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Aptamer-Assisted Delivery of Nucleotides with Tumor-Suppressing Properties for Targeted Cancer Therapies

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Abstract

Targeted cancer therapy is developing rapidly according to the fact that it has been demonstrated that this type of therapy can reduce various side effects and adverse events of the commonly available cancer treatment approaches such as chemotherapy and radiotherapy. This selective type of cancer therapy can mediate encouraging outcomes where the frontline cancer treatment methods have failed to do so. Aptamer-assisted delivery of various types of cargoes or the utilization of aptamer for the redirection of delivery vehicles is among various fields of targeted cancer therapy that have gained significant attention lately. Aptamers are single-stranded oligonucleotides or peptide molecules that harbor significant levels of specificity and affinity toward various types of targets such as cell surface antigens, ions, toxins, chemicals, etc. They have shown encouraging results in several types of targeted cancer therapy for the redirection of a variety of cargoes. In this review, we shed the light on the application of aptamers for the delivery of nucleotides such as MicroRNAs (miRNAs), short or small interfering RNAs (siRNAs), and short hairpin RNA or small hairpin RNAs (shRNAs) that harbor tumor suppression properties in various kinds of malignancies.

Keywords: Aptamer, Targeted Cancer Therapy, miRNA, siRNA, shRNA, Delivery Vehicles

1. Context

Novel approaches in the field of cancer treatment have revolutionized the way cancer patients are treated nowadays (1-3). The advent of targeted cancer treatment modalities, such as monoclonal antibodies or chimeric antigen receptor (CAR) T cells, has prompted the idea that targeted cancer therapies can ameliorate the side effects or enhance the therapeutic benefit of conventional cancer treatments. One of such targeted cancer therapies can be based on aptamer (1-3). Aptamers are short single-stranded oligonucleotides (either DNA- or RNA-based) or peptide molecules that have the ability to bind to a specific target molecule (4, 5). These oligonucleotides harbor significant binding affinity toward various targets, which can be of a wide range from cell surface antigens to soluble ligands (4, 5). Aptamers exhibit high affinity and specificity, similar to those of antibodies, because of their unique folding properties, which enable them to fold into tertiary structures (4, 5). However, aptamers suffer from several limitations, including their susceptibility to degradation in biological media or the high rate of renal clearance of naked aptamers, which is due to their small size.

The utilization of aptamers in various fields of research is mainly due to their multiple favorable properties that can be efficiently exploited for the redirection of various delivery platforms towards the tumor cells of interest with a great level of specificity (4, 5). Aptamers came to the spotlight of attention when in 2004, Macugen® (also known as Pegaptanib) became first aptamer approved by the US Food and Drug Administration (FDA) as an anti-angiogenic agent for the treatment of age-related macular degeneration (AMD) (6, 7). In comparison with antibodies, aptamers have a shorter generation time, exhibit more capability for modifications, harbor significant thermal stability, and their production is more cost-effective (4, 5).

To this date, aptamers have been utilized in many fields of investigation. The high affinity and specificity of aptamers allow for their application for clinical diagnostic purposes. They are also used in environmental protection and food safety fields. Aptamers are also used in the detection of pathogen microorganisms such as various types of viruses, bacteria, and parasites (8-23). Cancer recogni-

tion is another field in which aptamers are utilized for the detection of cancer-associated biomarkers such as mucin 1 (MUC1) and human epidermal growth factor receptor 2 (HER2) (24). For recognition purposes, aptamers are also used for the detection of the surface biomarkers of stem cells such as EpCAM, CD133, CD117, and CD44 (25). In addition to the abovementioned applications of aptamers, they are also used for monitoring environmental contaminations, including chemicals and toxins, for the production of biosensors capable of detecting various types of disease-related biomarkers, and they are also used as therapeutic agents (26, 27). Aptamers can also be conjugated to different types of molecules, such as cytotoxic drugs or nucleotides with tumor-suppressing properties (28). Moreover, they can be exploited for the redirection of cargo-loaded delivery vehicles such as liposomes, nanoparticles, and micelles (28). In this review, we discuss examples of aptamers used for the delivery of nucleotides that exhibit tumor-suppressing properties. We also discuss how these delivery platforms can be uniquely beneficial in the field of targeted cancer therapy.

2. Selective Delivery of Oligonucleotides Using Aptamer-Armed Platforms

2.1. Delivery of miRNAs

MicroRNAs, also known as miRNAs or miRs, are a class of 20 - 22 nucleotide-long non-coding small RNAs considered important regulators of various vital cellular functions such as proliferation, differentiation, and apoptosis (29-35). In addition, miRNAs exert such effects through the mechanism of complementary base-paring with their target mRNAs in a perfect or imperfect matching pattern leading to the subsequent degradation of the target; thus directly causing a transcriptional down-regulation or translational repression of the relative genes, which could include various tumor suppressor genes (29-35). Cancer therapeutic strategies leading to the loss of function of various cancer-specific miRNAs through their binding to miRNA-associated gene silencing complexes via fully-complementary base-pairing with synthetic oligonucleotides called “antagomir” or “antimiR” can lead to significantly reduced gene expression profiles of oncogenes as well as a considerably diminished cell viability of tumor cells (36-38).

Specific delivery of particular antimiR oligonucleotides to cancer cells through the targeting of nucleolin (the foremost nucleolar protein in growing eukaryotic cells, which is overexpressed in various types of malignancies) in cells that overexpress this cell surface protein can be utilized as a potent strategy to disrupt the miRNA-mediated oncogenic circuits in these cells (39, 40). Zhang and colleagues have reported the fabrication of a traceable and dual-targeted drug delivery system based on DNA-hybrid-capped mesoporous silica-coated quantum dots (MSQDs) in which the release of the loaded drug (doxorubicin) is controlled by miRNA (miR-21) (41). MiR-21 is one of the oncogenic miRNAs, which is overexpressed in various human cancers. Therefore, the suppression of its expression through delivering antisense oligonucleotides such as antimiR-21 can lead to the activation of caspase-dependent apoptosis and subsequent eradication of the tumor cells in a specific way. Moreover, the antimiR-21 strand can be coupled with a DNA aptamer, which leads to the formation of a DNA hybrid that can specifically recognize antigens overexpressed on the surfaces of the target tumor cells alongside having an exclusive response to miR-21 (which is proceeded through a complementary base-pairing mechanism) (41). In the study by Zhang and colleagues, the mentioned multifunctional MSQDs were loaded with doxorubicin, and they were capped with the DNA hybrid (synthesized by coupling antimiR-21 at the 3’ end of the AS1411 aptamer, a nucleolin-targeting aptamer) by forming 12 base pairs between parts of anti-miR-21 and the anchor-DNA on the nanoparticles resulting in the formation of a DNA gate for the prevention of doxorubicin leakage. These nanocarriers enter the tumor cells upon the recognition of nucleolin by AS1411. Since miR-21 is overexpressed in the cytoplasm of the tumor cells, they play the role of an exclusive key to unlock the doxorubicin gate and meditate its release from the delivery vehicle complex by competing with anchor-DNA for full hybridization with anti-miR-21. Additionally, further enhanced efficacy of the chemotherapy is achieved by the complementary base pairing of anti-miR-21 with miR-21 resulting in the suppression of miR-21 expression (41). In a nutshell, this platform might elevate the therapeutic efficacy while diminishing unwanted adverse effects (41).

Aptamer-redirection of miRNAs has been investigated in various types of cancers, including non-small cell lung cancer (NSCLC), glioblastoma, prostate cancer, breast cancer, and gastric cancer (42-44). Esposito et al. have investigated specific aptamers for the receptor tyrosine kinase Axl conjugated to the let-7g miRNA (42). They have demonstrated that these constructs selectively target Axl-expressing tumor cells and effectively suppress tumor growth in xenograft models of lung adenocarcinoma (42). Moreover, Russo et al. have investigated the Axl-specific aptamer-redirected reintroduction of miR-34c-3p to NSCLC cells (44). NSCLC cells exhibit a decreased level of miR-34c-3p as compared with normal lung cells (44). Therefore, the authors of this study hypothesized that this reintroduction might decrease the proliferation of NSCLC tumor cells in vitro (44). They demonstrated that this method can

suppress tumor cell growth in an efficient manner (44). Additionally, researchers have also investigated the Axl-aptamer-assisted delivery of miR-212 to NSCLC cells (43). TNF-related apoptosis-inducing ligand (TRAIL) is a well-recognized tumor suppressor pathway downregulated in many types of malignancies such as NSCLC (43). Recovering the activity of TRAIL can be achieved through reintroduction or overexpression of miR-212, which can lead to a targeted tumor cell apoptosis-mediated elimination (43). Iaboni et al. demonstrated that Axl-aptamer-assisted delivery of miR-212 to NSCLC cells could lead to selective tumor cell elimination (43).

2.2. Delivery of siRNAs

Short or small interfering RNAs (siRNAs) are a class of 20 - 25 base pair long synthetic double-stranded non-coding RNA molecules, which similar to endogenous microRNA, can operate within the RNA interference (RNAi) pathway to mediate highly efficient and specific post-transcriptional expression silencing of genes that are traditionally considered undruggable (45). Owing to their high therapeutic potential, siRNA-based approaches are being considered for various types of disease treatments, including several cancer types and viral infections (46-49). The application of siRNA-based therapeutics is still hindered by several drawbacks such as the instability of unmodified siRNAs in the bloodstream, their immunogenicity, and their weak cell-membrane crossing capabilities (45, 50). These limitations have encouraged researchers to develop safe siRNA delivery methods for redirecting them to their specific action sites without off-target toxicities or adverse effects (45, 50).

Targeted therapeutic agents consisting of aptamer-siRNA chimeras are currently being appraised for the treatment of several cancer types such as prostate cancer [by targeting prostate-specific membrane antigen (PSMA) and integrin alpha-V beta-3 ($\alpha V\beta 3$)], B cell non-Hodgkin lymphoma [by targeting B-cell-activating factor-receptor (BAFF-R)], and breast cancer (by targeting HER2) (51-56). Aptamer-siRNA chimeras have also been investigated in other fields such as drug hypersensitivity (57) and HIV-1 treatment (58-60).

In one study, an AS1411 aptamer-redirected nanoliposome-based delivery system has been utilized for the co-delivery of the chemotherapeutic drug paclitaxel (PTX) and Polo-like kinase 1-targeted siRNA (PLK1-targeted siRNA) to breast cancer cells (61). PLK1 is a highly conserved serine/threonine protein kinase with important regulatory mitotic effects whose high expression levels have been significantly associated with abnormal tumor cell proliferation, metastasis, angiogenesis, and tumor prognosis in various types of cancers, including breast cancer (62, 63). Therefore, PLK1 can be considered a promising primary target candidate for cancer treatment, including PLK1-targeting RNAi-based gene therapy (61, 64-66). The simultaneous co-delivery of PTX and siRNA proposed by the mentioned study could result in a synergistic incremental pattern of apoptotic cancer cells and diminished angiogenesis (61). Therefore, this method may exhibit various advantages over methods separately delivering PTX and siRNA (61). It could also demonstrate a valuable potential for suppressing the growth of breast cancer in preclinical models (61).

In another example, Zhou et al. have utilized anti-BAFF-R aptamers for the redirection and delivery of nanoparticles loaded with the STAT3 siRNAs (67). They have demonstrated that the BAFF-R aptamers can specifically redirect the nanoparticles toward various B cell lines (67). This action is followed by the internalization of the nanoparticles, which eventually leads to the disruption of STAT3 mRNAs (67).

Moreover, PSMA is a very popular target antigen targeted in investigations studying aptamer-assisted redirection platforms. In this regard, Wullner et al. conjugated siRNAs specific for eukaryotic elongation factor 2 mRNA (eEF2K) to PSMA-targeting aptamers (68). Inhibiting EEF2 can mediate protein synthesis blockade leading to apoptosis in the PMSA-expressing prostate cancer cells (68). Moreover, other researchers have generated aptamer-siRNA chimeras made of two anti-PSMA aptamers in between which two siRNAs, one specific for EGFR and the other one specific for survivin, are located (69). The authors have reported that these chimeras can suppress EGFR and survivin expression at the same time and mediate apoptosis both in vitro and in vivo in an efficient manner (69).

2.3. Delivery of DNAzymes

Deoxyribozyme (also known as DNA enzyme, DNAzyme, Dz, or catalytic DNA), is another example of nucleotides with therapeutic properties. They are synthetic single-stranded DNA molecules capable of mediating chemical or catalytic reactions on particular nucleic acid targets similar to those of other biological protein-based enzymes or ribozymes (70-72). DNAzymes have been at the center of attention mainly due to their outstanding advantages, including their affordability, stability properties, and easy biosynthesis process (70-72).

DNAzymes has been proven to be capable of cleaving β-catenin and survivin mRNA and BCR-ABL transcripts, which further proves their potent role in growth inhibition of tumor cells alongside justifying the numerous attempts made for the development of efficient DNAzyme delivery platforms such as Poly(lactic-co-glycolic acid)

(PLGA) microspheres, transferrin modified PEGylated polyplexes, poly-L-Lysine (PLL) microspheres, nanoparticulate systems, and dendrimers (73-77). These delivery platforms can be redirected towards tumor cells of interest using aptamers targeting tumor cell surface antigens. Such platforms can selectively deliver these delivery vehicles without targeting normal cells.

2.4. Delivery of shRNAs

Short hairpin RNAs or small hairpin RNAs (shRNAs), also known as hairpin vectors, are artificial RNA molecules biosynthesized exogenously or transcribed from RNA polymerase III promoters in vivo (78). These molecules are capable of inducing stable and heritable gene silencing effects with high specificity via RNAi pathway, thus allowing for the generation of continuous gene-modified cell lines or transgenic animals (78). After the generation of the shRNA transcripts, they are processed and loaded into RNA-induced silencing complex (RISC) in the cytoplasm undergoing further cytoplasmic RNAi processing (79). As the story is with plasmids, shRNAs encounter difficulties passing cellular membranes and migrating to the nucleus; therefore, their efficient delivery into target cells requires specific carriers such as nanocarriers or dendrimers capable of overcoming such obstacles (80-82).

One study has developed a novel targeted delivery platform for specific delivery of shRNA plasmids through the targeting of nucleolin ligand on target cancer cells (83). This targeted shRNA delivery system is composed of alkyl-modified polyamidoamine (PAMAM) dendrimers with 10-bromodecanoic acid (10C) and 10C-PEG to improve the efficiency of transfection, shRNA plasmid for specific knockdown of Bcl-xL protein, and the AS1411 aptamer for targeted delivery towards nucleolin over-expressing cancer cells (83). Dendrimers are star-shaped structures with numerous branches whose dimensions do not exceed nanometer scales. The fate of living cells is determined by the balance between the pro-apoptotic members of the Bcl-2 family, such as BAX, BAK, and BOK, which act by protecting the outer mitochondrial membrane and inhibiting the release of cytochrome c and the anti-apoptotic members, including Bcl-2, Bcl-xL, and MCL1. Selective silencing of Bcl-xL can be exploited as a strategy for apoptosis induction in cancer cells since the high level of Bcl-xL expression has been reported in numerous solid tumors such as bladder and gastric cancer (83-86). Without causing considerable cytotoxicity, the abovementioned targeted shRNA delivery system could efficiently downregulate the expression of Bcl-xL up to 25% and induce strongly selective late apoptosis in 14% of target cancer cells while exhibiting improved transfection efficiency in comparison to non-targeted vectors (83). In a nutshell, this strategic delivery system demonstrates that efficient and targeted apoptosis induction in various cancer cells through the knockdown of Bcl-xL expression using shRNAs can be achieved through aptamer-assisted redirection of delivery vehicles such as PAMAM dendrimers (83).

Moreover, Kim et al. have investigated the co-delivery of shRNAs specific for Bcl-xL and the chemotherapeutic agent doxorubicin using polyplexes redirected toward prostate cancer cells using anti-PSMA aptamers (87). They have reported that this construct effectively targets PSMA-expressing prostate cancer cells in a very selective manner (87). These results indicate that co-delivery of chemotherapeutic agents and shRNAs (such as the anti-Bcl-xL shRNA) can selectively target cancer cells and eliminate them with a significant level of specificity (87).

Furthermore, other researchers have generated aptamers harboring affinity and specificity for the HIV integrase (88). They have developed shRNA-aptamer fusions by joining the aptamers as the terminal loop of shRNAs targeting HIV Tat-Rev (88). These researchers have reported that the shRNA-aptamer fusions (using an aptamer named S3R3) can efficiently block HIV replication even in a long-term manner (88). They have also indicated that these shRNA-aptamer fusions exhibit similar suppression properties as those of the integrase inhibitor raltegravir (88). Such data can suggest that aptamer-shRNA fusions may have a bright future ahead of them and may be used for fighting against viral infections that can mediate malignancies such as the human papillomavirus (HPV).

3. Summary and Perspectives

Herein, we discussed the potential and application of aptamers specific for different targets utilized for the targeted delivery of various types of nucleotides with tumor growth suppression characteristics. Broadening the validity of the herein discussed platforms can be achieved through in-depth assessments and preclinical models of malignancies, especially where only in vitro assessments have been reported. Moreover, as we discussed throughout the article, the selective delivery capability of aptamers could be exploited for the treatment of viral infections and many other conditions as well. Special efforts should be made to be able to use innovative platforms for achieving such aims. Furthermore, alongside the types of malignancies popular in the field of aptamer-assisted cargo delivery investigations, other types of less investigated malignancies should also be considered since it is speculated that such outcomes can be achieved for their treatment as well. It is worth mentioning that there are still limitations surrounding this type of therapy. These limitations may in-

clude the off-tumor targeting toxicity of these platforms that can overshadow the potential of this type of cancer therapy. Therefore, discovering new strategies for tacking this hurdle is a factor of paramount importance.

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