Nucleic Acid Aptamers as Tools and Drugs: Recent Developments

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Nucleic acid aptamers are molecules that bind to their ligands with high affinity and specificity. Unlike other functional nucleic acids such as antisense oligonucleotides, ribozymes, or siRNAs, aptamers almost never exert their effects on the genetic level. They manipulate their target molecules such as gene products or epitopes directly and site specifically, leaving nontargeted protein functions intact. In a similar way to antibodies, aptamers bind to many different kinds of target molecules with high specificity and can be made to order, but as a result of their different biochemical nature and size they can also be used complementary to antibodies. In some cases, aptamers might be more suitable or more specific than antibody approaches or small molecules, both as scientific and biotechnological tools and as therapeutic agents. Recent examples of characterization of aptamers as tools for scientific research to study regulatory circuits, as tools in diagnostic or biosensor development, and as therapeutic agents are discussed.

1. Introduction

The word aptamer is derived from the greek word 'haptein' meaning 'to attach to.' Aptamers are typically single-stranded nucleic acids between 30 and 70 nucleotides in length. As a result of their capacity to form elaborate three-dimensional structures and shapes,[1] aptamers can bind with high specificity and affinity to a wide variety of target molecules, with dissociation constants down to picomolar values. So far the most effective way to find high-affinity nucleic acids with high specificity for a certain target is by systematic evolution of ligands by exponential enrichment (SELEX) technology.[2, 3] SELEX comprises an iterative process of in vitro selections of nucleic-acid target-binding events and partitioning events of unbound nucleic acids and is used to isolate high-affinity nucleic acid ligands from large pools of randomized nucleic acid sequence libraries usually containing more than 10^{15} different sequences initially (see Figure 1). Many variants and improvements of this technology have been used for various application needs (for examples, see refs. [4–12]). To make the process more efficient, automated SELEX protocols for protein targets have been developed and established as robotic systems.[13, 14] Novel methodologies for aptamer-development processes and new fields of application of these versatile molecules are constantly being explored in academic institutions and biotechnology companies.

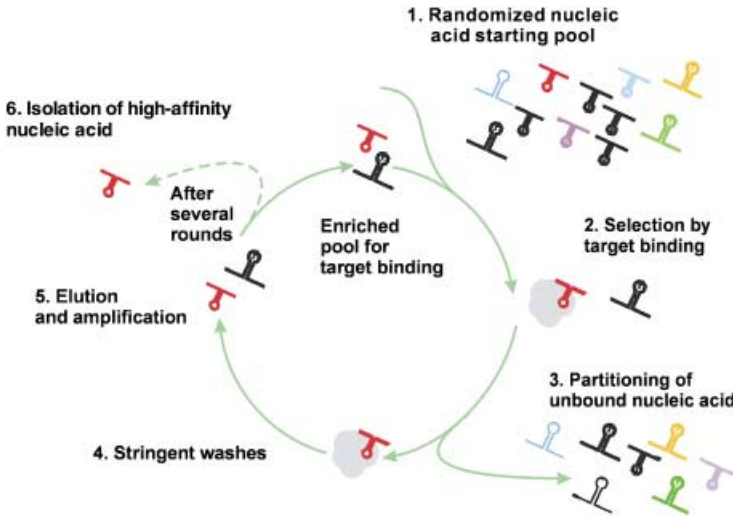

Figure 1. *Systematic evolution of ligands by exponential enrichment (SELEX).[2] By starting from a large pool of randomized nucleic acids (1), target-binding nucleic acids (2) are selected by partitioning them from nonbinding species (3). After washing (4), bound nucleic acids are eluted from the target, amplified (5), and used in the next round of selection as an enriched pool for target binding. After several rounds, high-affinity aptamers can be isolated (6).*

2. Aptamers as Tools

A continuously growing number of aptamers are being described as scientific and biotechnological tools to study specific cellular protein functions and protein–ligand interactions, and such aptamers have been excellently reviewed in the past.[5, 15–18] Aptamers are used to decipher biologically relevant regulatory circuits or to improve understanding of molecular mechanisms of disease processes. As tools for the characterization of

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molecular interaction pathways, they have been studied biochemically, in cell culture assay systems, and even in a multicellular organism.[19] This minireview focuses on some of the more recent developments in the aptamer field.

Aptamers against a variety of purified intracellular targets have been developed in vitro and then expressed intracellularly by various expression systems to exert their full function in the natural intracellular locations of their targets.[9, 16, 18, 20, 21] These so-called 'intramers'[16] have been discussed as promising new tools in functional proteomics and for target validation and small-molecule drug identification in high-throughput systems.[16, 18, 22] However, not every aptamer selected in vitro can be directly cloned and used as an in vivo highly active compound without the need to amend its properties in order to adjust it to the different conditions inside a living cell. Application of various modifications or postselection protocols might thus be necessary for in vivo activity.[23]

2.1. Aptamers to elucidate signal transduction pathways

One of the recent examples of the use of aptamers to define the functional interaction of proteins that mediate mammalian signal transduction pathways is the highly specific aptamers that have been reported to inhibit the Ras-induced Raf-1 activation signal transduction kinase cascade of cell proliferation and differentiation processes in a cell-free membrane fraction assay system.[24] The aptamers specifically inhibit only the Ras/raf-1 interaction and, unlike antibodies or antibody fragments, do not interfere with highly homologous protein interactions such as Ras/B-Raf binding. Also, unlike small-molecule inhibitors, the aptamers have no unspecific activating effects on the cascade. It will be interesting to see if these aptamers or derivatives thereof can also exert their function in living cells to enable researchers to dissect the specific Ras – Raf1 interaction signaling pathway.

Seiwert et al. have described the identification of aptamers that distinguish the phosphorylated from the unphosphorylated form of Erk 1/2, one of the mitogen-activated protein (MAP) kinases that mediate growth factor signaling.[25] Changes in the active status of a protein by phosphorylation is one of the main mechanisms by which signal transduction cascades are accomplished. Thus, the ability to specifically discriminate between a phosphorylated and unphosphorylated protein could lead to important advances in studying a pathway and could possibly allow interference at very specific sites. This work also nicely illustrates the high discriminatory capabilities of aptamers, which can bind differently to isoforms of proteins.

One of the most important steps in signal transduction pathways is receptor recognition. However, up to now there have not been many publications on aptamers targeted against receptor molecules, probably in part because of the instability of detergent-solubilized receptors as target molecules for aptamer selection.

One of the less common examples of aptamer development directly targeting a receptor molecule was described recently by Daniels et al. They generated the first aptamers against a G-protein-coupled receptor (GPCR), the receptor for Neurotensin-1, a stimulator of cell proliferation. They describe the aptamers as a valuable tool for probing the role of GPCRs in normal tissue and disease pathology, and also as a beneficial tool and aid for structural determinations of GPCRs.[26] Daniels et al. showed that their aptamers can bind specifically to the extracellular part of the receptor, but the aptamers could unfortunately not inhibit neurotensin binding and signaling.

These results demonstrate the finding of many researchers that aptamers seem not to be easily selected for every kind of target molecule or for every kind of interference with a specific target function, for example, the signaling function of a certain receptor. In some cases, more intricate development schemes have to be developed to find an aptamer with the desired properties.

2.2. Ligand-regulated aptamers as tools

Important applications of aptamers as scientific tools to study regulatory circuits include the recent descriptions of aptamers involved in gene-specific small-ligand-controlled translation. Ligand-specific aptamer sequences were introduced into the 5′-untranslated regions (UTR) of the genes of interest. When the small ligands were added, they bound to the aptamers and specific gene expression was hindered conditionally. When the ligands were not present, gene expression and the following regulatory processes, that is, cell cycle processes, occurred undisturbed. Ligand/aptamer-controlled gene expression has been shown to work in prokaryotic as well as in various eukaryotic cell systems.[27–29]

Interestingly, natural aptamers involved in different cellular processes also seem to be regulated through ligand or metabolite binding and serve as natural molecular switches in bacteria.[30, 31] In addition, an RNA of a bacterial virus was recently shown to play a vital part in adenosine triphosphate (ATP) binding as well as viral DNA packaging, and seems to be similar to ATP-binding RNA aptamers developed in vitro.[32]

Very intriguingly, Vuyisich and Beal describe ligand-regulated RNA aptamers that bind with high affinity to their protein target formamidopyrimidine glycosylase, but that are designed to let go of this protein target when their second target—the small ligand neomycin—is present.[11] The authors describe their approach as particularly useful in defining the function of proteins involved in phenomena for which the timing of events is critical, such as the cell cycle, the circadian clock, or embryonic development.

2.3. Aptamers as tools in antibiotics research

In the past, aptamer development has been used as an important tool for antibiotics research. RNA-acting antibiotics such as aminosides (aminoglycosides) bind to bacterial RNA causing premature termination of proteins and mistranslation in bacteria. By studying the structural interactions of such antibiotics with antibiotic-specific RNA aptamers, knowledge of antibiotic – RNA interactions might be gained that could lead to valuable insights for the rational design of new and highly active antibiotic compounds against which no bacterial resistance has yet emerged.[1, 33–35]

2.4. Aptamers as in vitro and in vivo diagnostic tools

The potential of aptamers as new diagnostic tools rivaling and complementing antibody approaches[5, 15] is constantly being advanced. As a recent example, aptamers have been described that are used in new assays for ultrasensitive detection of low-abundance target molecules down to zeptomol (10^{-20} mol) amounts through the proximity ligation and amplification assay of Fredriksson et al.[36, 37] In this assay, the coordinated binding of a target protein by two aptamers brings the aptamers into close proximity and promotes ligation of oligonucleotides linked to each aptamer affinity probe. By using a specific connector oligonucleotide, the two aptamer-linked oligonucleotides are ligated and give rise to an amplifiable DNA sequence that reflects the identity and amount of the target protein. Figure 2 depicts this assay.

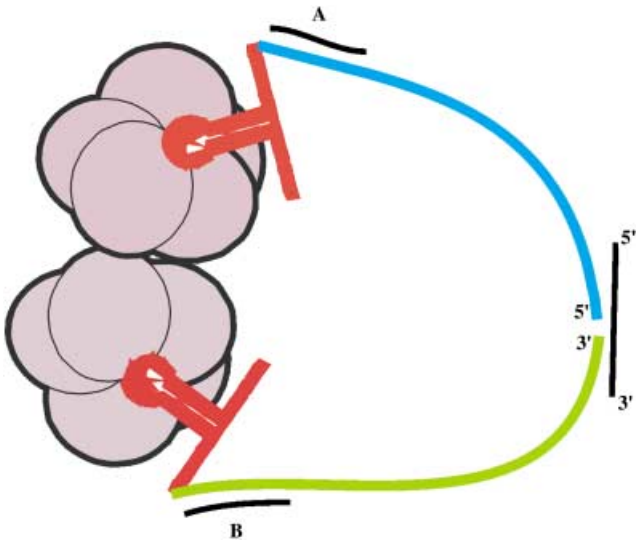

Figure 2. *Aptamers (red) that were developed against a homodimeric protein are extended by different sequences to give different proximity probes 1 (blue) and 2 (green). Upon binding to the homodimeric target, the proximity probes come close to each other. By using a common connector oligonucleotide (black), the proximal probes are ligated and are then amplified with PCR primers A and B and quantified by real-time detection.*

Aptamers have been shown to be useful diagnostic tools for recognizing complex targets such as human red blood cell ghosts, for distinguishing differentiated cells from parental cells in carcinoma cell diagnosis, and for application in HIV diagnosis.[12, 38, 39] Intriguingly, aptamers against the Alzheimer amyloid peptide were recently selected and shown to bind to amyloid fibrils.[40] These amyloid aptamers could prove useful in studying and diagnosing the disease.

A further interesting recent publication reports on the use of aptamers to help decipher the interaction sites of an autoantibody with its natural DNA target in systemic lupus erythematosus, which could also potentially be useful in diagnostic biosensor platforms for autoimmune disease.[41]

Closely related to in vitro diagnostics is in vivo imaging of disease-related targets such as cancer-related epitopes on cell surfaces and in tissues. Aptamers have also been employed in this field and have significantly advantageous properties, such as high tissue penetration ability and a high signal-to-noise ratio when compared to other specific imaging molecules such as antibodies or peptides (reviewed recently by Hicke and Stephens[42]). Lupold et al. have identified aptamers that bind to prostate cancer cells with low-nanomolar affinity through the extracellular portion of the prostate-specific membrane antigen (PSMA). PMSA aptamers may be used clinically and may be modified to carry imaging agents and therapeutic agents directed at prostate cancer cells.[43]

Hicke and co-workers have reported the development of aptamers against Tenascin C, an extracellular matrix protein that is overexpressed significantly in tumor tissue in comparison to expression in normal tissue.[44] The authors aim to conjugate the aptamers to radioisotope chelators or fluorescent dyes for Tenascin imaging of tumors, as well as for specific tumor targeting of radiotherapeutics. Charlton et al. have presented in vivo imaging of lung inflammation by a neutrophilic elastase-binding aptamer that was also described as having protecting effects in lung inflammatory injury.[45]

The imaging of thrombi formed in vitro by using thrombin aptamers has been recently shown to look promising, although in vivo imaging trials in a rat jugular vein model seem to still need improvement as fast blood clearance of the unmodified DNA aptamers was observed.[46]

2.5. Aptamers in biosensors

Aptamers are well suited to application in biosensors to specifically detect a large variety of target molecules like proteins, metabolites, amino acids, nucleotides, etc. Aptamers have also been shown to detect targets that are hard to address by using antibody biosensors, such as toxic agents or non-immunogenic analytes.[10, 37, 47, 48]

Recent examples include new biosensor methodology for detection of biotoxins such as cholera whole toxin and staphylococcal enterotoxin B by using aptamers developed by a magnetic-bead-based selection procedure,[8] as well as an aptamer-based quartz-crystal protein biosensor for IgE.[49a]

The goal of Rimmele and co-workers is to open a new field of application of aptamers: environmental analytics and chemical-process-controlling using biosensor approaches[49b]. They have identified aptamers that recognize a low-molecular-weight environmental toxin with high affinity and specificity and have developed a fiber-optic sensor system in a field biosensor prototype that uses these aptamers to conveniently detect the environmental toxin on-site with high sensitivity and within minutes. Figure 3 shows a schematic representation of the biosensor and an overlay plot of the binding curves of an aptamer and of an antibody to the toxin in real time. In the approach of Rimmele and co-workers, the aptamers not only proved to bind with higher affinity (Figure 3b), but also with more specificity than antibodies (data not shown) when detecting the environmental toxin.

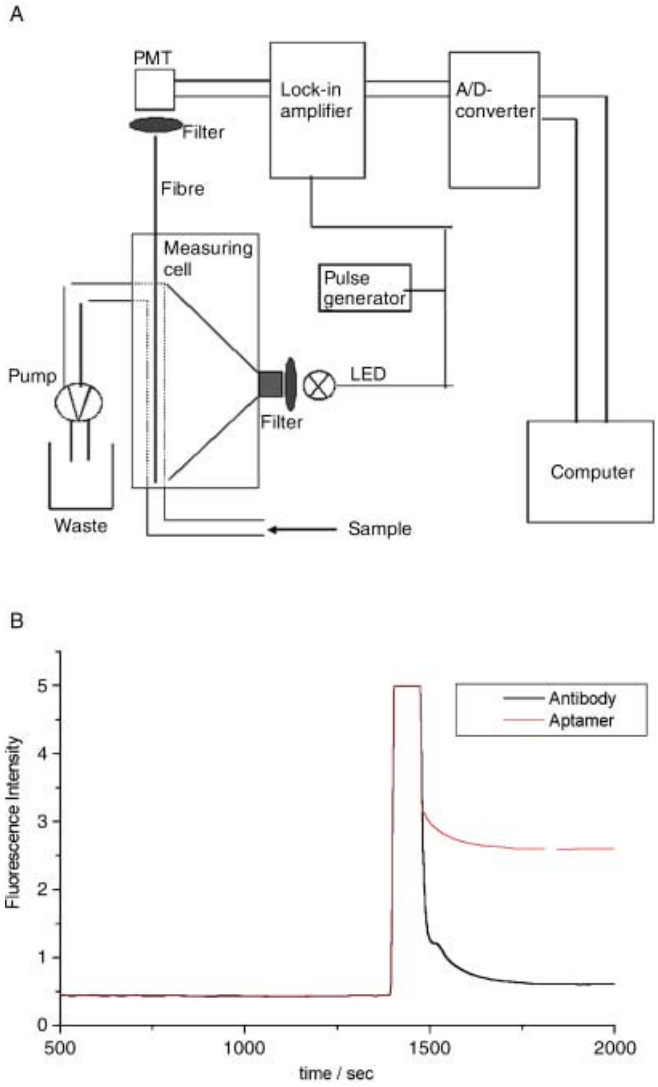

Figure 3. *Measurement of environmental toxin in a fiber-optic field biosensor. A) Measurement set-up of the sensor. The sample is pumped through the measuring cell with an embedded sensing glass fiber. The excitation light is passed through an interference filter and is guided by a fiber bundle to the measuring cell. The sensing glass fiber guides the fluorescence light through a filter to a photomultiplier tube (PMT). The PMT signal is collected by a lock-in amplifier, converted, and sent to a computer for data sampling. B) Overlay plot of real-time binding curves of aptamer versus antibody in the field sensor. The toxin was immobilized on a sensing glass fiber and binding of fluorescently labeled aptamer or antibody was measured in real time. Binding is shown as fluorescence intensity and the difference between the aptamer and antibody measurements mirrors a difference in toxin affinity, aptamer versus antibody, of more than 100-fold. Binding measurements were performed by Eva Ehrentreich-Förster, Fraunhofer Institute for Biomedicinal Technology.*

2.6. Aptamers as the sensing part of allosteric ribozymes

As an appropriate presentation of this field would go far beyond the scope of this minireview, only a few remarks about this very elegant additional biotechnological application of aptamers are made herein. 'Aptazymes,' or, 'allosteric ribozymes,' are aptamers in a functional conjunction with ribozymes. Ribozymes are RNA sequences with catalytic activity and mostly catalyze the cleavage of specific RNA substrates in either the same RNA molecule (self cleavage) or in a different target RNA (*trans*-cleavage). Directly connected to an aptamer RNA molecule, ribozymes can be engineered to become allosteric ribozymes, in which structural changes due to the binding of the aptamer ligand control the catalytic function of the ribozyme. Manifold improvements for genomic, proteomic, metabolomic, and molecular therapy approaches, as well as other implementations of aptazymes have been described.[10, 50–54] Aptazymes have been optimized for parallel diagnostic platforms or biosensor chip applications, for target validation applications, and for the fast identification of site-specific small-molecule drugs.[55]

The theoretical transition from aptamers as tools to aptamers as therapeutic agents is clearly seamless. Moreover, development of aptamers from serving as tools into aptamers as potential therapeutic agents and drugs is conceivable, necessary, and in many cases already being actively pursued.

3. Aptamers as Therapeutic Agents and Drugs-To-Be

Aptamers have been developed against many different disease-related targets to block specific sites on proteins or on other target molecules. Disease-promoting protein – protein interactions or protein – ligand interactions can thus potentially be hindered or signal-transducing cascades disturbed.[4–6, 17, 18, 24]

3.1. Aptamers targeting growth factors

One of the most advanced aptamers in drug development is an aptamer (NX 1838) against vascular endothelial growth factor (VEGF). VEGF has been described as a key factor in angiogenesis, vascular permeability, tumor growth, mesangioproliferative glomerulonephritis, and potentially also artherosclerosis, rheumatoid arthritis, psoriasis, and several retinopathies.[6]

EYEtech/Pfizer's 'Macugen' (pegaptanib sodium) is a pegylated form of NX 1838. A phase III trial recruitment for Macugen was recently completed to allow testing of the capacity of the drug to treat age-related macular degeneration (AMD) and to thus avoid AMD-induced blindness.[56–58] In phase I/II trials, 80% of patients treated with the anti-VEGF aptamer showed stable or improved vision after administration. Macugen will also be tested for efficiency in diabetic retinopathy.

The aptamer against VEGF (NX 1838) is currently also being tested in animal models in other disease settings to find out whether it can block VEGF binding to its receptor. The aptamer has been found to be inhibitory in tests in glomerular disease,[5, 59] in Wilms tumor growth,[60] and in a mouse xenograft model of neuroblastoma.[61] It has very recently been shown that, as in the antiangiogenesis action of aptamers against VEGF, aptamers against angiopoietin-2 (Ang2) could also act as an antiangiogenic agent in a rat corneal micropocket angiogenesis assay.[62]

Specific aptamers against yet another growth factor, platelet-derived growth factor (PDGF) B, were recently demonstrated to significantly reduce mesangial cell proliferation and glomerular matrix protein accumulation, which contribute to many pro-

gressive renal diseases, in a rat glomerulonephritis model.[63, 64] Moreover, experiments with these aptamers showed that PDGF effects can be separated from transforming growth factor B mediated effects in mesangioproliferative glomerulonephritides.

3.2. Aptamers targeting intracellular target molecules

In order to optimize functional therapeutic aptamers against intracellular targets, investigators have appended a variety of expression systems and used in vitro selection protocols that ameliorate intracellular aptamer function and expression or stability, or correct local presentation to allow the use of aptamers in gene therapy approaches.[9, 18, 20, 54]

Examples of aptamers against intracellular targets are RNA aptamers against protooncogenic transcription factors that take part in disease processes; such aptamers have been developed and have recently been further optimized. For example Maher and colleagues generated aptamers against transcription factor NFκB in vitro.[65] NFκB is involved in inflammation, in prevention of apoptosis in tumor cells, and in HIV-1 transcription, among other processes. The authors showed that their in vitro selected aptamers bind NFκB in vivo in a yeast system.[66] Recently, they re-optimized the aptamers by rounds of in vivo selection for optimal intracellular binding capacity to NFκB in a yeast three-hybrid system.[23] These aptamers are described as being potentially useful as decoys in reducing the activity of transcription factors involved in disease processes.

Beside the development of decoy aptamers by selection, genuine decoy aptamers for transcription factors have been directly developed by design based on their natural nucleic acid targets. In a phase I clinical trial, Kohn et al. recently retrovirally introduced the Rev responsive element (RRE) RNA of HIV-1 as a decoy into $CD34^+$ hematopoetic progenitor cells from the bone marrow of HIV-infected pediatric patients. The researchers could re-infuse the changed cells into the patients without adverse effects.[67] Previously, they had shown that expressing RRE RNA in such cells leads to the inhibition of HIV-1 replication in the cells.

The application and advantages of different rationally designed genuine decoy aptamers have recently been discussed by Mann and Dzau.[68] For example, one decoy aptamer was studied for positive effects that alleviate restenosis of venous bypass grafts used for surgical revascularization of occlusive disease. The process underlying accelerated restenosis is intimal hyperplasia, the abnormal proliferation and migration of small muscle cells in the inner layer of vessel walls in response to vessel injury. Intimal hyperplasia occurs in all patients over the months following a bypass operation and makes the overlaying endothelium dysfunctional and the patients highly susceptible to subsequent accelerated artherosclerosis. The pivotal cell cycle transcription factor E2F has been shown to regulate many different cell cycle genes. E2F decoy double-stranded DNA aptamers that bear the consensus E2F binding site were shown to markedly inhibit intimal hyperplasia in rat carotid artery injury models and it recently was shown that ex vivo transfection of human bypass vein grafts with E2F-decoy oligodeoxynucleotides during the operation process significantly lowers failure rates of human primary bypass vein grafting.[68] Intimal hyperplasia could also be reduced significantly in a rat carotid restenosis model by using intraperitoneal administration of platelet-derived growth factor(PDGF) RNA aptamers that had been selected by SELEX technology.[69]

The therapeutic use of aptamers as escort aptamers to bring radiotherapeutics or toxic agents site-specifically and with high tissue retention to their target cells is discussed in Section 2.4 and by Hicke and Stephens.[42]

Antiviral aptamers against reverse transcriptase have recently been shown to be effective against viral spread of HIV-1 in human T-lymphoid cell lines.[70] Bai et al. even went further and demonstrated resistance to HIV-1 infection of human thymografts in an in vivo human thymopoesis mouse model. The authors used gene therapeutics to change human thymus progenitor cells to express Rev aptamers. The cells were re-introduced into a SCIDhu in vivo human thymopoesis mouse model, where they differentiated correctly, expressed the aptamers properly, and rendered human thymus grafts in the mouse resistant to HIV-1 infection.[71] But aptamers against viral disease have not only been developed to combat HIV. The inhibitory activity of aptamers against hepatitis C virus NS3-protease activity was recently demonstrated in a Hela cell system.[72]

3.3. Aptamers targeting factors in autoimmune and inflammatory disease

Their apparent lack of immunogenicity[6, 73] makes aptamers excellent potential agents in autoimmune disease therapeutic approaches. For myosthenia gravis, aptamers selected to bind to specific acetylcholesterin-receptor-targeting antibodies have recently been shown to block antibody binding to the receptor in postsynaptic muscle cell membranes.[74] However, these aptamers could not efficiently block heterogenous populations of autoantibodies in patient sera. For a therapeutic approach to myosthenia gravis, combinations of RNA aptamers against a greater fraction of autoantibodies need first to be developed.

Aptamers binding to autoantibodies in systemic lupus erythematosus[41] are described in Section 2.4.

RNA aptamers have also been developed against Oncostatin M (OSM), a multifunctional member of the interleukin-6 cytokine family that has been implicated as a key proinflammatory modulator of the chronic inflammatory disease rheumatoid arthritis, which leads to destruction of articular cartilage, ligaments, and subchondral bone.[75] Inhibition of OSM binding to its receptors by antibodies had previously been shown to have ameliorating effects in murine rheumatoid arthritis models, and Rhodes et al. discuss a possible ameliorating effect for the newly developed aptamers as well, but the authors have not yet been able to show such an effect.

Other aptamers against targets involved in different inflammatory disorders have already been tested successfully in animal models.[76, 77] As an example, highly specific aptamers have been developed against L-selectin, a member of a family of cell-surface lectins that mediates cell adhesion by recognition of cell-specific carbohydrate ligands and that plays an important role in inflammation and reperfusion injury. Small molecule inhibitors of

L-selectin show only low affinity and little specificity for a particular selectin. The highly specific aptamers targeted at human L-selectin inhibited binding of small-molecule ligands to cell-surface L-selectin and L-selectin-mediated lymphocyte rolling on endothelial cells in vitro and also inhibited successfully L-selectin-mediated homing of human lymphocytes to lymph nodes in a SCID mouse model of inflammatory disease.[76]

3.4. Aptamers as regulatable therapeutics

A fascinating application advantage of aptamers as therapeutics has recently been described by Rusconi et al. The authors report the development of aptamers against coagulation factor IXa as potent anticoagulant agents beside heparin and previously studied aptamers against coagulation factor VIIa and thrombin. Rusconi et al. showed how rationally designed oligonucleotides complementary to these aptamers can act as antidotes to efficiently reverse the aptamer activity in plasma taken from healthy volunteers and heparin-intolerant patients, and thus how aptamers can serve as regulatable therapeutics accessible by rational design.[78] According to this rationale, we could also picture the ligand-regulated aptamer approach of Vuyisich and Beal described in Section 2.2.[11] as applicable for therapeutic on/off switching of specific protein activity by aptamers. Table 1 summarizes some of the current examples of therapeutic aptamers in clinical and preclinical trials.

4. Advantages and Limitations of Aptamers—Necessary Future Developments

The advantages of aptamers in comparison to antibodies lie mostly in their size and nonproteinaceous nature, and in many ways aptamers are robust chemicals rather than biologicals.[15] Their size is between that of a peptide and a single-chain Fab fragment and their affinity for a targeted protein on average higher than that of Fab fragments,[6] while at the same time aptamers are less vulnerable to the reducing conditions of the cytoplasm. Even if aptamers are larger than small molecules, they are able to penetrate well into target and extravascular tissue[42] or the extracellular matrix.[64] As a result of their fast blood clearance, aptamers show a more favorable signal-to-noise ratio in in vivo imaging than antibodies.[45] Blood clearance of therapeutic RNA molecules for other therapeutic settings needs, however, to be adequately slowed down. The pharmacokinetics and blood clearance of aptamers as potential drugs have been shown to be positively influenced by pegylation or by embedding aptamers in liposomes,[5, 6, 63] or by 3′-biotinylation.[79]

One disadvantage of aptamers as well as antibodies is that they unfortunately do not seem to be readily made for any target molecule, but their development depends greatly on the properties of a target and its availability in high enough amounts. There is also not one best method for aptamer development for any kind of target molecule. Some targets work better for aptamer development than others and some work with a distinct selection method only. In many cases, the success of developing an aptamer with the desired properties seems to depend greatly on the use of the exact conditions during development in which the aptamers should later exert their function, for example, the temperature at which target inhibition should occur.[26]

Most aptamers need a certain amount of cations to be able to fold correctly into their active state. For some application conditions, this requirement could pose a challenge to aptamer usage, which needs to be taken into consideration during aptamer development.

Also, the pI value of a target molecule clearly influences the capacity of negatively charged nucleic acids to bind to it, and has to be taken into consideration in target epitope selection and selection of buffer conditions. By choosing the right conditions, Breaker and co-workers were recently able to develop aptamers against thiamine pyrophosphate and showed that, surprisingly, in particular the phosphate group of the target was recognized and was bound most efficiently by the aptamers.[31] Many very helpful discussions on different selection possibilities and methods have been written in the past (see, for example, ref. [80]).

One of the advantages of aptamers versus small-molecule drugs is their exquisite specificity. Aptamers are designed and developed for high affinity to only a certain target molecule or

Table 1. *Examples of aptamers in preclinical studies and clinical trials.*

Aptamer target and therapeutic effect of aptamer	Actual trial status	Reference
Anti-VEGF aptamer ('Macugen') has been tested successfully in clinical trials against age-related macular degeneration (AHD)	Phase III	[57, 58]
Rev RRE decoy aptamers in hematopoetic stem cells inhibit HIV-1 replication and re-infusion of cells into patients has no adverse effects	Phase I	[67]
E2F decoy aptamers significantly lower failure rates of human primary bypass vein grafting	Phase I	[68]
Rev aptamers in human thymus progenitor cells have been introduced into a SCID human thymopoesis mouse model and induce resistance to HIV-1 infection of human thymus grafts in the mouse	Preclinical	[71]
Anti-VEGF aptamers effectively suppress primary Wilms tumor growth in experimental animals with no observed adverse effects	Preclinical	[60]
Aptamers against angiopoietin-2 (Ang2) can act as an antiangiogenic agent in the rat corneal micropocket angiogenesis assay	Preclinical	[62]
PDGF aptamers lead, in a rat restenosis model, to significant reduction of intimal hyperplasia, a severe complication in bypass patients	Preclincal	[69]
Highly specific aptamers inhibit L-selectin-mediated homing of human lymphocytes to lymph nodes in a SCID mouse model of inflammatory disease	Preclincal	[76]
RNA aptamers against coagulation factor IXa and their direct antidote molecules have been successfully tested in human plasma samples	Preclincal	[78]

only a particular isoform of a target protein and can distinguish between different conformational states of the same protein.[25, 81] Their high specificity is reflected in their many different possible modes of target recognition. Excellent work to elucidate the molecular structure of aptamer – ligand interactions has had a great impact on our understanding of aptamer function.[1, 82] Most aptamers are very adaptive and flexible when unbound. Different aptamers show totally different binding motifs, which range from stacking of flat ligand moieties between bases to binding of target moieties or amino acids deeply inside the groove of twisted double-helix parts of the aptamer. Figure 4 shows an illustrative example of the structure of an RNA aptamer folding around its antibiotic target molecule.[1]

Aptamers can form specific hydrogen bonds or lie perpendicular to the target. They have been shown to bind only to the surface of antiparallel beta sheets or to encapsulate their target molecule by adopting unusual L-shaped architectures.[1, 35, 82]

In addition and complementary to structural analysis, mutagenesis experiments with aptamers have been shown to be crucial to determine the minimal length of the essential substructures of functional aptamers or to determine important interacting structures within an aptamer as well as single nucleotides that are essential for specific binding to a certain target molecule.[83–86]

The nonproteinaceous nature of nucleic acid aptamers renders their production at a consistent quality convenient, with efficient and exact modifications and labels, and allows them to be applied easily in immobilization procedures, and to be easily regenerated by heat or ionic changes when used as a tool.[49]

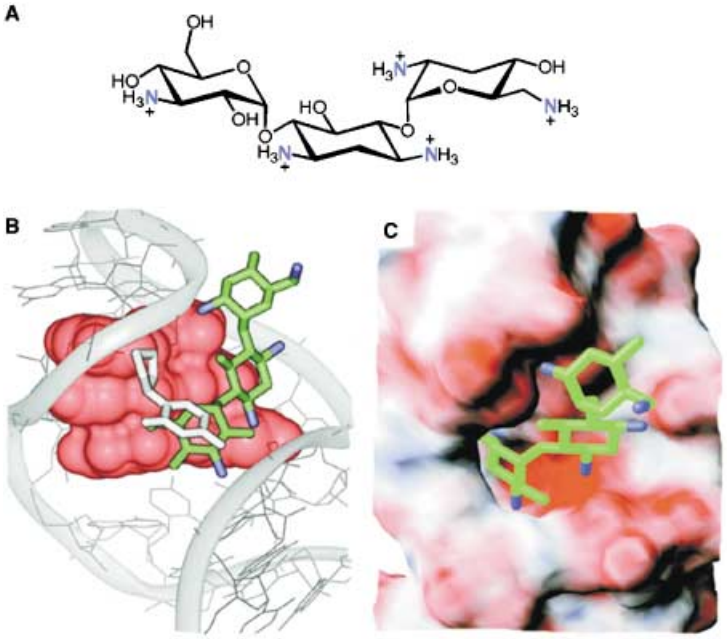

Figure 4. *Molecular recognition of the aminoglycoside antibiotic tobramycin (A) by an RNA aptamer. B) In the aptamer complex, the RNA encapsulates the tobramycin ligand (green), which packs against the base edges (red) within the deep groove. A base flap (gray sticks) closes the groove above the bound drug. C) The ligand-binding pocket provides a negatively charged environment displaying shape complementarity between electronegative sites (red) in the cavity and the positions of the cationic ammonium groups (blue) in the aminoglycoside. The RNA surface is colored according to the electrostatic potential, with red indicating negative charge and blue indicating positive charge. Reprinted with permission from T. Hermann, D. J. Patel,* Science ***2000****, 287, 820 – 825. Copyright American Association for the Advancement of Science. Part C was first published in A. Nicholls, K. A. Sharp, B. A. Honig,* Proteins*, **1991**, 11, 281 and is reproduced with permission from John Wiley & Sons, Inc.*

One of the not-yet-understood features of nonnatural RNA aptamer sequences is their apparent lack of immunogenicity,[73] which makes them a favorable drug for avoidance of immune responses, especially in allergy or autoimmune disease scenarios.[41, 74, 75] Even trials intended to elicit an immune response against different specific aptamers have failed so far.[6, 87]

Clearly, an emerging considerable advantage of aptamers as therapeutic drugs is the possibility to easily design and efficiently use antidotes, and thus to be able to selectively reverse the activity of the drug.[78]

Resistance of aptamers against nuclease attack is achieveable nowadays, albeit still expensive, by chemical modifications at the 2′-position of the ribonucleotide moieties, by circularization or capping of RNAs to avoid exonuclease attack, or by 'spiegelmer' technology.[6, 88–92] 'Spiegelmers' are mirror-image, high-affinity oligonucleotide ligands consisting of L-ribose or L-2′-deoxyribose units instead of the natural D-ribose or D-2′-deoxyribose units. The chiral inversion confers high resistance against enzymatic degradation of the molecules.[87, 90] Spiegelmers are developed by an elegant 'mirror-image' SELEX approach.[93] Chemical stabilization modifications are, where possible, made from the beginning of aptamer development to keep the high-affinity-binding characteristics of aptamers once they have been identified, although there are examples where extensive stabilization modifications could be introduced later without diminishing binding activity.[75]

A limitation of aptamers that also applies to antibody fragments or peptides seems to be that, unlike small molecules, they cannot enter animal cell culture cells unaided. Most researchers in industry therefore focus on extracellular aptamer targets, because of the much easier access of the aptamer drugs to either target molecules.

In vivo delivery of aptamers to intracellular targets has mostly been addressed up to now by incorporating them into liposome vesicles.[6, 68] In clinical trials of another functional therapeutic class of RNA molecules, ribozymes, the drugs seem however to be able to penetrate into patient cells without additional transfection or delivery reagents. The clinical formulations required to date seem only to be simple saline solutions.[94] The same could be true for aptamer therapeutics, but this still needs to be examined. Alternatively, aptamers as 'intrameric' entities are being more and more successfully explored in cell culture systems and animal models by using gene therapeutic approaches and vector delivery systems.[18, 20, 54, 70]

Lastly, we would like to apologize to those whose contributions are not mentioned directly here, as this minireview can only give a flavor of recent developments in this fast-evolving, intriguing field of RNA technology.

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