Recent advances of DNAzyme-based nanotherapeutic platform in cancer gene therapy

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Received: 26 June 2020 / Accepted: 8 October 2020 / Published online: 20 November 2020

Abstract Deoxyribozyme (or denoted as DNAzyme), which is produced by *in vitro* screening technology, has gained extensive research interest in the field of biomedicine due to its high catalytic activity and structure identification. This review introduces the structural characteristics of RNA-cleaving DNAzyme and its application potential in cancer gene therapy, which plays a significant role in cancer-related gene inactivation by specifically cleaving target mRNA and inhibiting the expression of the corresponding protein. However, the low delivery efficiency and cellular uptake hindered the widespread usage of DNAzyme in gene therapy of cancers. Emerging nanotechnology holds great promise for DNAzyme to overcome these obstacles. This review mainly focuses on DNAzyme-based nanotherapeutic platforms in gene therapy of cancers, including oncogene antagonism therapy, treatment resistance gene therapy, immunogene therapy, and antiangiogenesis gene therapy. We also revealed the potential of DNAzyme-based nanotherapeutic platforms as emerging cancer therapy approaches and their security issues.

Keywords DNAzyme, Cancer therapy, Gene-silencing, Nanocarrier

INTRODUCTION

DNAzymes have been demonstrated to specifically perform the functions of the degradation of target mRNA, ligation, or phosphorylation of DNA, and so on. They are obtained by *in vitro* screening technology and show high structure recognition ability (Fu and Sun 2015; Hollenstein 2015). Since the first proposition in 1994 by Breaker, various DNAzymes have been discovered. Among these DNAzymes, the one with RNA-cleaving activity has attracted extensive

research interests due to its great potential in cancer therapy (Zhou *et al.* 2017). After the catalytic cleaving target mRNA, the expression of corresponding proteins is then downregulated. To realize the gene-silencing, the structure of the DNAzyme should be integrated. Each DNAzyme has a core catalytic zone which flanked by two-side recognition arms. The longer the recognition arms, the more stable the DNAzyme binds to its substrates by Watson-Crick basis pairing (Dass *et al.* 2008; Fokina *et al.* 2012; Santoro and Joyce 1997; Silverman 2005). In this way, the special sequence of recognition arms determines the specificity of the DNAzyme and stabilizes the catalytic core of the DNAzyme to realize the hydrolysis reaction (Khachigian 2019). Of all types of DNAzymes, 10-23 DNAzyme is the most popular one

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because it can cleave almost any phosphodiester bond between the unpaired purines and the paired pyrimidine (Xu *et al.* 2012). In the presence of specific metal ions such as Mg^{2+} (Cao *et al.* 2020), Pb^{2+} (Lan *et al.* 2010), Mn^{2+} (Wang *et al.* 2019a), Cu^{2+} (Liu and Li 2007), and Na^{+} (Zhou *et al.* 2016), DNAzyme can successfully cut off the target mRNA by catalyzing the hydrolysis of the phosphodiester of target RNA (Fig. 1). Therefore, DNAzyme may become a potential and powerful tool for gene-regulating and provide a novel gene therapy approach for cancer therapy.

As an important tool of gene therapy, DNAzyme shows some inherent advantages compared with other enzymes. Firstly, DNAzyme has unparalleled structural stability than protein and ribozyme. Secondly, it can cut target mRNA more accurately than the ribozyme without any immune responses and significant cytotoxicity. Furthermore, DNAzyme has the characteristics of high specificity, easy modification and functionalization, and low cost (Li *et al.* 2018; Ren *et al.* 2019; Silverman 2016). Theoretically, the DNAzymes can be used to silence any genes as we expect which are preferable for cancer treatment. The specific catalytic activity of DNAzymes makes them applicable for precise gene therapy. Following the study of bcr-abl oncogene-targeted DNAzyme for acute lymphoblastic leukemia, DNAzyme acts as a novel class of biotechnology-derived drugs and has shown great significance in the field of cancer gene therapy (Wu *et al.* 1999). Although some breakthroughs have been made, there are still some unavoidable challenges in applying DNAzyme in cancer treatment. The first key issue is how to efficiently deliver DNAzymes to cancer cells. DNAzymes have to overcome multiple physiological barriers during transportation from the administration site to cancer tissues, including high interstitial pressure of the tumor tissue, the abundant tumor extracellular matrix (ECM), and high risk of being degraded by nucleases (Farrokhi *et al.* 2018;

Xiao *et al.* 2019). Therefore, it is necessary to explore stable vectors to improve the efficiency of DNAzymes for overcoming these obstacles. Another key issue is that the cofactors in organisms are insufficient to support the catalytic activity of DNAzymes, which reduces their gene-silencing efficiency (Feng *et al.* 2018; Roma-Rodrigues *et al.* 2020).

In recent years, emerging nanomedicine brings new opportunities for cancer gene therapy. The nanocarriers with a proper size can prevent DNAzymes from degradation and deliver the DNAzymes to tumor cells after intravenous administration by “enhanced permeability and retention (EPR) effect” and positive targeting (Davis *et al.* 2008; Maeda *et al.* 2000). Thus, a nanotherapeutic platform is an effective means to improve the stability, catalytic capacity, and the delivery efficiency of DNAzymes (Roma-Rodrigues *et al.* 2020; Xiao *et al.* 2019). In this review, we summarized recent progress in the development of DNAzyme-based nanoplatform for oncogene antagonism, reversing therapeutic resistance, immunogene therapy, and antiangiogenesis in gene therapy of cancers and made an outlook on their clinical application in the future.

THE APPLICATION OF DNAZYME-BASED NANOPLATFORMS IN CANCER GENE THERAPY

Cancer progression is a complex and multi-step process, which involves the mutation or abnormal expression and regulation of multiple cancer-associated genes including activation of oncogenes, high expression of apoptosis suppressor genes, and drug-resistant genes in tumor cells, and so on. A series of gene therapy strategies have been designed to target these cancer-associated genes, such as oncogene antagonistic therapy, antiangiogenic therapy, immune gene therapy, and drug-resistant gene therapy. Based on the remarkable advantages of DNAzyme, gene intervention strategy using an RNA-cleaving DNAzyme has been one promising alternative for cancer therapy. Recent progress on DNAzyme focuses on how to develop smart nanoplatforms for efficiently delivering DNAzyme for cancer gene therapy.

DNAzyme-based nanoplatforms for oncogene antagonism

More and more studies show that both oncogenes and antioncogenes play key roles in regulating the proliferation and metastasis of tumor cells. In general, various stimuli including virus infection, chemical carcinogens, and radiation can activate the oncogenes to

Fig. 1 The action mechanism of DNAzyme with RNA-cleaving activity

promote the progression of cancers. Therefore, the oncogenes can be used as ideal targets for DNAzymes to enhance the therapeutic effect.

Unlimited proliferation is one characteristic that distinguishes cancer cells from normal cells. C-jun is a member of the activating protein 1 (AP-1) transcription factors that participate in cellular proliferation, transformation, and apoptosis. It was reported that the overexpression of c-jun in cancer cells could increase the activity of AP-1, which was closely related to the highly invasive property of malignant cancers (Peng *et al.* 2016). Tan *et al.* established a c-jun mRNA-targeting DNAzyme (Dz13) enfolded chitosan nanoparticles (Dz13-NP) with a size of 50–300 nm. The *in vivo* and *in vitro* results demonstrated the high stability of Dz13-NP as well as the activity of cleaving c-jun mRNA thus inhibiting tumor growth (Tan *et al.* 2010). Meng *et al.* demonstrated the effectiveness and feasibility of DNA tetrahedron as DNAzyme nanocarriers for gene-silencing therapy. These nanostructures have high cellular uptake ability to downregulate the c-jun mRNA, resulting in the inhibition of cell proliferation (Fig. 2) (Meng *et al.* 2019). The miR-21 is an endogenous non-coding short single-stranded RNA, and emerging evidence shows that the overexpression of miR-21 is related to the proliferation and invasion of cancer cells. Recently, Ren *et al.* demonstrated that DNA tetrahedron could be used to co-delivery (anti-miR-21) 17E DNAzyme, ZY11-targeting aptamer, and doxorubicin (DOX) for synergistic cancer therapy (Ren *et al.* 2019). This nanoplatform shows favorable tumor targeting and pH responsiveness property to achieve remarkable gene-silencing efficiency. The results indicated that the DNA tetrahedron was a promising delivery nanocarrier for tumor-targeted multimodal therapy. In addition, a study by Wang and coworkers reported a DNA methyltransferase (DNMTs)-specific DNAzyme which was used to significantly suppress tumor growth in the

model of bladder cancer mouse (Wang *et al.* 2015). Li *et al.* designed a multifunctional DNA nanoscorpion functioned with specific aptamers and human epidermal growth factor receptor (HER2) mRNA-targeting DNAzyme. The results displayed that the DNA nanoscorpion had the capability of efficiently targeting and downregulating specific targets to suppress tumor growth (Li *et al.* 2018). To enhance the catalytic efficiency of DNAzyme due to the lack of cofactors, a study by He and his colleagues revealed the availability of *in situ* cofactor generation strategy for gene-silencing therapy (He *et al.* 2016). They exploited a dual-functional nanocomposite equipped with gold nanoparticles (GNPs), AS1411 aptamer, anti-miR-21 DNAzyme, DOX, and acid-decomposable ZnO quantum dots as a self-generation pool of Zn^{2+} ions, to realize the high efficiency of intracellular gene-silencing. Similarly, Wang *et al.* synthesized DNAzyme nanosponges (NSs) which were composed of multivalent tandem aptamer sequences, DNAzymes, ZnO NPs, and DOX. The NSs collapsed in the acidic condition to generate Zn^{2+} , which acted as not only the DNAzyme cofactors to mediate the self-catabolic activity of DNAzyme, but also the therapeutic reactive oxygen species generators. This novel and intelligent self-driven drug delivery system might be engineered with more therapeutic agents and shows great promise and versatility for biomedicine applications. (Wang *et al.* 2019b). In addition, nano-scaled zeolitic imidazolate framework-8 (ZIF-8) NPs, which collapsed in the acid conditions to generate Zn^{2+} , were used as delivery vehicles of DNAzymes to overcome the limitation mediated by insufficient of cofactors and promote the early growth response-1 (EGR-1)-associated antiproliferation efficacy (Wang *et al.* 2019a). These smart nanocarriers not only protect DNAzymes from digestion by endogenous nucleases, but also increase the targeted oncogenes silencing activity of DNAzyme in the cytoplasm, leading to enhanced antiproliferation effect.

Fig. 2 **A** The self-assembled TDNs modified with a c-jun targeting DNAzyme realize higher cellular uptake efficiency and gene-silencing activity (Meng *et al.* 2019). **B** A multifunctional nanocomposite probe consisted of GNPs, DNAzyme, Dox, AS1411 aptamer, and ZnO QDs for achieving intracellular gene regulation and anticancer drug delivery (He *et al.* 2016)

Apoptosis has an essential role in the occurrence, progression, and metastasis of cancers. In most cancers, the inhibitors of apoptosis proteins (IAP) that contribute to tumor cells apoptosis resistance are overexpressed. Therefore, the development of nanotherapeutic platforms targeting IAP has attracted much attention. Survivin is a powerful antiapoptotic gene and overexpressed in most cancers. For the last few years, our laboratory has been working on developing DNAzyme-based multifunctional nanocarriers that target apoptosis-related genes to enhance cancer therapy outcomes. Recently, we synthesized a biodegradable, dual-target nanoflowers (DNFs) functionalized with antisurvivin DNAzyme, anti-EGR-1 DNAzyme, and AS1411 aptamer using rolling circle amplification (RCA) strategy. The DNFs degrade at acidic condition due to the decomposition of the co-assembled magnesium pyrophosphate framework, generating abundant Mg^{2+} . The additive Mg^{2+} could serve as the cofactor of the DNAzyme to enhance its efficiency to cleave apoptosis-related target mRNAs (Fig. 3). We predicted that this biodegradable, multifunctional therapeutic system has great potential for high efficiency gene therapy (Jin *et al.* 2017). In addition, antisurvivin DNAzyme could also be used to

enhance the efficiency of photodynamic therapy (PDT). We found that the upconversion nanoparticles (UCNPs) could work as energy donors of photosensitizers (TMPyP₄) to activate the PDT in the presence of NIR light. However, the overexpressed antiapoptotic proteins would increase the difficulty of PDT treatment. In this work, the loaded DNAzymes could efficiently silence the survivin gene and amplify the therapeutic outcome. As a result, this nanoplatform would be used as an admirable alternative to enhance the efficiency of PDT with the help of adjuvant gene therapy (Jin *et al.* 2020). These studies demonstrated the feasibility and effectiveness of the IAP targeting DNAzymes to synergistically enhance therapeutic performance. EGR-1 is another key factor in regulating cell proliferation, differentiation, and apoptosis of malignant tumors. Wang *et al.* employed MnO_2 nanosheets to deliver EGR-1 DNAzymes and photosensitizers toluidine blue (TB) (Wang *et al.* 2019c). The MnO_2 nanosheets were employed as both delivery carriers and the pool of DNAzyme cofactor due to their appealing physicochemical properties. The MnO_2 nanosheets were degraded by the intracellular GSH to produce Mn^{2+} , which served as cofactors of DNAzyme to enhance the silencing efficiency. Meanwhile, the

Fig. 3 DNAzyme-based nanotherapeutic platform in oncogene antagonistic therapy. **A** The DNFs target the cancer cell to achieve efficient dual gene-silencing (Jin *et al.* 2017). **B** The fol-DNAzyme-MnPDA nanoplatform as a versatile vehicle for multimodal imaging-guided gene regulation and photothermal therapy. (Feng *et al.* 2018). **C** The multifunctional DNAzyme-assisted upconversion nanoplatform for enhanced PDT by DNAzyme-mediated gene-silencing of survivin (Jin *et al.* 2020)

consumption of GSH further improved the PDT efficiency. Similarly, the Ce6-DNAzyme-MnO₂ nanosystem was synthesized by Fan *et al.* to overcome the limitation stemmed from the inefficiency and poor biostability of DNAzyme (Fan *et al.* 2015). Apart from these researches, a recent study by Feng *et al.* utilized the polydopamine-Mn²⁺ nanoparticles (MnPDA) as the vehicles of EGR-1 targeting DNAzyme (Feng *et al.* 2018). Results showed that the MnPDA not only downregulated the EGR-1 mRNA efficiently but also served as magnetic resonance imaging (MRI) contrast agents, thus realizing the integration of tumor detection and treatment.

Metastasis, as a prominent feature of malignant tumors, is a major factor affecting prognosis. Silencing metastasis-related mRNA will attenuate the probability of tumor metastasis. Xing *et al.* used the N-acetyl-L-leucine-polyethylenimine (N-Ac-L-Leu-PEI) as the DNAzyme vectors to targeted silence aurora kinase A mRNA. The nanocomplex presented excellent cellular uptake ability and great potential in cleaving target mRNA, which would induce apoptosis and inhibit tumor metastasis (Xing *et al.* 2015). Integrin is a member of cell adhesion molecules (CAM) which affects the growth, proliferation, and invasion of tumors through mediating the interaction between cancer cells and the ECM. Wiktorska *et al.* employed liposomes to transfect β 1-targeting DNAzyme for specifically cleaving β 1 integrin mRNA and eradicating the invasive cancer cells (Wiktorska *et al.* 2010). All these reports clearly demonstrated the effectiveness of DNAzyme in attenuating the probability of tumor metastasis.

DNAzyme-based nanoplatforms for treatment resistance

Treatment resistance, whether inherent or acquired, is a major problem leading to the failure of cancer therapy. Crafty cancer cells have evolved various resistance mechanisms that protect them from being killed by chemotherapy drugs, heat, and radiation. For example, the blockage of the apoptosis pathway, the overexpression of multidrug resistance-related proteins, and the stress protective function of cancer cells contribute to treatment resistance (Ramos and Bentires-Alj 2015). Therefore, it is necessary to develop effective approaches to restore the sensitivity of cancer cells to various treatments, which might provide new therapeutic options for cancer therapy. In this respect, our laboratory has done some work on developing multifunctional nanoplatforms for overcoming the therapeutic resistance. We synthesized a DNAzyme nanosponges by assembling a cationic polymer polyetherimide (PEI) and

a long single-stranded DNA encoded with multivalent DNAzymes for avoiding thermo-resistance in photothermal therapy (PTT). The results showed the capability of these nanosponges to downregulate the heat shock protein 70 (HSP70) mRNA for reversing the resistance of cancer cells to hyperthermia (Fig. 4) (Jin *et al.* 2018). Due to its simple synthesis, good biocompatibility, and high efficiency, nanosponge-ICG is a promising therapeutic drug for PTT. Triple-negative breast cancer (TNBC) is the most difficult type of breast cancer to treat due to its severe therapeutic resistance to chemotherapy or radiotherapy. To increase the sensitization of TNBC cells to chemotherapy drugs, we constructed a NIR-light activated combination therapeutic nanoplatform using mesoporous silica-coated gold nanorods (Au@MSNs) modified with survivin targeting DNAzyme. Once exposed to NIR-light irradiation, the absorbed NIR light by gold nanorods was converted into heat, which triggered the release of DNAzyme to downregulate survivin mRNA for sensitizing MDA-MB-231 cells, resulting in enhanced PTT effect (Sun *et al.* 2018).

Multidrug resistance (MDR) has been the primary obstacle that contributes to the failure of chemotherapy. It was reported that more than 90% of the death with malignant tumors are related to MDR. Up to now, a variety of drug resistance-related factors have been discovered. For example, the multidrug resistance gene 1 (MDR1) encodes the plasma membrane P-glycoprotein (P-gp), which is a member of the ATP-binding cassette (ABC) transporter, functions as the energy-dependent “drug pump” to flush out the lipophilic chemotherapy drugs, resulting in the decrease of intracellular drugs (Gao *et al.* 2006). Zokaei *et al.* synthesized an MDR1-targeting DNAzyme delivery platform using chitosan β -cyclodextrin nanocomplexes. After the silencing of the MDR1 gene, DOX-resistant breast cancer cells MCF-7/DR showed enhanced sensitivity toward DOX, achieving a high antitumor therapy effect (Zokaei *et al.* 2019). In addition, MDR can also be reversed by reducing non-MDR-associated proteins, such as pro-apoptotic c-jun protein. Sun *et al.* used mesoporous silica nanoparticles (MSNs) to deliver c-jun targeting DNAzyme to attenuate the drug efflux because silencing c-jun mRNA mediated the downregulation of multidrug resistance-associated protein 1 (MRP1), which is bound up with the acquired MDR in metastatic prostate cancer (Sun *et al.* 2017). Dass *et al.* also indicated the role of DNAzymes-mediated silencing c-jun gene in reversing the drug resistance of osteosarcoma. The *in vitro* experiments demonstrated its role in restoring the chemosensitivity as well as

Fig. 4 The synthesis of DNAzyme-based nanosponges (A) and enhanced PT effect by silencing HSP70 mRNA (B) (Jin *et al.* 2018)

inhibiting tumor metastasis (Dass *et al.* 2008). Bcl-xl has also been demonstrated to be associated with apoptosis and drug resistance (Amundson *et al.* 2000). Yu *et al.* developed a Bcl-xl targeting DNAzyme system to restore the sensitivity of the drug resistance cells to taxol (Yu *et al.* 2014). Although treatment resistance is an intractable issue that could not be completely avoided, DNAzymes show great potential in silencing treatment resistance-related mRNA to recover the sensitivity of cancer cells. All the above reports revealed the excellent efficiency of DNAzyme-based nanoplateforms in enhancing the overall therapeutic effect.

DNAzyme-based nanoplateform for immunogene therapy

Cell-mediated adaptive immunity plays an important role in the antitumor immune response. T lymphocytes mainly consist of CD8⁺ cytotoxic T cells and effector CD4⁺ T cells. CD8⁺ cytotoxic T cells can be activated by the stimulus signals from dendritic cells (DCs) and CD4⁺ T cells, directly killing tumor cells, while CD4⁺ T cells have a role in the proliferation and activation of CD8⁺ cytotoxic T cells and the establishment of memory. Meanwhile, CD4⁺ T cells can not only kill tumor cells by the cytokines that they secrete, such as TNF- α , but also directly destroy tumors by recognizing major histocompatibility complex (MHC) molecules on the surface of tumor cells (Raphael *et al.* 2020; Haabeth *et al.* 2014). Therefore, selectively silencing specific tumor-related genes by DNAzyme can be used to directly or indirectly activate the adaptive immune of the body for enhanced antitumor effect.

Previous researches have demonstrated that Dz13 could induce adaptive immunity (Khachigian *et al.* 2012). The preclinical safety and *in vivo* efficiency of

Dz13 were confirmed in various tumor-bearing mice. Subsequently, the safety and tolerability of Dz13 in human beings were assessed in phase 1 clinical study in 2013. The results demonstrated that the tumor regressed significantly without any side effect after the intratumoral administration of DNAzyme-loaded lipid. Immunohistochemical staining showed that the antitumor effect was ascribed to the increased expression of apoptosis markers caspases 3, 8, 9, and P53, and the decreased expression of Bcl-2 and MMP-9. Meanwhile, the expression of CD4 and CD8, markers of T-lymphocyte infiltration into basal-cell carcinoma, was increased, indicating stimulated infiltration of immune cells (Cho *et al.* 2013). Cai *et al.* demonstrated that Dz13 could induce adaptive immunity in skin cancers. The DNAzyme increased the content of CD8⁺ and CD4⁺ T cells three-to-four-fold in immunocompetent mice, resulting in enhanced antitumor immunity, which contributes to the enhanced antitumor effect (Cai *et al.* 2012). In subsequent work, they tested the antitumor immunotherapy effect of a c-jun targeting DNAzyme. They demonstrated the capability of DNAzyme-loaded lipid to downregulate the expression of c-jun and increase the content of CD4⁺ T cells in the tumors. Thus, the DNAzyme-loaded lipid can effectively inhibit B16F10 tumor growth and abscopal tumor relapse through adaptive immunity (Cai *et al.* 2018). To improve the intracellular delivery efficiency of DNAzyme, Peng *et al.* used nanosize metal-organic frameworks (MOFs) to deliver DNAzyme into RAW264.7, CD4⁺ T cells, and B cells. These MOFs not only exhibit excellent transfection efficiency in mammalian immune cells than conventional transfection agents, but also reversibly uptake and release DNAzyme in cancer cells, and protect them from digestion by nuclease (Peng *et al.* 2018). In addition, innate immunity also plays a role in defending against cancer. Jang *et al.*

developed a treatment platform containing DNAzyme and CpG sequence to simultaneous silence epidermal growth factor receptor (EGFR) to promote the apoptotic cell death and activate Toll-like receptor 9 (TLR9)-dependent p38 MAPK signaling pathway via the innate immune response and resulted in a stronger inhibition of non-small-cell lung cancer proliferation (Jang *et al.* 2018). Compared with traditional antibody therapy and adoptive cell therapy, DNAzyme-based immunogene therapy is more economical and safe. Therefore, the DNAzyme-based nanoplatforms provide a new approach for tumor immunotherapy.

DNAzyme-based nanoplatform for antiangiogenesis

Angiogenesis plays a crucial role in the growth and metastasis of malignant tumors (Cully 2017). The newly formed blood vessels could transport plentiful oxygen and nutrients deep into the tumor to promote the rapid proliferation of tumor cells. Thus, the antiangiogenesis strategy is determined to be a promising approach for blocking the progression of malignant tumors. At present, the antiangiogenic drugs approved by the U.S. FDA show serious side effects. Therefore, it is necessary to develop more safe and effective antiangiogenic adjuvant therapy strategies. Antiangiogenesis gene therapy strategies involve the expression inhibition of proangiogenic molecules, as well as the apoptosis induction of vascular endothelial cells (Lugano *et al.* 2020).

As we know, tumor angiogenesis involves multiple signaling molecules, such as VEGF, VEGFRs, and matrix metal proteinases (Goel and Mercurio 2013). Many researchers have reported that the inhibition of angiogenesis could be realized by the downregulation of VEGF or its tyrosine kinase receptor through DNAzyme-based gene-silencing. Zhang *et al.* used transcatheter arterial chemoembolization (TACE) technology to efficiently deliver the VEGFR-1 targeting DNAzyme into tumors. Results showed that the expression level of VEGFR-1 mRNA in rabbit corneal endothelial cells and tumor tissue decreased. After treating with DNAzyme, the permeability of tumor microvascular was suppressed through non-invasive dynamic contrast enhanced magnetic resonance imaging (DCE-MRI) (Zhang *et al.* 2016). Shen *et al.* designed a VEGFR-1 mRNA-targeting DNAzymes, which could efficiently inhibit the VEGFR-1 expression, tube formation, and tumor growth in both murine B16 melanoma and human xenograft nasopharyngeal carcinoma (NPC) models (Shen *et al.* 2013). Another remarkable research of antiangiogenesis was proposed by Yang and his colleagues. They designed a latent membrane protein 1

(LMP1)-targeted DNAzyme (DZ1) to interfere with the tube formation ability of the endothelial cell and downregulated the secretion of VEGF of tumor cells. *In vivo* experiments, results showed that the DNAzyme significantly inhibited the growth of tumors and reduced the permeability of tumor vessels (Yang *et al.* 2015). Researches have shown that matrix metalloproteinase-9 (MMP-9) is involved in multiple tumor events such as angiogenesis, the proliferation, and metastasis of tumor cells (Egeblad and Werb 2002). Hallett *et al.* used an anti-MMP-9 DNAzyme (AM9D) for breast cancer treatment. AM9D inhibited the invasion of breast cancer cells and reduced the volume of the tumor. Moreover, AM9D reduced the vascularization of tumors and induced more apoptosis of tumor cells compared with the untreated group (Hallett *et al.* 2013). Although DNAzyme-based gene therapy for antiangiogenesis is at the preliminary stage of experimental research, the results are encouraging.

CONCLUSION AND PROSPECTS

RNA-cleaving DNAzymes have been considered as promising therapeutic reagents in cancer treatment due to their excellent properties, which include easy synthesis and modification, high stability, high cleavage specificity, and catalytic activity. However, inefficient delivery hinders the widespread application of DNAzymes in cancer therapy. Therefore, it is a big challenge for DNAzyme to transport from the administration site to blood circulation, go through the tumor stroma and specifically recognize the cancer cells. For the past few years, remarkable progress has been made in the development of DNAzyme-based nanotherapeutic platforms for cancer gene therapy, which brings hope for the clinical application of these DNAzymes. In this review, we briefly discussed the DNAzyme-based nanotherapeutic platform as promising gene therapy approaches in cancer therapy, including oncogene antagonism therapy, treatment resistance gene therapy, immunogene therapy, and antiangiogenesis gene therapy. New exciting researches for DNAzyme-based gene suppression of adjuvant cancer therapy are underway. First, we would like to point out that more focus could be paid on developing self-supported multifunctional nano-delivery systems to promote the delivery of DNAzymes for enhancing the antitumor therapeutic efficacy. Second, immunotherapy has been demonstrated to an effective therapeutic strategy to combat with cancer. However, only a few successful examples that employed DNAzymes to enhance the overall immunotherapy outcome.

Therefore, more attention could be paid to silence immunosuppression genes using DNAzymes for amplifying the immunotherapy effect. In addition, the biosafety of the DNAzymes or the nanocarriers should be put into consideration in further studies. Although the DNAzyme-based nanotherapeutic platform is still in its infancy as a cancer gene therapy approach, the development of multifunctional nanocarriers for DNAzyme delivery and cancer gene therapy is likely to open new avenues for cancer therapy in the future.

Acknowledgements The work was supported by the National Natural Science Foundation of China (21603051), the Natural Science Foundation of Hebei Province (B2018201221, B2018201157), Hebei Youth top talent project, Key Projects of Education Department of Hebei Province (ZD2018036), and Undergraduate Innovation and Entrepreneurship Training Program of Hebei University (2020351).

Compliance with Ethical Standards

Conflict of interest Wendi Huo, Xiaona Li, Bei Wang, Haoran Zhang, Jinchao Zhang, Xinjian Yang, and Yi Jin declare that they have no conflict of interest.

Human and animal rights and informed consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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