As an alternative to the above procedure, Rajendran and Ellington (2003) developed a SELEX strategy using a DNA library, wherein a fluorescent reporter was introduced to a 5′-primer, and in every selection round, the fluorescent ssDNA pool was annealed to capture oligonucleotides possessing biotin molecules and Dabcyl. Obtained molecules possessing Dabcyl were in the proximity of the fluorescein, and these duplexes were captured with a streptavidin agarose column. After thorough washing, the bound products were extracted and amplified for another round of selection (Fig. 3). After performing nine SELEX rounds, the selected aptamers had a 17-fold enhancement in fluorescence intensity with the target molecules. Furthermore, ligand-dependent fluorescence was obtained with the target, and the fluorescent mechanism mimics the strategy reported by Nutiu and Li (2003).

Post-SELEX modifications to enhance half-life of internalizing aptamers

Nucleic acids are usually degraded by nucleases and cleared rapidly from circulating plasma, resulting in a short in vivo half-life. As stability is mandatory under cellular milieu, modifications of aptamers may increase half-life. Common strategies for post-SELEX modifications include 2′ fluoro, amino, methoxy, and end protection, although other strategies have been followed to enhance the half-life of aptamers during or after SELEX processing (Eaton et al. 1997; Willis et al. 1998; Rusconi et al. 2002; Healy et al. 2004; Peng and Damha 2007; Mi et al. 2010; Gopinath and Kumar 2013). Eaton et al. (1997) have shown post-SELEX optimization of aptamers, and Healy et al. (2004) have indicated that chemical conjugation, especially with polyethylene glycol, is effective for circulation of aptamers. Similarly, stable aptamers generated to target a vascular endothelial growth factor were found to possess a resistance to plasma containing nuclease (Willis et al. 1998). The usage of a 2′fluoropyrimidine-modified RNA library for the generation of an antifactor IXa aptamer (in vitro SELEX) and antihepatic colon cancer aptamer (in vivo SELEX) has been demonstrated successfully (Rusconi et al. 2002; Mi et al. 2010). In the case of the antifactor IXa aptamer, additional end modifications on the aptamer were incorporated after the SELEX process to further improve the half-life. As an another approach, Gopinath et al. (2012) demonstrated 2′ fluoropyrimidine modification with generated RNA aptamer that targeted the gD protein of herpes simplex virus-1, in which they stabilized the aptamer after the SELEX process (post-SELEX modification). Furthermore, in their study, a similar modification was found to be suitable for the minimized aptamer (minimized based on mapping analysis) (Gopinath et al. 2012). Other modifications, such as 3′ capping, locked nucleic acid, and Spiegelmers, have also been demonstrated to stabilize aptamers (Esposito et al. 2011). Additionally, 2′-deoxy-2′-fluoroarabinonucleotide residues on G-quadruplexes derived from DNA aptamers bind to thrombin, phosphorothioate aptamers bind HIV, and DNA telomeric sequences have been shown to be stable (Peng and Damha 2007).

The important issue with modifications is that they should not interfere with the secondary/tertiary structures of molecules. Occasionally, modifications of aptamers will cause the aptamers to lose binding affinity or completely lose their ability to make conformational changes upon complex formation with the appropriate target. In contrast, longer aptamers need more efficient stabilization than shorter aptamers, making it necessary for full-length aptamers to have protection against

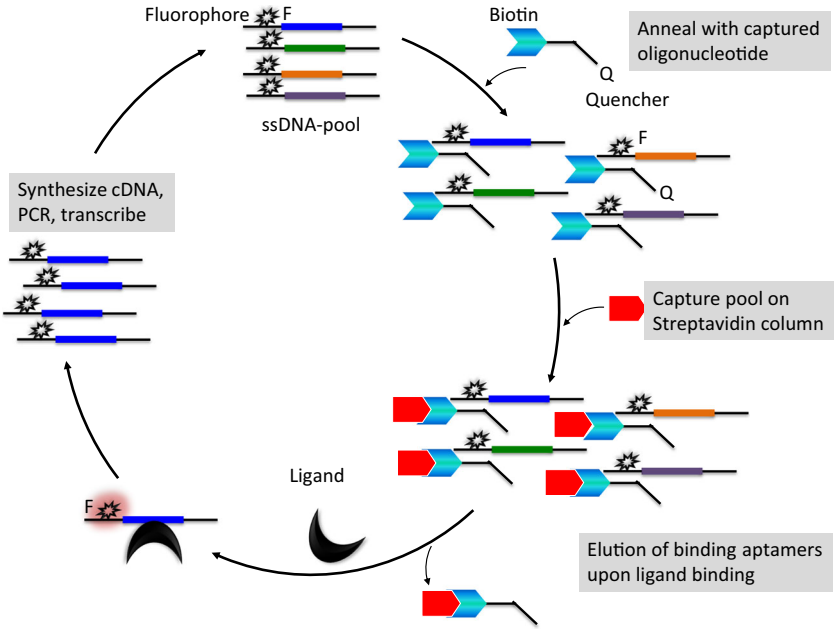

Fig. 3 Illustration for steps involved in fluorescent-SELEX process. The three mandatory steps are complex formation, partition, and amplification. Fluorophore is incorporated in the pool molecules. Separations of bound molecules have been carried out via the affinity process of streptavidin and biotin

nucleases. Modifications to aptamers are also limited by the stability of the nucleic acid polymerases used to incorporate the modified nucleoside triphosphates. It has been shown that incorporating the triphosphates 4′-thiouridine and 4′-thiocytidine into a T7 polymerase reaction yields stable RNA aptamers (Kato et al. 2005). All of these post-SELEX modifications create easier ways for aptamers to be used in theranostic applications with the targeted biomarkers.

Potential biomarkers for cell-SELEX

The cell membrane is the barrier for the import and export of molecules/oligonucleotides to and from the cell. Surface/cell membrane proteins (both intrinsic and extrinsic) from bacteria, viruses, and mammalian cells play an important role in pathogenesis and may be potential targets for the next generation of aptamers. Several proteins from bacterial cell membranes have been identified, including S-layer proteins (bacilli and clostridia), protein A (*Staphylococcus aureus*), M proteins (*Streptococcus pyogenes*), Pneumococcal surface protein A (*Streptococcus pneumoniae*), and internalin B (*Listeria monocytogenes*) (Navarre and Schneewind 1999). Similarly, potential viral capsid protein targets for cell-SELEX are hemagglutinin, neuraminidase, gD protein, and surface and transmembrane proteins (Gopinath 2007b).

The antigen CD30, also known as TNFRSF8, is a transmembrane receptor protein from the tumor necrosis factor (TNF) family that is expressed in activated B and T cells and overexpressed in cancer cells. It is therefore an important biomarker in the diagnosis of lymphomas and other tumors. Even though anti-CD30 antibodies are commonly used for clinical purposes to detect CD30 expression, an anti-CD30 aptamer would be an additional molecule with which to further explore CD30 antigens. Anti-CD30 RNA aptamers were generated and identified by the extracellular domain of the receptor activator of nuclear factor-κB. Generated aptamers have shown strong and specific binding to CD30 proteins, with an affinity 1000 times that of other proteins from the TNF family (Mori et al. 2004). The CD33 antigen, a type 1 transmembrane glycoprotein with an apparent molecular weight of 67 kDa, is categorized under sialic acid-binding immunoglobulin-related lectins (superfamily) and is another potential target. This antigen is expressed on early multilineage hematopoietic progenitors, myelomonocytic precursors, mature myeloid cells, monocytes, macrophages, and dendritic cells (Orava et al. 2010). Other targets include the carcinoembryonic antigen, CA15-3 antigen, MUC1 peptides, and Tn antigens (Orava et al. 2010). Kim et al. (2014) generated an aptamer for epithelial cell adhesion molecules. A transmembrane protein could be a biomarker for stem cells.

Influenza and other viruses possess hemagglutinin and neuraminidase as their major membrane proteins. In the influenza virus, there are two major influenza viral types (A and B). The major surface antigens, hemagglutinin and neuraminidase, are involved in the interaction of the influenza virus with its host, through glycan molecules. Hemagglutinin and neuraminidase are also involved in differentiating influenza A into subtype levels. A number of subtypes of influenza have emerged that appear to change due to seasonal variations. Gopinath et al. (2006b) used whole influenza virus belonging to the H3N2 strain (A/Panama/2007/1999) for the generation of aptamers, and the desired aptamer was able to discriminate among other viruses belonging to influenza types

A and B. The same team was later involved in the generation of aptamers targeting different viral membrane proteins (Gopinath et al. 2012; Gopinath and Kumar 2013).

Nucleolin, a major nucleolar protein, participates in ribosome biosynthesis and maturation and has been reported to also be involved in various other cellular regulations, including cell proliferation and growth, cytokinesis, replication, embryogenesis, and nucleogenesis. It is also a transcriptional repressor, a switch region-targeting factor (Srivastava et al. 1999) and a shuttling vehicle for transferring molecules between the cell surface and the nucleus. Nucleolin has also been identified as a cellular receptor molecule for human respiratory syncytial virus (Tayyari et al. 2011). With this wide range of functions, the nucleolin molecule is a promising target for aptamers. Soundararajan et al. (2008) analyzed the aptamer AS1411, which can bind nucleolin, and found that it affects Bcl-2 mRNA regulation. Following these developments with AS1411, several studies have investigated the potential usage of AS1411 (Li et al. 2014a; Rosenberg et al. 2014; Reyes-Reyes et al. 2015). These studies include phase II clinical trials with renal carcinoma cells and delivery systems and indicate promising developments with the AS1411 aptamer.

Aptamers as cell surface targeting and intracellular delivery vehicles

Aptamers are capable of binding to receptor molecules, which could lead to aptamer-based delivery systems and the usage of chemical tags alongside currently available chemical strategies to improve cellular uptake. Both active and passive mechanisms are involved in cellular transport, an example of which is drug export in cancer cells during treatment. Different molecules with the above mechanisms, including monoclonal antibodies (Adams and Weiner 2005), aptamers (Levy-Nissenbaum et al. 2008), short peptides (Brown 2010), and other small molecules (Sudimack and Lee 2000), have been proposed for different purposes, such as localization and transportation (nanocarrier/drug carrier). Passive mechanisms are also involved with the topical application of drugs and their delivery to cells, and oligonucleotides/aptamers have also shown to be involved in the passive mechanism (Regnier et al. 1999). Currently, two antibody-conjugated drugs have been approved by the Food and Drug Administration (FDA) to treat cancers, and one of those is in clinical use. Gemtuzumab ozogamicin (Pfizer) was the first FDA-approved antibody-conjugated drug designed for acute myeloid leukemia patients, but it had significant side effects and was discontinued (Stasi 2008; Firer and Gellerman 2012). Perera et al. (2007) have shown that an antiepidermal growth factor receptor antibody has a novel internalization, intracellular trafficking, and biodistribution. Even though an antibody-based drug carrier has been reported (Adair et al. 2012), there will be great batch-to-batch variations. This is not the case with aptamers and aptamer conjugations, which do not show batch-to-batch variations.

Through the process of "induced fit" (Fig. 4), aptamers are able to differentiate between cognate and non-cognate ligands and dissociate slowly. Other advantages of aptamers in comparison with antibodies are low immunogenicity, small size, low cost, high affinity, and the ability to penetrate solid tumors. Aptamers that bind with high specificity and affinity to their target molecules have been shown to inhibit the functions of their bound targets under both in vivo and in vitro conditions. Moreover, the accumulation of the reporter molecules around the cells leads to an increase in signals in comparison with the surrounding media. Furthermore, engineered aptamers yield strong output signals that can be quantitated and ligand-induced aptamers have various applications, including detecting disease-causing protein markers in vivo. Oligonucleotide analogs bearing a P–C bond have been investigated as promising molecules for nucleic acid-based delivery. The appealing feature of C-phosphonate derivatives as aptamers is their lack of a negative charge facilitates cellular uptake by increasing the passive transport mechanism of cellular membranes.

Penetrating aptamers play a vital role in internalization processes and in selective capturing or killing and are capable of traveling with nanoparticles, drugs, and other nucleic acids as chimeras (Fig. 5a, b). Shum et al. (2013) used the term "smart bomb" to describe the role of aptamers in selectively killing targets. Based on internalization, an uptake selection was performed against prostate cancer that can differentiate it from non-prostate cancers. An aptamer was loaded with an encapsulated drug, docetaxel, which improved cytotoxicity once it was internalized (Xiao et al. 2012). Orava et al. (2010) compiled a number of aptamers involved in the internalization process, such as sgc8c, A10, 5TR1, 5TRG2, GalNAc3, Ag9, and TTA1, which acted as transports to target PTK7, PSMA, MUC1, Tn antigen, N-acetylgalactosamine, PSMA, and Tenascin C, respectively.

Aptamer-based chimeras

Several nanotechnological applications for forming chimeras with engineered aptamers have been demonstrated by different subtypes of RNA, such as small interfering RNA, small nuclear RNA, small nucleolar RNA, packaging RNA, and microRNA (Fig. 5b). These RNA molecules play functional roles by forming complexes characterized as ligand-induced RNAs, RNA-induced ligands, or coinduced conformational changes (Gopinath 2009). In this regard, several shortened aptamers have been proposed with different analytes (Gopinath et al. 2006a), as smaller aptamers perform better as chimeras because longer aptamers may undergo degradation. In recent years, there has been an increasing interest in

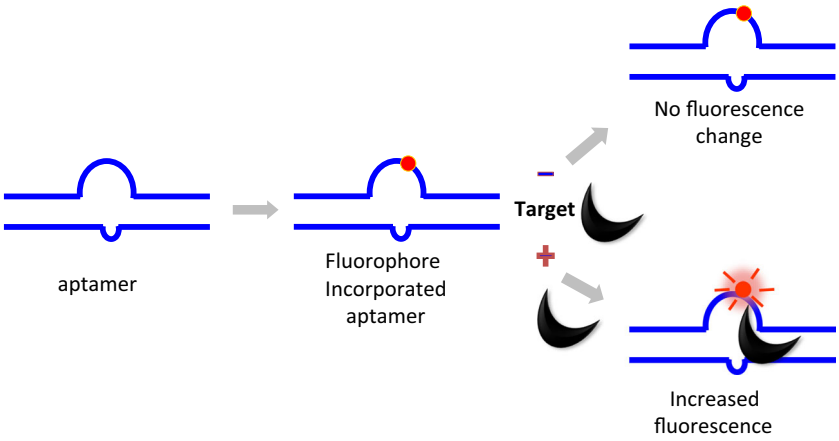

Fig. 4 Ligand-induced aptamer. Fluorophore-tagged aptamer fluoresces when target molecule is present

the applications of aptamer chimeras in the field of therapeutics (Chen et al. 2015; Rohde et al. 2015; Subramanian et al. 2015; Wang et al. 2015; Diao et al. 2016). Rohde et al. (2015) demonstrated a transferrin receptor and microRNA aptamer chimera (mir-126) that promoted sprouting of endothelial cells and suppressed recruitment of breast cancer cells. An aptamer-siRNA polymeric nanocomplex was designed for targeting epithelial cell adhesion molecules, which are overexpressed in solid tumors (Subramanian et al. 2015), and Wang et al. (2015) used the aptamer-siRNA chimera to silence survivin in cancer cells and enhance the effect of doxorubicin, even when used in small amounts. Very recently, using an antiprostate specific membrane antigen aptamer-siRNA chimera, Diao et al. (2016) specifically controlled the growth of a prostate tumor. In another interesting study, Chen et al. (2015) modified tissue-engineered blood vessels with an aptamer-siRNA chimera and achieved cell-specific delivery and capture. Furthermore, that study showed that PEGylation and 2'fluoro modification of the aptamer increased its stability and specificity. These kinds of stabilization are supporting aptamers when they carry nanoparticles or other molecules.

Aptamer-conjugated nanoparticles

Nanoparticles have been considered for a wide range of applications, both in soluble and insoluble forms. An advantage of soluble forms of nanoparticles is that they can encapsulate antibiotics/drugs and then release them when they reach the cellular milieu, making them highly applicable for drug delivery systems. Anthracycline, mitoxantrone, and etoposide are some of the drugs that can be encapsulated insoluble nanoparticles for treating cancers. Studies on aptamer-based enhanced drug delivery have been reported for prostate cancer (Farokhzad et al. 2004, 2006; Dhar et al. 2008) and lymphoblastic leukemia cells (Huang et al. 2008). Yu et al. (2011) generated an aptamer

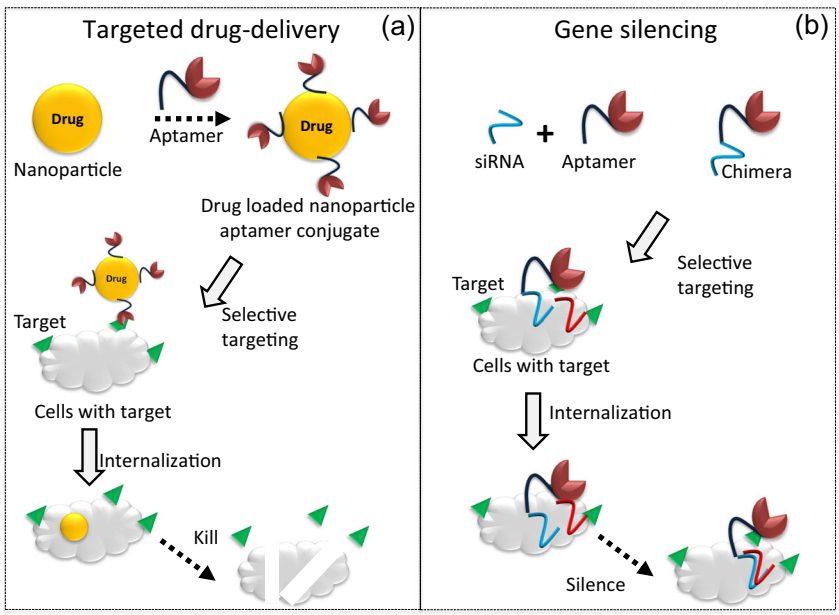

Fig. 5 Internalizing aptamer with carrier. **a** Targeted drug-delivery. Drug-loaded nanoparticle and aptamer conjugate are shown for selective killing. **b** Gene silencing. Aptamer-siRNA chimera is shown for silencing of targeted sequence

against the MUC1 protein from adenocarcinoma cells. The aptamer was conjugated with Paclitaxel-loaded poly (lactic-co-glycolic-acid) nanoparticles, approximately 225 nm in size, and improved effects against adenocarcinoma cells were demonstrated by these conjugations. One issue with aptamer-conjugated nanoparticle-mediated drug delivery is a need to evaluate non-specific toxicity. These particles should not show an adverse effect on any cells other than the target. The passive delivery of a nanocarrier has also been found to be effective for chemotherapy, but this strategy is not targeted to a specific location and will affect locations other than the one targeted.

In contrast, insoluble nanoparticles such as gold can be used to carry multiple molecules (Ali et al. 2011; Ali et al. 2012). The gold nanoparticle (GNP) is an ideal material that is widely used, owing to its unique characteristics, such as easy dispersal in water, compatibility with surface chemical functionalization, biological non-reactivity, and the ability to be tailored with uniform and varied nanosizes (Gopinath et al. 2013). Previously, prostate-specific membrane antigen (PSMA)-specific aptamer-GNP conjugation has been studied for cancer therapy (Kim et al. 2010b), with the aptamer being functionalized on GNP and loaded with doxorubicin. This composition was shown to have more than a 4-fold higher action. In another study, aptamer-functionalized GNPs were codelivered with two different anticancer drugs, achieving an improvement in drug efficacy (Shiao et al. 2014). Huang et al. (2008) demonstrated that ~80 aptamers could be linked covalently to the surface of Au–Ag nanorods using thiolated functional groups on the aptamers. Recent studies have attested to the therapeutic success of aptamer–nanoparticle conjugates with higher efficiencies (Azhdarzadeh et al. 2016; Hong et al. 2016; Leach et al. 2016; Liu et al. 2016). These interesting studies include antiepithelial cell adhesion molecule aptamer-mediated detection of cancer biomarkers at the level of femtomoles (Hong et al. 2016).

Internalizing aptamers in cell theranostics

"Theranostics" refers to the combined study of therapeutic and diagnostic aspects. On this front, aptamers have been suggested for different theranostic applications combining imaging and therapeutic aspects. The imaging aspect predominantly includes fluorescence-based organic dyes, inorganic quantum dots, and nuclear imaging agents, all of which can be tagged on to the drugs or aptamers to be used for therapeutic purposes, such as gene therapy, radiation, photodynamic therapy, hyperthermia and chemotherapy (Kelkar and Reineke 2011). One potential way to achieve greater benefits with wide theranostic applications of aptamers is to generate internalizing aptamers. The idea/logic behind this is that when producing aptamers against a receptor on the cell surface or transmembrane peptides or molecules that can enter the cells, receptors on the cell surface are usually involved in the cellular metabolic pathway. As a consequence, the receptor will enter into the cell (internalization) and reach the binding partner (intracellular components) to initiate the function. If the aptamer is created for a surface receptor/antigen, the receptor can behave like a transporter to carry the aptamer to its binding partner. If one wished to destroy or locate the binding partner for the purpose of therapeutics or diagnosis, this can be achieved by tagging the aptamer with drug-loaded particles or with the appropriate tags (Fig. 6a, b). As the receptor travels to the cell, it carries the aptamer along with the tags and reaches the desired target (Fig. 7). Aptamers can also specifically bind to internalized surface biomarkers (cellular portals) and act as delivery vehicles. In this context, the membranome plays a vital role. The membranome is a combination of the proteome and the lipidome, or an entire set of proteins on biological membranes, and is usually referred to in epigenetics (Chang et al. 2013a). The membranome has been shown to play important roles, as it can provide potential biomarkers and targets for aptamers generated by SELEX.

Currently, applications for aptamers also include in-depth participation in in vivo and in vitro diagnoses involved in the development of novel diagnostic platforms (Gopinath et al. 2008a; Blind and Blank 2015; Kadioglu et al. 2015). Furthermore, with the support of fluorescent labeling and molecular beacons, several aptasensors have been generated (Yamamoto-Fujita and Kumar 2005; Gopinath et al. 2008a). In the fields of medicine and therapeutics, aptamers for detecting a wide range of analytes are being developed at a fast pace. Several aptamers have been reported to have affinities in the range of 1 pM to 1 μM (Yamamoto-Fujita and Kumar 2005; Kadioglu et al. 2015), and Gopinath and Kumar (2013) have generated a stable anti-influenza aptamer with a dissociation constant of 67 fM. Recent achievements in aptamer-mediated therapeutics have been considered as treatments. Several higher-affinity aptamer candidates have entered various clinical trials, which could take aptamers to the next level.

Prospects: clinical phase trials with internalizing aptamers

All of the achievements with aptamers mentioned above have created fast tracks by which to enter the different phases of preclinical and clinical trials, and several aptamers are in the pipeline at different stages for future drug developments. With the emergence of many aptamer candidates, different aptamers have entered clinical assessments. This is due to the fact that no animals are involved in the generation of aptamers and the availability of the abovementioned modifications that increase the half-lives of aptamers under physiological conditions. A list of aptamers currently undergoing various phases of trials for the development of future drugs can be found in Table 1

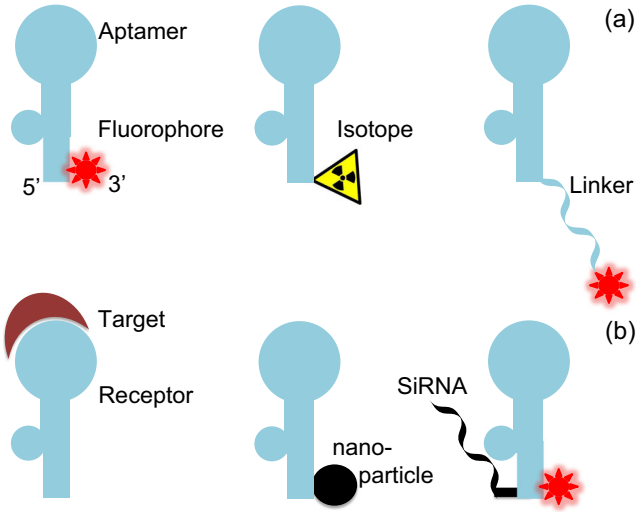

Fig. 6 Modifications on aptamer. **a** Types of tagging. Fluorophore, radio isotope tagging, and tagging with linker are shown. **b** Carriers with aptamer. Complexes of aptamer-target, aptamer-nanoparticle, and aptamer-siRNA chimera are shown

(Que-Gewirth and Sullenger 2007; Esposito et al. 2011; Ashrafuzzaman 2014). An aptamer-based drug called "Macugen" (Pegaptanib, by Pfizer and Eyetech) has been accepted by the FDA to treat age-related macular degeneration for the elderly, although clinical studies were initially begun with a different aptamer (ARC 183) that was generated to target thrombin and was tested on canine cardiopulmonary bypass models. Following the lead of Macugen, several aptamers are currently being tested for future medicine (Fig. 8).

Other aptamer-based drugs being developed for age-related macular degeneration, besides Macugen, include E10030 (phase II), ARC1905 (phase I), and EYE 001 (phase II). The aptamer drug ARC1779 is under phase II trial for the treatment of thrombotic thrombocytopenic purpura, while NU172, another antithrombin aptamer drug, and REG-1, an aptamer for the human blood clotting protein factor IXa, are both under phase II testing. Other aptamer-based drugs include NOX-A12 for lymphoma patients (phase I), NOX-E36 for type-2 diabetes and diabetic nephropathy (phase I), AS1411 for cancer (phase II), Avrina for eczema (phase III), LY218308 for cancer (phase III), E2F for inhibiting the proliferation of mesangial cells (phase III), NOX-H94 for anemia (phase I), and BAX499/ARC19499 for hemophilia (phase I).

In addition, there are several potential aptamers that could be drugs but have not yet been trialed for clinical assessments (Esposito et al. 2011). Research on the production and efficient usage of internalizing aptamers or short peptides is currently ongoing (Lindgren et al. 2000; Li et al. 2014b; Sánchez-Luque et al. 2014; Wu et al. 2014; Darmostuk et al. 2015). Through the use of sensing strategies with aptamers, cell tracking has been used to monitor the status and functions of cancer cells. All other aptamers generated are soon to undergo diagnostic tests (Gopinath et al. 2006a; Shangguan et al. 2006; Gopinath 2007b; Gopinath et al. 2008b) and are expected to be future drug candidates (Tables 2 & 3).

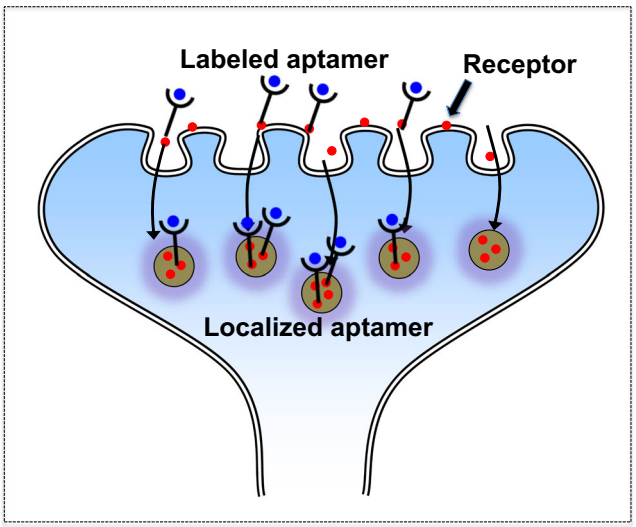

Fig. 7 Localization of aptamer in cell. Involvement of receptor in transport of the aptamer is shown

Potential molecules on which to focus for aptamer generation—future directions

Even though different aptamers have been shown to function in cell surface targeting and as intracellular delivery vehicles, some important molecules have been ignored. These molecules include exosomes, vault particles, and bacteriocins. Among these targets, exosomes and vault particles possess a shuttling behavior of normal and cancer-causing cells, whereas bacteriocin is a protein that diffuses from certain bacterial cells and inhibits neighboring strains.

Exosomes

Exosomes are bioactive vesicles approximately 30–100-nm diameters in size and were first described by Rose Johnstone in the 1970s. They are released constitutively by cells through the fusion of multivesicular endosomes on the plasma membrane and subsequently release the vesicles into the extracellular regions (Schorey and Bhatnagar 2008; Tan et al. 2010). Exosomes contain different functional proteins, mRNAs, and microRNAs (Skog et al. 2008; Mathivanan et al. 2010) which provide a signaling mechanism during tumor progression. Exosomes have been identified in different body fluids, indicating that their role is to exchange information, irrespective of the distance between cells (Qazi et al. 2010).

Table 1 Aptamer candidates under different phases of clinical trials

Aptamer	Target	Treatment	Stage
Macugen	VEGF-165	Age-related macular degeneration	Approved phase III
E10030	PDGF	Age-related macular degeneration	Phase II
ARC1905	C5	Age-related macular degeneration	Phase I
ARC1779	vWF	Thrombotic thrombocytopenic purpura	Phase II
NU172	Thrombin	Acute coronary bypass surgery	Phase II
REG-1	Factor IXa	Acute coronary syndrome	Phase II
NOX-A12	SDF-1	Lymphoma (patients undergoing autologous stem cell transplantation)	Phase I
NOX-E36	CCL2	Type-2 diabetes and diabetic nephropathy	Phase IIa
AS1411	Nucleolin	Cancer	Phase II
Edifoligide (E2F decoy)	E2F/CABG	Surgery	No improvement over placebo in Phase III
Avrina (NF-kB decoy)	NF-kB	Eczema	Phase III
ARC183	Thrombin	Anticoagulation	Phase I completed, but not in development
EYE001	VEGFR	Age-related macular degeneration	Phase II/III
LY2181308	Survivin mRNA	Anticancer	Phase III
E2F decoy oligonucleotides	Mesangial cells	Inhibition of the proliferation of mesangial cells	Phase III
NOX-H94	Hepcidin	Anemia	Phase I
BAX499/ ARC19499	TFPI	Hemophilia	Phase I

They are also involved in communication between the tumor and the surrounding stromal tissue in tumor progression (King et al. 2012).

In a clinical scenario, exosome-mediated transportation could form a strong basis for the generation of a novel cancer therapy, via a system that recognizes and kills cancer cells. With coupled nanotechnology, engineered exosomes are novel avenues for cancer therapy (Tan et al. 2010). Aptamers are suitable substitutes to carry drugs or nanoparticles and travel with exosomes into cancer cells. Moreover, aptamers are amenable to being labeled with fluorescent tags such as FITC, rhodamine, and cyanine for the purpose of diagnosis and will keep their original functions despite modifications. With these advantages, the flexible properties of aptamers hybridized with exosomes could be used for diagnosis and therapy. Since exosomes can serve as transports to deliver genetic material or drugs, gene knock-down in cancer cells through exosomes is highly possible and could be accomplished by tagging the sequences on the exosome. The diagnosis of a target via exosome-mediated aptamers could be performed by tagging the fluorescent directly onto the aptamer/exosome, and further localization in the cancer cells could be visualized via imaging studies. The feasibility of mouse dendritic cell derived-exosomes has already been evaluated in phase clinical trials, which is an encouraging step toward the future development of exosomes in cancer therapy.

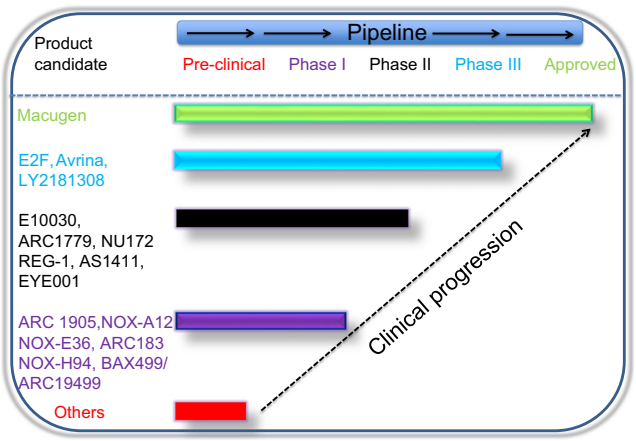

Fig. 8 Aptamers in clinical trials. Aptamers approved by the FDA, preclinical, and different phases of clinical trials are indicated as pipeline candidates

Table 2 Involvement of cell-SELEX in aptamer applications

Target	Aptamer	Aptamer size	Aptamer type	Binding strength	Applications	Reference
Leukemia	DNA	88	Stem-loop	Kd 800 pM	Specific leukemia-targeted delivery	Shangguan et al. (2006)
Cancer	DNA	26	G-quadrat	–	Cancer treatment	Tucker et al. (2012)
Ramos cells	DNA	–	Stem-loop	Kd-Nanomolar order	Specific binding	Tang et al. (2007)
MCF7	DNA	24	Stem-loop	–	Drug-delivery	Yu et al. (2011)
Prostate cancer	RNA	77	Stem-loop	Affinity 117 nM	Cancer therapy	Xiao et al. (2012)
African trypanosomes	RNA	79	Pseudo-knot	Kd 60 ± 17 nM	Drugs for African trypanosomiasis	Homann and Göringer (1999)
HepG2	DNA	78	Stem-loop	Kd-micromolar order	Stem cell biomarker	Kim et al. (2014)
Colorectal cancer	DNA	73	Stem-loop	27.4 nM	Diagnosis of colorectal cancer	Hung et al. (2015)

Vault particles

Similar to exosomes, vault particles could facilitate the travel of conjugate aptamers into cells. Vaults are the largest ribonucleoprotein complex, barrel-shaped particles with a higher molecular mass (13 MDa) in eukaryotic cells, including those of mammals, avians, and amphibians. Vault particles were isolated by Nancy Kedersha and Leonard Rome in the 1980s, and the split coordinates of these particles are available in the Protein Data Bank (entries 2zuo, 2zv4, and 2zv5). Vault particles appear to reside both in the cytoplasm and the nucleus and have been implicated in nucleocytoplasmic and intracellular transport. A previous study with multidrug resistance cell lines showed increased major vault protein levels, demonstrating that the vault non-coding RNAs and vault proteins are coordinately regulated and suggesting that the entire vault particle is upregulated in multidrug resistance (Gopinath et al. 2010). In a clinical scenario, it has been suggested that vaults may play a major role in intracellular detoxification processes and thus may function in the multidrug resistance of cancer cells (Kitazono et al. 1999; Scheffer et al. 2000; Azmi et al. 2013). The first drug-resistant phenotype was developed in Chinese hamster ovary cell lines and correlated with the drug resistance of several cell lines (Juliano and Ling 1976).

Table 3 Aptamer-based diagnoses of whole cell/surface proteins

Diagnostic method	Aptamer type	Target	Reference
Surface plasmon resonance (SPR)	RNA	Influenza A (H3N2)	Gopinath et al. (2006a)
SPR	RNA	Influenza A (H1N1)	Regnier et al. (1999)
Flow cytometry	DNA	T-cell lymphoblastic leukemia	Chang et al. (2013b)
ELISA and SPR	DNA	Glioblastoma derived cell line U251	Chang et al. (2013b)
Imaging (fluorescence confocal microscopy)	DNA	A cellular membrane protein nucleolin	Chang et al. (2013b)
Imaging	DNA	Liver cancer cell line-MEAR	Xiao et al. (2008)
Surface plasmon fluorescence spectroscopy (SPFS)	RNA	Influenza B (Tokio)	Lakshmipriya et al. (2013)
Waveguide mode sensor	RNA	Influenza B (Tokio)	Lakshmipriya et al. (2013)
Surface plasmon fluorescence spectroscopy (SPFS)	RNA	Influenza B (Jilin)	Lakshmipriya et al. (2013)
Waveguide mode sensor	RNA	Influenza B (Jilin)	Lakshmipriya et al. (2013)
Imaging	RNA	Hepatic tumor	Mi et al. (2010)
Imaging	RNA	Brain tissue	Cheng et al. (2013)
SPR	RNA	Herpes simplex virus	Gopinath et al. (2012)

Bacteriocins

Bacteriocins are diffusing molecules found in bacteria. They are protein-based inhibitors that inhibit closely related or similar strains and act as narrow spectrum antibiotics. Bacteriocins guide the development of antibiotics to kill particular bacteria, so industries are highly interested in investigating the use of bacteriocins for preventing infectious diseases. The bacteriocins of lactic acid-producing bacteria play a vital role in food preservation. Different categories of bacteriocins depend on the species and include colicins, microcins, and warnerins. Many bacteriocins come from Gram-positive bacteria and kill species from the same ecological niche (Jack et al. 1995). Bacteriocins can be an alternative to traditional antibiotics when resistant bacterial species arise (Cotter et al. 2013). The generation of aptamers that target bacteriocins could control or accelerate the action of bacteriocins.

Conclusion

As stated above, cell-internalizing aptamers can be generated predominantly with cell-SELEX and other in vivo SELEX strategies (Table 2). The applications of these cell-internalizing aptamers for diagnosis and drug-delivery purposes, through cell surface targeting and intracellular delivery, are enhanced when combined with nanotechnology. For diagnoses, aptamers can be chemically conjugated with fluorophores such as fluorescein, rhodamine, and cyanine. For drug delivery, a drug-loaded nanoparticle may be attached to the aptamer and can then be delivered to the site of action, as a so-called aptamer-guided nanocarrier. Designing aptamer-siRNA complexes or chimeras may lead to enhancements in the application of internalizing aptamers. Furthermore, two or more aptamers generated for a single target could be conjugated with an appropriate length of linker, and producing aptamers aimed at both a target and its receptor elevate the efficiency of inhibition. Currently, internalizing aptamers are being actively tested for a wide range of applications in theranostics.

Acknowledgments Subash Gopinath acknowledges the supports rendered by Universiti Malaysia Perlis. Y. Chen thanks the support from High Impact Research grants—"MoE Grant UM.C/625/1/HIR/MOE/DENT/09" and "UM-MoHE HIR UM.C/625/1/HIR/MOHE/MED/16/5". T. Lakshmipriya thank Universiti Malaysia Perlis for providing the Post-doctoral position.

Compliance with ethical standards

Funding This study was funded by the High Impact Research grants ("MoE Grant UM.C/625/1/HIR/MOE/DENT/09" and "UM-MoHE HIR UM.C/625/1/HIR/MOHE/MED/16/5).

Conflict of interest The authors declare that they have no competing interests.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

References

Adair JR, Howard PW, Hartley JA, Williams DG, Chester KA (2012) Antibody–drug conjugates—a perfect synergy. Expert Opin Biol Ther 12:1191–1206. doi:10.1517/14712598.2012.693473

Adams GP, Weiner LM (2005) Monoclonal antibody therapy of cancer. Nat Biotechnol 23:1147–1157. doi:10.1038/nbt1137

Ali ME, Hashim U, Mustafa S, Che Man YB, Islam KN (2012) Gold nanoparticle sensor for the visual detection of pork adulteration in meatball formulation. J Nanomater 1–7. doi:10.1155/2012/103607

Ali ME, Hashim U, Mustafa S, Man YBC, Yusop MHM, Bari MF, Islam KN, Hasan MF (2011) Nanoparticle sensor for label free detection of swine DNA in mixed biological samples. Nanotechnology 22: 195503. doi:10.1088/0957-4484/22/19/195503

Ashrafuzzaman M (2014) Aptamers as both drugs and drug-carriers. Biomed Res Int 2014:1–21. doi:10.1155/2014/697923

Azhdarzadeh M, Atyabi F, Saei AA, Varnamkhasti BS, Omidi Y, Fateh M, Ghavami M (2016) Shanehsazzadeh S, Dinarvand R. Theranostic MUC-1 aptamer targeted gold coated superparamagnetic iron oxide nanoparticles for magnetic resonance imaging and photothermal therapy of colon cancer. Colloids Surf B: Biointerfaces 143:224–232

Azmi AS, Bao B, Sarkar FH (2013) Exosomes in cancer development, metastasis, and drug resistance: a comprehensive review. Cancer Metastasis Rev 32:623–642. doi:10.1007/s10555-013-9441-9

Blind M, Blank M (2015) Aptamer selection technology and recent advances. Mol Ther Acids 4:e223. doi:10.1038/mtna.2014.74

Brown KC (2010) Peptidic tumor targeting agents: the road from phage display peptide selections to clinical applications. Curr Pharm Des 16:1040–1054

Camorani S, Esposito CL, Rienzo A, Catuogno S, Iaboni M, Condorelli G, de Franciscis V, Cerchia L (2014) Inhibition of receptor signaling and of glioblastoma-derived tumor growth by a novel PDGFRβ aptamer. Mol Ther 22:828–841

Chang YM, Donovan MJ, Tan W (2013a) Using aptamers for cancer biomarker discovery. J Nucleic Acids 2013:817350. doi:10.1155/2013/817350

Chang YM, Donovan MJ, Tan W (2013b) Using aptamers for cancer biomarker discovery. J Nucleic Acids. doi:10.1155/2013/817350

Chen HW, Medley CD, Sefah K, Shangguan D, Tang Z, Meng L, Smith JE, Tan W (2008) Molecular recognition of small-cell lung cancer cells using aptamers. ChemMedChem 3:991–1001. doi:10.1002/cmdc.200800030

Chen W, Zeng W, Sun J, Yang M, Li L, Zhou J, Wu Y, Sun J, Liu G, Tang R, Tan J, Zhu C (2015) Construction of an aptamer-siRNA chimera-modified tissue-engineered blood vessel for cell-type-specific capture and delivery. ACS Nano 23:6069–6076

Cheng C, Chen YH, Lennox KA, Behlke MA, Davidson BL (2013) In vivo SELEX for identification of brain-penetrating aptamers. Mol Ther Nucleic Acids 2:e67. doi:10.1038/mtna.2012.59

Cotter PD, Ross RP, Hill C (2013) Bacteriocins—a viable alternative to antibiotics? Nat Rev Microbiol 11:95–105. doi:10.1038/nrmicro2937

Darmostuk M, Rimpelová S, Gbelcová H, Ruml T (2015) Current approaches in SELEX: an update to aptamer selection technology. Biotechnol Adv. doi:10.1016/j.biotechadv.2015.02.008

Dhar S, Gu FX, Langer R, Farokhzad OC, Lippard SJ (2008) Targeted delivery of cisplatin to prostate cancer cells by aptamer

functionalized Pt(IV) prodrug-PLGA-PEG nanoparticles. Proc Natl Acad Sci U S A 105:17356–17361. doi:10.1073/pnas.0809154105

Diao Y, Liu J, Ma Y, Su M, Zhang H, Hao X (2016) A specific aptamer-cell penetrating peptides complex delivered siRNA efficiently and suppressed prostate tumor growth in vivo. Cancer Biol Ther

Eaton BE, Gold L, Hicke BJ, Janjić N, Jucker FM, Sebesta DP, Tarasow TM, Willis MC, Zichi DA (1997) Post-SELEX combinatorial optimization of aptamers. Bioorg Med Chem 5:1087–1096. doi:10.1016/S0968-0896(97)00044-8

Esposito CL, Catuogno S, de Franciscis V, Cerchia L (2011) New insight into clinical development of nucleic acid aptamers. Discov Med 11:487–496

Farokhzad OC, Cheng J, Teply BA, Sherifi I, Jon S, Kantoff PW, Richie JP, Langer R (2006) Targeted nanoparticle-aptamer bioconjugates for cancer chemotherapy in vivo. Proc Natl Acad Sci U S A 103:6315–6320. doi:10.1073/pnas.0601755103

Farokhzad OC, Jon S, Khademhosseini A, Tran TNT, LaVan DA, Langer R (2004) Nanoparticle-aptamer bioconjugates: a new approach for targeting prostate cancer cells. Cancer Res 64:7668–7672. doi:10.1158/0008-5472.CAN-04-2550

Firer MA, Gellerman G (2012) Targeted drug delivery for cancer therapy: the other side of antibodies. J Hematol Oncol 5:70. doi:10.1186/1756-8722-5-70

Gopinath SCB (2007a) Methods developed for SELEX. Anal Bioanal Chem 387:171–182. doi:10.1007/s00216-006-0826-2

Gopinath SCB (2007b) Antiviral aptamers. Arch Virol 152:2137–2157. doi:10.1007/s00705-007-1014-1

Gopinath SCB (2009) Mapping of RNA-protein interactions. Anal Chim Acta 636:117–128. doi:10.1016/j.aca.2009.01.052

Gopinath SCB (2011) Aptamers. In: Meyers RA (ed) Encyclopedia of analytical chemistry. John Wiley, Chichester, pp. 1–27

Gopinath SCB, Awazu K, Fujimaki M, Shimizu K, Shima T (2013) Observations of immuno-gold conjugates on influenza viruses using waveguide-mode sensors. PLoS One 8:1–10. doi:10.1371/journal.pone.0069121

Gopinath SCB, Awazu K, Fujimaki M, Sugimoto K, Ohki Y, Komatsubara T, Tominaga J, Gupta KC, Kumar PKR (2008a) Influence of nanometric holes on the sensitivity of a waveguide-mode sensor: label-free nanosensor for the analysis of RNA aptamer-ligand interactions. Anal Chem 80:6602–6609. doi:10.1021/ac800767s

Gopinath SCB, Hayashi K, Kumar PKR (2012) Aptamer that binds to the gD protein of herpes simplex virus 1 and efficiently inhibits viral entry. J Virol 86:6732–6744. doi:10.1128/JVI.00377-12

Gopinath SCB, Kumar PKR (2013) Aptamers that bind to the hemagglutinin of the recent pandemic influenza virus H1N1 and efficiently inhibit agglutination. Acta Biomater 9:8932–8941. doi:10.1016/j.actbio.2013.06.016

Gopinath SCB, Misono TS, Kawasaki K, Mizuno T, Imai M, Odagiri T, Kumar PKR (2006a) An RNA aptamer that distinguishes between closely related human influenza viruses and inhibits haemagglutinin-mediated membrane fusion. J Gen Virol 87:479–487. doi:10.1099/vir.0.81508-0

Gopinath SCB, Misono TS, Kumar PKR (2008b) Prospects of ligand-induced aptamers. Crit Rev Anal Chem 38:34–47. doi:10.1080/10408340701804558

Gopinath SCB, Sakamaki Y, Kawasaki K, Kumar PKR (2006b) An efficient RNA aptamer against human influenza B virus hemagglutinin. J Biochem 139:837–846. doi:10.1093/jb/mvj095

Gopinath SCB, Wadhwa R, Kumar PKR (2010) Expression of noncoding vault RNA in human malignant cells and its importance in mitoxantrone resistance. Mol Cancer Res 8:1536–1546. doi:10.1158/1541-7786.MCR-10-0242

Gourronc FA, Rockey WM, Thiel WH, Giangrande PH, Klingelhutz AJ (2013) Identification of RNA aptamers that internalize into HPV-16 E6/ E7 transformed tonsillar epithelial cells. Virology 446:325–333

Healy JM, Lewis SD, Kurz M, Boomer RM, Thompson KM, Wilson C, McCauley TG (2004) Pharmacokinetics and biodistribution of novel aptamer compositions. Pharm Res 21:2234–2246. doi:10.1007/s11095-004-7676-4

Homann M, Göringer HU (1999) Combinatorial selection of high affinity RNA ligands to live African trypanosomes. Nucleic Acids Res 27:2006–2014. doi:10.1093/nar/27.9.2006

Hong Y, Lee E, Ku M, Suh JS, Yoon DS, Yang J (2016) Femto-molar detection of cancer marker-protein based on immuno-nanoplasmonics at single-nanoparticle scale. Nanotechnology 27:185103

Huang Y-F, Chang H-T, Tan W (2008) Cancer cell targeting using multiple aptamers conjugated on nanorods. Anal Chem 80:567–572. doi:10.1021/ac702322j

Hung L, Wang C-H, Che Y, Fu C, Chang H, Wang K, Lee G (2015) Screening of aptamers specific to colorectal cancer cells and stem cells by utilizing on-chip cell-SELEX. Sci Rep 5:10326. doi:10.1038/srep10326

Jack R, Tagg J, Ray B (1995) Bacteriocins of gram-positive bacteria. Microbiol Rev 59:171–200

Jhaveri S, Rajendran M, Ellington AD (2000) In vitro selection of signaling aptamers. Nat Biotechnol 18:1293–1297. doi:10.1038/82414

Juliano RL, Ling V (1976) A surface glycoprotein modulating drug permeability in Chinese hamster ovary cell mutants. Biochim Biophys Acta Biomembr 455:152–162. doi:10.1016/0005-2736(76)90160-7

Kadioglu O, Malczyk AH, Greten HJ, Efferth T (2015) Aptamers as a novel tool for diagnostics and therapy. Investig New Drugs. doi:10.1007/s10637-015-0213-y

Kato Y, Minakawa N, Komatsu Y, Kamiya H, Ogawa N, Harashima H, Matsuda A (2005) New NTP analogs: the synthesis of 4′-thioUTP and 4′-thioCTP and their utility for SELEX. Nucleic Acids Res 33:2942–2951. doi:10.1093/nar/gki578

Kelkar SS, Reineke TM (2011) Theranostics: combining imaging and therapy. Bioconjug Chem 22:1879–1903

Kim S, Lee J, Lee SJ, Lee HJ (2010a) Ultra-sensitive detection of IgE using biofunctionalized nanoparticle-enhanced SPR. Talanta 81:1755–1759. doi:10.1016/j.talanta.2010.03.036

Kim D, Jeong YY, Jon S (2010b) A drug-loaded aptamer−gold nanoparticle bioconjugate for combined CT imaging and therapy of prostate cancer. ACS Nano 4:3689–3696. doi:10.1021/nn901877h

Kim JW, Kim EY, Kim SY, Byun SK, Lee D, Kim WK, Han BS, Chi S, Lee SC, Bae K (2014) Identification of DNA aptamers toward epithelial cell adhesion molecule via cell-SELEX

King HW, Michael MZ, Gleadle JM (2012) Hypoxic enhancement of exosome release by breast cancer cells. BMC Cancer 12:421. doi:10.1186/1471-2407-12-421

Kitazono M, Sumizawa T, Takebayashi Y, Chen Z-S, Furukawa T, Nagayama S, Tani A, Takao S, Aikou T, Akiyama S (1999) Multidrug resistance and the lung resistance-related protein in human colon carcinoma SW-620 cells. J Natl Cancer Inst 91:1647–1653. doi:10.1093/jnci/91.19.1647

Lakshmipriya T, Fujimaki M, Gopinath SCB, Awazu K (2013) Generation of anti-influenza aptamers using the systematic evolution of ligands by exponential enrichment for sensing applications. Langmuir 29:15107–15115. doi:10.1021/la4027283

Leach JC, Wang A, Ye K, Jin S (2016) A RNA-DNA hybrid aptamer for nanoparticle-based prostate tumor targeted drug delivery. Int J Mol Sci. doi:10.3390/ijms17030380

Levy-Nissenbaum E, Radovic-Moreno AF, Wang AZ, Langer R, Farokhzad OC (2008) Nanotechnology and aptamers: applications in drug delivery. Trends Biotechnol 26:442–449. doi:10.1016/j.tibtech.2008.04.006

Li L, Hou J, Liu X, Guo Y, Wu Y, Zhang L, Yang Z (2014a) Nucleolin-targeting liposomes guided by aptamer AS1411 for the delivery of siRNA for the treatment of malignant melanomas. Biomaterials 35:3840–3850

Li WM, Bing T, Wei JY, Chen ZZ, Shangguan DH, Fang J (2014b) Cell-SELEX-based selection of aptamers that recognize distinct targets on metastatic colorectal cancer cells. Biomaterials 35:6998–7007. doi:10.1016/j.biomaterials.2014.04.112

Lindgren M, Hällbrink M, Prochiantz A, Langel U (2000) Cell-penetrating peptides. Trends Pharmacol Sci 21:99–103. doi:10.1016/S0165-6147(00)01447-4

Liu J, Wei T, Zhao J, Huang Y, Deng H, Kumar A, Wang C, Liang Z, Ma X, Liang XJ (2016) Multifunctional aptamer-based nanoparticles for targeted drug delivery to circumvent cancer resistance. Biomaterials 91:44–56

Mallikaratchy P, Tang Z, Kwame S, Meng L, Shangguan D, Tan W (2007) Aptamer directly evolved from live cells recognizes membrane bound immunoglobin heavy mu chain in Burkitt's lymphoma cells. Mol Cell Proteomics 6:2230–2238. doi:10.1074/mcp.M700026-MCP200

Mathivanan S, Ji H, Simpson RJ (2010) Exosomes: extracellular organelles important in intercellular communication. J Proteome 73:1907–1920. doi:10.1016/j.jprot.2010.06.006

Mi J, Liu Y, Rabbani ZN, Yang Z, Urban JH, Sullenger BA, Clary BM (2010) In vivo selection of tumor-targeting RNA motifs. Nat Chem Biol 6:22–24. doi:10.1038/nchembio.277

Mori T, Oguro A, Ohtsu T, Nakamura Y (2004) RNA aptamers selected against the receptor activator of NF-κB acquire general affinity to proteins of the tumor necrosis factor receptor family. Nucleic Acids Res 32:6120–6128. doi:10.1093/nar/gkh949

Navarre WW, Schneewind O (1999) Surface proteins of gram-positive bacteria and mechanisms of their targeting to the cell wall envelope. Microbiol Mol Biol Rev 63:174–229

Nutiu R, Li Y (2003) Structure-switching signaling aptamers. J Am Chem Soc 125:4771–4778. doi:10.1021/ja028962o

Orava EW, Cicmil N, Gariépy J (2010) Delivering cargoes into cancer cells using DNA aptamers targeting internalized surface portals. Biochim Biophys Acta Biomembr 1798:2190–2200. doi:10.1016/j.bbamem.2010.02.004

Peng CG, Damha MJ (2007) G-quadruplex induced stabilization by 2'-deoxy-2' -fluoro-d-arabinonucleic acids (2'F-ANA). Nucleic Acids Res 35:4977–4988. doi:10.1093/nar/gkm520

Perera RM, Zoncu R, Johns TG, Pypaert M, Lee F-T, Mellman I, Old LJ, Toomre DK, Scott AM (2007) Internalization, intracellular trafficking, and biodistribution of monoclonal antibody 806: a novel anti-epidermal growth factor receptor antibody. Neoplasia 9:1099–1110. doi:10.1593/neo.07721

Qazi KR, Torregrosa Paredes P, Dahlberg B, Grunewald J, Eklund A, Gabrielsson S (2010) Proinflammatory exosomes in bronchoalveolar lavage fluid of patients with sarcoidosis. Thorax 65:1016–1024. doi:10.1136/thx.2009.132027

Que-Gewirth NS, Sullenger BA (2007) Gene therapy progress and prospects: RNA aptamers. Gene Ther 14:283–291. doi:10.1038/sj.gt.3302900

Rajendran M, Ellington AD (2003) In vitro selection of molecular beacons. Nucleic Acids Res 31:5700–5713. doi:10.1093/nar/gkg764

Regnier V, De Morre N, Jadoul A, Préat V (1999) Mechanisms of a phosphorothioate oligonucleotide delivery by skin electroporation. Int J Pharm 184:147–156

Reyes-Reyes EM, Šalipur FR, Shams M, Forsthoefel MK, Bates PJ (2015) Mechanistic studies of anticancer aptamer AS1411 reveal a novel role for nucleolin in regulating Rac1 activation. Mol Oncol 9:1392–1405

Rohde JH, Weigand JE, Suess B, Dimmeler S (2015) A universal aptamer chimera for the delivery of functional microRNA-126. Nucleic Acid Ther 25:141–151

Rosenberg JE, Bambury RM, Van Allen EM, Drabkin HA, Lara PN Jr, Harzstark AL, Wagle N, Figlin RA, Smith GW, Garraway LA, Choueiri T, Erlandsson F, Laber DA (2014) A phase II trial of AS1411 (a novel nucleolin-targeted DNA aptamer) in metastatic renal cell carcinoma. Investig New Drugs 32:178–187

Rusconi CP, Scardino E, Layzer J, Pitoc GA, Ortel TL, Monroe D, Sullenger BA (2002) RNA aptamers as reversible antagonists of coagulation factor IXa. Nature 419:90–94. doi:10.1038/nature00963

Sánchez-Luque FJ, Stich M, Manrubia S, Briones C, Berzal-Herranz A (2014) Efficient HIV-1 inhibition by a 16 nt-long RNA aptamer designed by combining in vitro selection and in silico optimisation strategies. Sci Rep 4:6242. doi:10.1038/srep06242

Scheffer GL, Schroeijers AB, Izquierdo MA, Wiemer EAC, Scheper RJ (2000) Lung resistance-related protein/major vault protein and vaults in multidrug-resistant cancer

Schorey JS, Bhatnagar S (2008) Exosome function: from tumor immunology to pathogen biology. Traffic 9:871–881. doi:10.1111/j.1600-0854.2008.00734.x

Sefah K, Meng L, Lopez-Colon D, Jimenez E, Liu C, Tan W (2010) DNA aptamers as molecular probes for colorectal cancer study. PLoS One 5:e14269. doi:10.1371/journal.pone.0014269

Sefah K, Tang ZW, Shangguan DH, Chen H, Lopez-Colon D, Li Y, Parekh P, Martin J, Meng L, Phillips JA, Kim YM, Tan WH (2009) Molecular recognition of acute myeloid leukemia using aptamers. Leuk Off J Leuk Soc Am Leuk Res Fund UK 23:235–244. doi:10.1038/leu.2008.335

Shangguan D, Li Y, Tang Z, Cao ZC, Chen HW, Mallikaratchy P, Sefah K, Yang CJ, Tan W (2006) Aptamers evolved from live cells as effective molecular probes for cancer study. Proc Natl Acad Sci U S A 103:11838–11843. doi:10.1073/pnas.0602615103

Shangguan D, Meng L, Cao ZC, Xiao Z, Fang X, Li Y, Cardona D, Witek RP, Liu C, Tan W (2008) Identification of liver cancer-specific aptamers using whole live cells. Anal Chem 80:721–728. doi:10.1021/ac701962v

Shiao Y-S, Chiu H-H, Wu P-H, Huang Y-F (2014) Aptamer-functionalized gold nanoparticles as photoresponsive nanoplatform for co-drug delivery. ACS Appl Mater Interfaces 6:21832–21841. doi:10.1021/am5026243

Shum K-T, Zhou J, Rossi JJ (2013) Nucleic acid aptamers as potential therapeutic and diagnostic agents for lymphoma. J Cancer Ther 4:872–890. doi:10.4236/jct.2013.44099

Skog J, Würdinger T, van Rijn S, Meijer DH, Gainche L, Curry WT, Carter BS, Krichevsky AM, Breakefield XO (2008) Glioblastoma microvesicles transport RNA and proteins that promote tumour growth and provide diagnostic biomarkers. Nat Cell Biol 10:1470–1476. doi:10.1038/ncb1800

Soundararajan S, Chen W, Spicer EK, Courtenay-Luck N, Fernandes DJ (2008) The nucleolin targeting aptamer AS1411 destabilizes Bcl-2 messenger RNA in human breast cancer cells. Cancer Res 68:2358–2365. doi:10.1158/0008-5472.CAN-07-5723

Srivastava M, Srivastava M, Pollard HB, Pollard HB (1999) Molecular dissection of nucleolin's role in growth and cell proliferation: new insights. FASEB J 13:1911–1922

Stasi R (2008) Gemtuzumab ozogamicin: an anti-CD33 immunoconjugate for the treatment of acute myeloid leukaemia. Expert Opin Biol Ther 8:527–540. doi:10.1517/14712598.8.4.527

Subramanian N, Kanwar JR, Kumar AP, Janakiraman N, Khetan V, Kanwar RK, Eluchuri S, Krishnakumar S (2015) EpCAM aptamer mediated cancer cell specific delivery of EpCAM siRNA using polymeric nanocomplex. J Biomed Sci 22:4. doi:10.1186/s12929-014-0108-9

Sudimack J, Lee RJ (2000) Targeted drug delivery via the folate receptor. Adv Drug Deliv Rev 41:147–162. doi:10.1016/S0169-409X(99)00062-9

Taghdisi SM, Abnous K, Mosaffa F, Behravan J (2010) Targeted delivery of daunorubicin to T-cell acute lymphoblastic leukemia by aptamer. J Drug Target 18:277–281. doi:10.3109/10611860903434050

Tan A, de la Peña H, Seifalian AM (2010) The application of exosomes as a nanoscale cancer vaccine. Int J Nanomedicine 5:889–900. doi:10.2147/IJN.S13402

Tang Z, Shangguan D, Wang K, Shi H, Sefah K, Mallikratchy P, Chen HW, Li Y, Tan W (2007) Selection of aptamers for molecular recognition and characterization of cancer cells. Anal Chem 79: 4900–4907. doi:10.1021/ac070189y

Tayyari F, Marchant D, Moraes TJ, Duan W, Mastrangelo P, Hegele RG (2011) Identification of nucleolin as a cellular receptor for human respiratory syncytial virus. Nat Med 17:1132–1135. doi:10.1038/nm.2444

Thiel WH, Bair T, Peek AS, Liu X, Dassie J, Stockdale KR, Behlke MA, Miller FJ Jr, Giangrande PH (2012) Rapid identification of cell-specific, internalizing RNA aptamers with bioinformatics analyses of a cell-based aptamer selection. PLoS One 7:e43836

Tucker WO, Shum KT, Tanner JA (2012) G-quadruplex DNA aptamers and their ligands: structure, function and application. Curr Pharm Des 18:2014–2026

Tuerk C, Gold L (1990) Systematic evolution of ligands by exponential enrichment: RNA ligands to bacteriophage T4 DNA polymerase. Science 249:505–510. doi:10.1126/science.2200121

Van Simaeys D, López-Colón D, Sefah K, Sutphen R, Jimenez E, Tan W (2010) Study of the molecular recognition of aptamers selected through ovarian cancer cell-SELEX. PLoS One 5:e13770. doi:10.1371/journal.pone.0013770

Wang T, Gantier MP, Xiang D, Bean AG, Bruce M, Zhou SF, Khasraw M, Ward A, Wang L, Wei MQ, AlShamaileh H, Chen L, She X, Lin J, Kong L, Shigdar S, Duan W (2015) EpCAM aptamer-mediated survivin silencing sensitized cancer stem cells to doxorubicin in a breast cancer model. Theranostics 20:1456–1472

Willis MC, Collins B, Zhang T, Green LS, Sebesta DP, Bell C, Kellogg E, Gill SC, Magallanez A, Knauer S, Bendele RA, Gill PS, Janjic N (1998) Liposome anchored vascular endothelial growth factor aptamers. Bioconjug Chem 9:573–582. doi:10.1021/bc980002x

Wu J, Zhu Y, Xue F, Mei Z, Yao L, Wang X, Zheng L, Liu J, Liu G, Peng C, Chen W (2014) Recent trends in SELEX technique and its application to food safety monitoring. Mikrochim Acta 181:479–491. doi:10.1007/s00604-013-1156-7

Xiao Z, Levy-Nissenbaum E, Alexis F, Lupták A, Teply BA, Chan JM, Shi J, Digga E, Cheng J, Langer R, Farokhzad OC (2012) Engineering of targeted nanoparticles for cancer therapy using internalizing aptamers isolated by cell-uptake selection. ACS Nano 6: 696–704. doi:10.1021/nn204165v

Xiao Z, Shangguan D, Cao Z, Fang X, Tan W (2008) Cell-specific internalization study of an aptamer from whole cell selection. Chem Eur J 14:1769–1775. doi:10.1002/chem.200701330

Yamamoto-Fujita R, Kumar PKR (2005) Aptamer-derived nucleic acid Oligos: applications to develop nucleic acid chips to analyze proteins and small ligands. Anal Chem 77:5460–5466. doi:10.1021/ac050364f

Yan Q (2014) Pharmacogenomics in drug discovery and development. Methods Mol Biol 1175:v–vii

Yu C, Hu Y, Duan J, Yuan W, Wang C, Xu H, Yang XDA (2011) Novel aptamer-nanoparticle bioconjugates enhances delivery of anticancer drug to MUC1-positive cancer cells in vitro. PLoS One 6:1–8. doi:10.1371/journal.pone.0024077

Zhang K, Sefah K, Tang L, Zhao Z, Zhu G, Ye M, Sun W, Goodison S, Tan W (2012) A novel aptamer developed for breast cancer cell internalization. ChemMedChem 7:79–84. doi:10.1002/cmdc.201100457

Zhao Z, Xu L, Shi X, Tan W, Fang X, Shangguan D (2009) Recognition of subtype non-small cell lung cancer by DNA aptamers selected from living cells. Analyst 134:1808–1814. doi:10.1039/B904476K

Zhou J, Bobbin ML, Burnett JC, Rossi JJ (2012) Current progress of RNA aptamer-based therapeutics. Front Genet 3:1–14