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Application and prospects of nucleic acid nanomaterials in tumor therapy

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Cancer poses a great threat to human life, and current cancer treatments, such as radiotherapy, chemotherapy, and surgery, have significant side effects and limitations that hinder their application. Nucleic acid nanomaterials have specific spatial configurations and can be used as nanocarriers to deliver different therapeutic drugs, thereby enabling various biomedical applications, such as biosensors and cancer therapy. In recent decades, a variety of DNA nanostructures have been synthesized, and they have demonstrated remarkable potential in cancer therapy related applications, such as DNA *origami* structures, tetrahedral framework nucleic acids, and dynamic DNA nanostructures. Importantly, more attention is also being paid to RNA nanostructures, which play an important role in gene therapy. Therefore, this review introduces the developmental history of nucleic acid nanotechnology, summarizes the applications of DNA and RNA nanostructures for tumor treatment, and discusses the development opportunities for nucleic acid nanomaterials in the future.

1. Introduction

Cancer has become a significant threat to human health because of its extremely high fatality rate. According to a report by the International Agency for Research on Cancer, approximately 10 million people succumbed to cancer in 2020, and the number of cancer patients will increase by 60% globally in the next 20 years.[1–3] Current clinical treatments for cancer mainly include radiotherapy, chemotherapy, and surgery; however, these treatments have shortcomings. Surgical treatment is only suitable for the early stages of cancer and the recurrence rate of cancer after surgery remains high, whereas radiotherapy and chemotherapy have toxic side effects on normal tissues and are prone to drug resistance. Therefore, the development of new tumor treatment regimens has become an important research topic in recent years. Compared with traditional antitumor drugs, nucleic acid nanomaterials possess excellent programmability, narrow particle size distribution, low cytotoxicity, and stable spatial structures; therefore, have the potential to improve the stability, delivery efficiency, and targeting ability of traditional chemotherapy drugs.[4,5]

DNA is an important biological material that carries genetic information. Due to the stability of complementary base pairing, DNA is regarded as a bionanomaterial with extremely wide application prospects.[6,7] In recent years, a variety of nucleic acid nanomaterials, such as DNA *origami* structures, tetrahedral framework nucleic acids (tFNAs), and dynamic DNA nanostructures have been synthesized and widely used in the field of targeted delivery of antitumor drugs. Because of their biocompatibility and modified specific targeting characteristics, nucleic acid nanomaterials can be effectively used to transport drugs to the tumor site, thereby improving the efficacy of drug delivery systems.[4] More attention has also been paid to the role of RNA in cancer treatment, with siRNA and mRNA being representative molecules in gene therapy. Some RNA-based therapies have been approved by the U. S. Food and Drug Administration and European Medicines Agency,[8] but their limited stability, high cost, and complexity remain significant challenges.[9] Theoretically, designed RNA nanostructures can reduce RNA degradation by RNase H and improve transfection efficiency.[10]

Many nucleic acid nanomaterials have shown outstanding results in tumor chemotherapy, photodynamic therapy, gene therapy, and immunotherapy, and they are considered promising potential treatment methods.[11–13] In this review, we outline the application and prospects of nucleic acid nanomaterials in tumor therapy and demonstrate their advantages and disadvantages by introducing their functions and properties.

2. Development of nucleic acid nanomaterials

Nucleic acid nanotechnology was first proposed by Nadrian Seeman in 1982, who first used DNA fragments to build structures, thereby creating a new field of research.[14] Over the past 40

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years, nucleic acid nanotechnology has developed rapidly, producing various spatial nanostructures from one to three dimensions. In 2006, Paul W. K. Rothemund first reported DNA *origami* technology, which allows for precise regulation of DNA nanostructures and assembly of nucleic acid nanomaterials of various shapes.[15] Over the past ten years, advances in technology have led to the development of software to assist in the design of DNA nanostructures, such as caDNAno, oxDNA, and CanDo, thereby making it possible to use DNA *origami* technology to design more complex and delicate structures.[16–18] For example, Hendrik *et al.* designed a 3D DNA nanostructure with complex curvatures by tuning the specific positions and patterns of the crossovers between adjacent DNA double helices.[19] In 2018, Zhang *et al.* used DNA *origami* blocks to construct a "tensegrity triangle" with the assistance of caDNAno and further assembled it into a 3D rhombohedral crystalline structure.[20]

In addition to its structural diversity, DNA *origami* technology can be used to precisely modify DNA nanostructures based on its ability to locate each base of the nanostructure. In 2015, Qiao enhanced the antitumor effect of DNA *origami* nanomaterials by modifying the surface of gold nanorods, thereby providing a promising candidate material for cancer diagnosis and treatment.[11]

In 2018, Fan Chunhai *et al.* first proposed the concept of framework nucleic acids (FNAs), which represented a new type of nucleic acid nanomaterial with unique physicochemical and biological properties.[21] Among the various framework nucleic acid structures, tFNAs are widely used due to their simple synthesis, high yield, and good economic performance. tFNA is a nucleic acid nanomaterial with excellent anti-inflammatory and antioxidant capacities, which plays an important role in various disease models.[22–24] For example, Qi discovered that tFNAs can exhibit protective effects by inhibiting apoptosis and alleviating oxidative stress, thereby making it suitable for treating acute kidney injury.[25] Additionally, Zhu *et al.* reported that tFNAs can alleviate cellular inflammation by activating the AKT signaling pathway.[26] As a drug delivery vehicle, tFNAs can enhance the efficacy of chemotherapy drugs; Fig. 1 shows several tFNAs drug delivery systems for cancer treatment.[27–31]

In recent years, dynamic DNA nanostructures have been the focus of nucleic acid nanomaterial development. For example, Juul *et al.* designed a special three-dimensional DNA nanostructure that can realize reversible encapsulation and release of enzyme cargo without any form of covalent or noncovalent linkage for heat-sensitive drug delivery.[32] In 2021, Tian Taoran designed a melittin-loaded DNA nanostructure for targeted therapy, which undergoes a conformational change after binding to the target protein, thereby releasing the drug. All of the aforementioned aspects highlight the broad potential of dynamic DNA structures in biomedicine.[33]

As DNA nanotechnology is rapidly developing, some researchers have also turned their attention to RNA, which shares many similarities with DNA.[8] Early research on RNA nanotechnology began with the bacteriophage phi29,[34] which can assemble inactive mutant pRNA into dimers, trimers, and hexamers, such as RNA dendritic polymers, of which RNA trimers represent a suitable carrier for drug delivery. In the early 2000s,[35] tectoRNA and RNA units were intensively studied, and the generation of specific RNA nanoassemblies was made possible by computational design.[36–39] Inspired by DNA *origami*, Geary *et al.*[40] proposed a method of designing single-stranded RNA structures (RNA *origami*) using thermal annealing and co-transcriptional folding. In summary, canonical Watson-Crick base pairing, noncanonical base pairing, base stacking, and networks of tertiary contacts have laid the foundation for creating diverse RNA structures.[40] The versatile structure and suitable pharmacokinetic and pharmacodynamic profiles of RNA nanotechnology showcase their potential in cancer therapy. These structures play an irreplaceable role in expanding the application of nucleic acid nanomaterials in cancer treatment. In the next section, we will focus on the applications of DNA and RNA structures in cancer therapy.

3. Properties and applications of nucleic acid nanomaterials

3.1 DNA nanotechnology

3.1.1 tFNAs. tFNAs are highly editable, and small-molecule drugs, polypeptides, oligonucleotides, and antibodies can bind to tFNAs through covalent bonding or charge attraction.[41,42] Moreover, these functionally modified tFNAs can still carry cargo into the cell. The strong carrying capacity and editability of tFNAs have enabled their wide applicability in multiple aspects of tumor therapy. It is possible to load tFNAs with chemotherapeutic drugs to enhance their efficacy and reduce tumor drug resistance.[43] Small molecule chemotherapeutics can be conjugated to tFNAs in a variety of ways, although the most common method is direct incubation. Even using the same incubation method, different small-molecule drugs can bind to tFNAs *via* different mechanisms. For example, paclitaxel can bind to chimeric DNA in the form of monomers in the grooves directed away from the three benzene rings.[44] Flavonoids, on the other hand, intercalate their hydroxyl groups with hydrogen bonds between the guanine and cytosine residues in tFNAs.[45] The combination of the tFNA delivery system and the drug can overcome the low water solubility and difficult entry of some drugs, and endocytosis mediated by tFNAs can also increase the concentration of the drug in the cell.[46] For example, Xie *et al.* found that tFNAs loaded with paclitaxel can significantly enhance the water solubility of PTX and greatly reduce drug resistance in lung cancer models.[47] In addition to carrying chemotherapeutic drugs, tFNAs can also carry aptamers that enhance drug targeting. Aptamers are nucleotide sequences that have a unique affinity for a particular molecule. For example, in response to the overexpressed transmembrane growth factor receptor HER2 on the surface of breast cancer cells, Ma *et al.* linked an anti-HER2 aptamer called HApt-tFNA to the apex of tFNAs and showed that HApt-tFNA accumulated massively in HER2-positive SK-BR-3 cells and induced apoptosis in nearly 50% of the cells.[48] Similarly, AS1411, an aptamer targeting nucleolin overexpressed on the surface of tumor cells, can help tFNAs to rapidly target tumor cells, and the tFNA

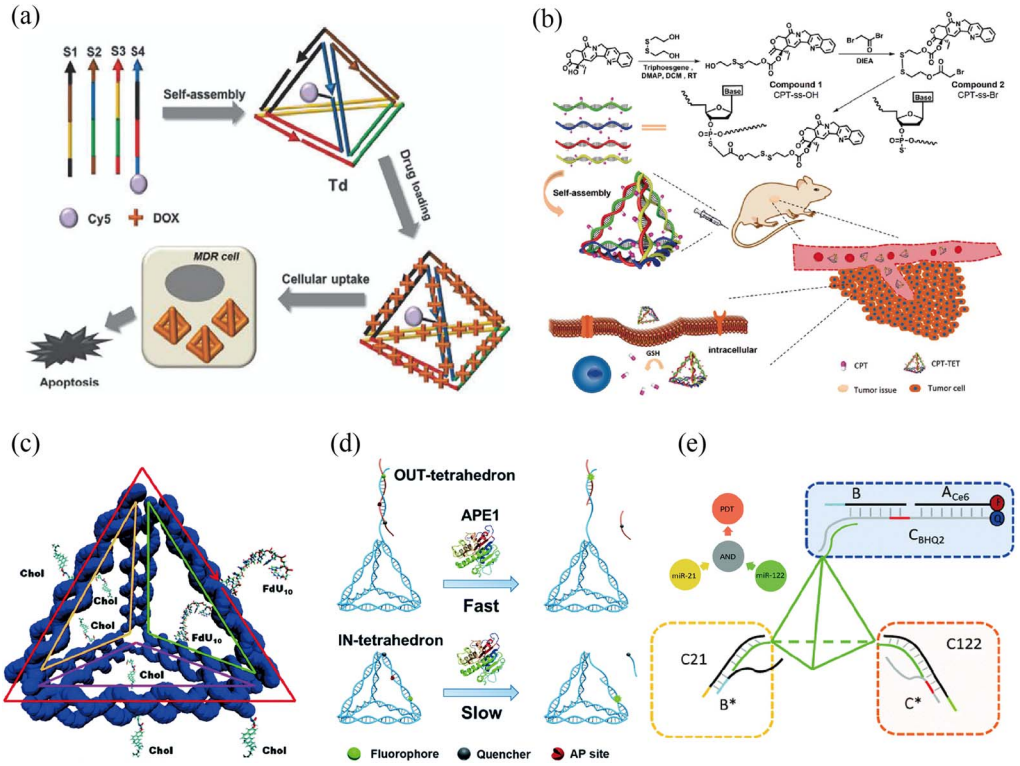

Fig. 1 tFNA nanostructure functionalization for tumor therapy. (a) Self-assembled DNA tetrahedron for the treatment of multidrug-resistant cancer cells. Adapted with permission from ref. 27. Copyright 2013, Royal Society of Chemistry. (b) Camptothecin-grafted DNA tetrahedron for the inhibition of tumor growth. Adapted with permission from ref. 28. Copyright 2019, Wiley-VCH. (c) Cell targeting was achieved by locating multi-functional components *via* intercalation or insertion at one end of the tFNA. Adapted with permission from ref. 29. Copyright 2018, Royal Society of Chemistry. (d) Through intracellular ape1 binding, an apurinic/apyrimidinic endonuclease (ape1)-site-modified tFNA was employed to interfere with enzymatic catalysis, increasing tumor cell sensitivity to chemotherapeutic treatments. Adapted under the terms of the CC-BY Creative Commons Attribution 3.0 Unported license (https://creativecommons.org/licenses/by/3.0) from ref. 30. Copyright 2019, Royal Society of Chemistry. (e) A DNA-tetrahedron-based logic device was utilized to recognize microRNA for specific cell selection, after which a photosensitizer was activated for synergetic chemotherapy and photodynamic effects in the treatment of solid tumor tissue and metastasis. Adapted with permission from (ref. 31). Copyright 2021, Royal Society of Chemistry.

complex linked to AS1411 and the anticancer drug 5-fluorouracil (5-FU) can simultaneously enhance the targeting of 5-FU and promote the apoptosis of nucleolin-positive MCF-7 cells.[49]

In addition, tFNAs can be used for gene therapy due to their ability to link oligonucleotides to regulate tumor-related genes. The connection between tFNAs and oligonucleotides is relatively conservative. Oligonucleotides can be hydrogen-bonded with sticky ends extending from the apex of tFNAs or directly covalently bonded to one end of the single strand as an extended cantilever. In addition to the aptamers described above, oligonucleotides have been studied extensively and include microRNAs (miRNAs) and small interfering RNAs (siRNAs). miRNA is a small nucleotide with a length of approximately 20–25 bp, that is mainly produced by cells to regulate various physiological activities. siRNA is a synthetic double-stranded RNA that can interfere with the transcription of mRNA to regulate the physiological processes of cells. It is difficult for both types of oligonucleotides to cross cell membranes without carriers because of their negative charges. tFNAs can enhance the transmembrane capacity of oligonucleotides, such as miRNAs and siRNAs, and protect them from degradation. Li *et al.* found that tFNAs help microRNA214-3p enter A549 cells to silence the SURVIVIN gene and promote apoptosis.[50] Similarly, tFNAs can also be loaded with siRNA and aptamers to target and interfere with the normal physiological processes of tumor cells to promote their apoptosis. For example, Xiao *et al.* linked tFNAs with AS1411 to siBraf, which interferes with Braf gene expression in melanoma; moreover, the complex showed strong cleavage of Braf mRNA and promoted apoptosis in melanoma cells.[13,51]

In addition to directly acting on tumor cells, tFNAs can be involved in indirect tumor therapy in various ways. Tumor immunotherapy refers to enhancing the immunogenicity of tumor cells or the killing effect of the immune system in various ways to achieve antitumor activity. Cytosine-phosphate-guanine oligodeoxynucleotides (CpG ODNs) are commonly used to stimulate dendritic cells and macrophages to secrete pro-inflammatory factors, such as tumor necrosis factor-α and interleukin-1, resulting in enhanced innate immune strength. Liu *et al.* co-linked antigens and CpGs on tFNAs because CpGs can help the immune system generate stronger and longer-lasting immune effects than tFNAs linked to antigens alone.[52]

In addition, tFNAs linked to miRNAs can achieve similar effects. Qin *et al.* demonstrated that tFNAs linked to microRNA-155 can promote the polarization of macrophages to a proinflammatory type and enhance innate immunity.[53] In photodynamic therapy (PDT), specific wavelengths of light, photosensitizers, and oxygen are used to generate reactive oxygen species during treatment, in which tumor cells are killed through reactive oxygen species-induced apoptosis and necrosis.[54] The intensity of PDT action is positively correlated with intracellular photosensitizer and oxygen concentrations. However, typical photosensitizers are hydrophobic and difficult to transport into the cells.[55] Therefore, similarly to small-molecule drugs, tFNAs enhance the hydrophilicity of photosensitizers, promote their entry into cells, and increase their concentrations, thereby enhancing PDT efficacy. Kim *et al.* combined methylene blue with tFNAs to enhance light-induced cytotoxicity.[56] Wang *et al.* combined IR780, a photosensitizer with photothermal and photosensitizing effects, with tFNAs and showed that tFNAs not only enabled IR780 to achieve a stronger PDT killing effect but also significantly increased the accumulation of IR780 in tumor sites; moreover, under infrared light irradiation, IR780 loaded with tFNAs exhibited stronger tumor-imaging effects than IR780 alone.[57]

Interestingly, tFNAs have four vertices; that is, the mixing of multiple therapeutic strategies will be the future development direction of tFNAs in the field of tumor therapy. For example, when chemotherapy drugs are combined with immunotherapy, tFNAs can be linked to doxorubicin (DOX) and CpG to achieve higher efficacy.[58] Ren *et al.* reported tFNAs with multiple functions of targeting aptamers, *e.g.*, DOX and DNase, which further expanded the therapeutic scope of tFNAs for tumor treatment.[59] Therefore, in the future, multiple functional modifications of tFNAs will make tFNA-based treatments more comprehensive and complex, which can further improve the efficiency of treatment.

3.1.2 DNA *origami*. In 2006, Rothemund first proposed a new DNA self-assembly method called DNA *origami* technology,[15] which applies the principle of complementary base pairing to fold specific regions on long-chain DNA (scaffold) and then fix the short-chain DNA (staples) to construct the expected structure. Because the experimental conditions for DNA *origami* technology are relatively simple and the assembly is highly efficient, nanomaterials or molecules assembled using DNA *origami* technology can be used to prepare nanodevices or drug carriers with certain optoelectronic properties. Based on DNA *origami* technology, nanoarchitectures of various shapes and sizes have been synthesized, such as diverse two-dimensional nanostructures (rectangles, triangles, pentacles, smiling faces, *etc.*),[15] nanotubes,[60] polyhedral nanostructures,[19,61] and other complicated DNA *origami* nanostructures (DONs).[62–64]

In 1986, Matsumura *et al.*[65] reported the enhanced permeability and retention (EPR) effects of these nanostructures. Since then, the development and research of macromolecular anticancer drugs have increased, and various nanomaterials have performed well in tumor targeting based on EPR.[66,67] In addition to their targeting properties, DNA *origami* nanostructures have good stability and biocompatibility, excellent cellular membrane penetration, and multiple modification sites for a series of biomolecules. Hence, DNA *origami* nanostructures are promising platforms for creating multifunctional nanomaterials for cancer therapy.[68]

To serve as a drug delivery system, DNA *origami* nanostructures must exhibit excellent stability in circulation conditions. Many studies have shown that DNA nanostructures are more stable than single- or double-stranded DNA. The complex structure of DNA *origami* may induce improved stability by hindering the function of nucleases.[69] Moreover, encapsulation modifications have been performed to improve the stability of DNA nanostructures. Perrault *et al.*[70] utilized a lipid bilayer to encapsulate DNA *origami* nanostructures, and this updated form led to longer blood circulation times compared with the plain form. Other molecules, such as PEG, polypeptides, and proteins, have been used for the same purpose.[71,72]

DOX is an antitumor micromolecule that can inhibit the synthesis of RNA and DNA, and has been applied a cell cycle nonspecific drug with a broad antitumor spectrum. However, DOX may produce toxic effects, such as leukopenia, thrombocytopenia, cardiotoxicity, nausea, and loss of appetite. Due to its ability to intercalate into DNA double strands, DOX serves as a classic drug that can be incorporated into DNA *origami* nanostructures, which are characterized by higher tumor-targeting efficiency and fewer toxic effects. Zhao *et al.*[73] encapsulated DOX in screwy DNA nanotubes and studied their release rates, and found that DNA nanotubes could improve the cellular uptake of DOX to kill cancer cells. Jiang *et al.*[74] also reported that DNA nanocarriers exhibit an enrichment effect and reduce drug resistance to a certain extent (Fig. 2), and also used triangular DNA nanocarriers to deliver DOX.[75] The study revealed that DOX-loaded DNA *origami* nanostructures have improved antitumor effects and lower systemic toxicity in mouse models compared with free DOX molecules.

Currently, an increasing number of researchers are focusing on gene therapy, which applies exogenous nucleic acid strands, such as siRNA, microRNA, and CpG,[77] to inhibit cancer cells. However, these therapeutic sequences are unstable in a complicated *in vivo* environment, and therefore there is an urgent need to find suitable delivery systems for these molecules. DNA *origami* nanostructures can be used to extend the length of usable DNA strands to ensure a connection with exogenous nucleic acid strands by protecting the nucleic acid strands and making it possible to target certain tissues. Schuller *et al.*[78] utilized DNA *origami* nanostructures to deliver CpG sequences, resulting in a strong immune response and fewer side effects. Rahman *et al.*[79] reported DNA bricks modified with siRNA that could knock down the antiapoptotic protein Bcl-2. These results indicated that DNA bricks could stabilize siRNA and effectively inhibit the growth of lung tumors.

Aptamers are short, simple-stranded DNA or RNA oligonucleotides that specifically bind to target molecules.[80] Aptamers tend to fold and form stable three-dimensional structures through base pairing, electrostatic interactions, or hydrogen bonding, and can wrap around target molecules with high affinity. To obtain a high uptake efficiency of DNA *origami* nanostructures, aptamers can be added to certain strands of the DNA nanostructures. Song *et al.*[81]

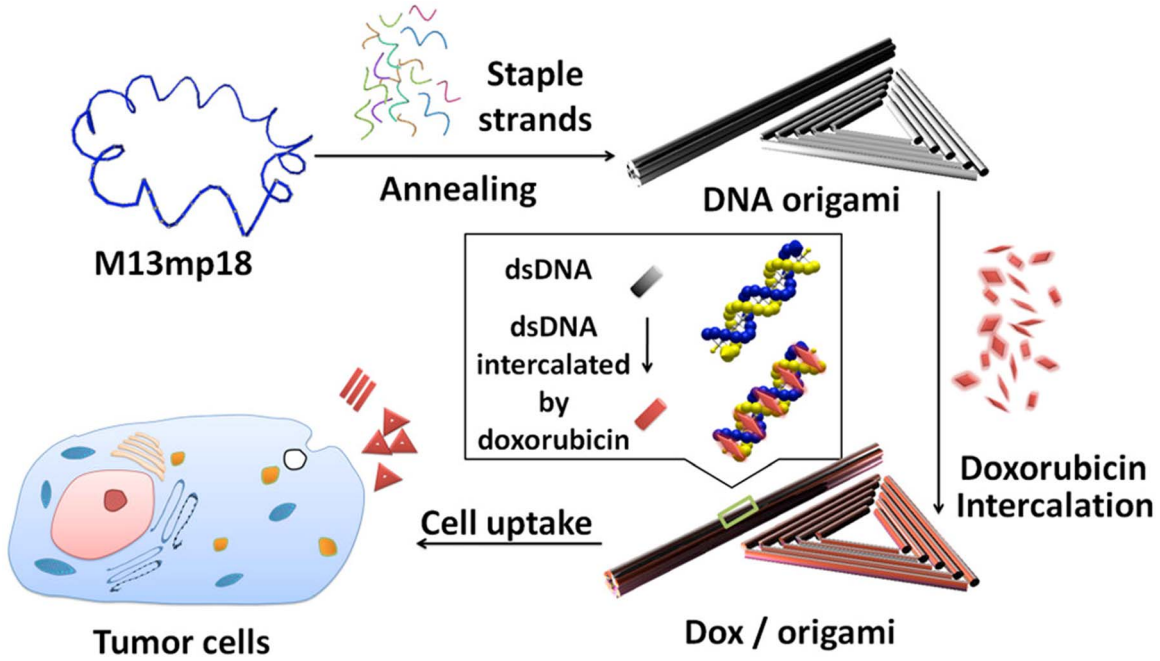

Fig. 2 DNA *origami* and doxorubicin *origami* delivery system assembly. The long single-strand M13mp18 genomic DNA scaffold strand (blue) is folded into the triangle and tube structures through the hybridization of rationally designed staple strands. Watson–Crick base pairs in the double helices serve as docking sites for doxorubicin intercalation. After incubation with doxorubicin, the drug-loaded DNA nanostructure delivery vessels were administered to MCF 7 cells, and the effects were investigated. Adapted with permission from ref. 76. Copyright 2012, American Chemical Society.

incorporated DOX, AuNRs, and the tumor-specific aptamer MUC-1 into DNA *origami* nanostructures to kill drug-resistant cancer cells, which significantly enhanced the internalization of the nanostructure by cancer cells. Schaffert *et al.*[82] synthesized a two-dimensional DNA nanostructure with the modified transport protein transferrin (TF). The results showed that transferrin improved the cellular uptake of the DNA nanostructures, and the internalization efficiency was influenced by the number of modified transferrins.

In recent years, multidrug resistance has become a major obstacle in cancer therapy. To solve this problem, researchers have combined multiple chemotherapeutic drugs within the same nano-delivery system, which is called combination therapy. Liu *et al.*[83] designed a multifunctional DNA *origami* nanostructure to deliver DOX and shRNA to destroy multidrug-resistant breast cancer cells (MCF-7R), and found that the application of shRNA successfully reversed drug resistance in MCF-7R cells, which led to an enhanced therapeutic effect of DOX. Pan *et al.*[84] established a versatile DNA *origami* platform to combine chemotherapy with RNA. In this study, two different antisense oligonucleotides (ASOs) were used to target P-glycoprotein (P-gp) and Bcl-2. Meanwhile, they connected the aptamer MUC-1 to the surface of the DNA platform to target cancer cells. These results demonstrated that this DNA platform increased the therapeutic effect of DOX with the assistance of ASOs and aptamers. These studies indicated that DNA *origami* nanostructures perform well in drug delivery and combination therapy.

In addition to drug delivery, DNA *origami* nanostructures are promising platforms for cancer detection.[85] With the rise in cancer incidence and the development of tumor-targeting therapies, the early diagnosis of cancer has become increasingly crucial. Various DNA *origami* detectors have been constructed for cellular imaging by modification with fluorescent molecules. For example, Shen *et al.*[86] incorporated biscyanine into DNA nanotubes to visualize live cells directly, and Samanta *et al.*[87] combined DNA *origami* with infrared-emitting QDs for biophotonics applications.

3.1.3 Dynamic DNA nanostructure. Although tFNAs and DNA *origami* have demonstrated significant potential in the field of drug delivery and cancer therapy, the complicated tumor microenvironment places higher requirements on drug delivery systems. In recent years, dynamic DNA nanostructures have attracted considerable attention from researchers; Table 1 shows the timeline of the representative advances in the field of dynamic DNA nanostructures. Based on nucleic acids, proteins, and physical factors such as light, temperature, and pH, a range of stimuli-responsive dynamic DNA nanostructures have been designed and applied in various biomedical fields. In addition, dynamic DNA nanostructures can serve as special modifications in other nanomaterials. Kahn *et al.*[88] combined dynamic DNA nanostructures with polyacrylamide to construct an environmentally sensitive hydrogel.

As single strands of DNA forming double-stranded structures through hydrogen bonds, DNA nanostructures are affected by changes in pH or ion concentration. Moreover, a wide range of ions have been reported to be suitable for insertion into DNA

Table 1 Timeline of the representative advances in the field of dynamic DNA nanostructures

Year	Dynamic DNA nanostructures	Ref.
1999	Paired double crossover structure	106
2001	Toehold-mediated strand displacement (TMSD) based DNA tweezers	107
2003	pH-based DNA nanostructure	108
2005	DNA walker	109
2007	DNA box actuated by TMSD	110
2011	DNA beacon which detects biomolecules	111
2012	Logic-gated DNA box which displays molecular cargo	112
2015	DNA structure connected with shape complementarity	63
2015	DNA-based hinges, sliders, and hybrid mechanisms	64
2016	Rotary device made from multiple tightly-fitted components	113
2018	Gold nanocrystal-mediated slider	114
2019	Molecular algorithm that executes logic and outputs literal Arabic numerals	115
2019	DNA box actuated by changing pH	116
2019	Thermally-actuated nanovalve	117
2019	Self-regulating DNA nanotubes	118
2021	Robotic nanobee	33

strands and can induce the formation of complicated structures.[89,90] In addition, some DNA strands are easily affected by environmental pH. For instance, the i-motif sequence includes abundant cytosine and tends to maintain a linear strand in a neutral environment. However, as the pH decreases, the i-motif folds and forms a secondary structure.[91] Meanwhile, the guanine-rich sequence, which is paired with the i-motif, can construct a G-quadruplex in the same plane.[92] Therefore, the i-motif and G-quadruplex are often used as switch structures. Keum *et al.*[93] developed a pH-sensitive DNA nanocarrier to control the release of proteins based on the i-motif sequence. Park *et al.*[94] utilized the i-motif and G-quadruplex to deliver DOX through a triggered release. Moreover, they combined photodynamic and photothermal therapy with chemotherapy, which led to enhanced antitumor effects and fewer toxic side effects.

Aptamers bind tightly to target molecules by folding into secondary structures, such as G-quadruplexes and DNA loops, and the target molecules are mainly proteins, including cytokines, kinases, cell adhesion factors, and cell surface receptors. Through their combination with certain proteins, aptamers can perform various biological functions. Researchers believe that proteins cannot approach other biomolecules after binding with aptamers, which inhibits the usual effects of the proteins. AS1411 is a classic G-quadruplex aptamer targeting the surface receptors of cancer cells (nucleolin), and reports have indicated that AS1411 can inhibit the growth of cancer cells, especially blood cancer cells.[95] Considering the multiple functions of aptamers, they have been widely applied in diverse DNA nanodelivery systems.[96] In addition to promoting the target efficacy and therapeutic effect of nanomedicine, aptamers can serve as switches in dynamic DNA nanostructures. Tian *et al.*[33] constructed a nucleolin-triggered dynamic DNA tetrahedron based on the AS1411 switch (Fig. 3). This tetrahedron remains intact in a nucleolin-free environment, and when it approaches cancer cells, the AS1411 strand separates and binds to nucleolin, resulting in structural disintegration and drug release. This design significantly reduces the damage to normal tissues caused by chemotherapy drugs.

The basic unit of the DNA double strand is a base pair bound by hydrogen bonds. However, by regulating the affinity of different single DNA strands, competitive binding of these DNA strands is expected, which will lead to dynamic sequence replacement. The replaced single strand may subsequently induce the replacement of other DNA strands.[98] This theory is based on various DNA reactions, such as hybridization chain reaction (HCR), catalytic DNA hairpin (CDH), and catalytic DNA circuits (CDC). A hairpin-based HCR is a classic example of a DNA replacement reaction. Traditionally, hairpin-based HCRs include two DNA hairpins and an initiator. The initiator induces Hairpin-1 to release a low affiliative DNA strand, which demonstrates a high affinity to Hairpin-2 and initiates strand displacement of Hairpin-2. Subsequently, the redundant strand from Hairpin-2 further triggers the displacement reaction of Hairpin-1.[99,100] Through this cascading reaction, an initial sequence can be amplified for detection, which builds a theoretical foundation for the use of DNA biosensors to detect proteins, small molecules, and nucleic acids.[101] In addition, dynamic DNA nanostructures demonstrate several merits as biosensors, such as their outstanding biocompatibility and cellular internalization, high structural editability, and diverse detection targets. Based on HCR, Zhang *et al.*[102] constructed a DNA tetrahedron modified with DNA hairpins and showed that the DNA tetrahedron could rapidly sense tumor cells by amplifying the number of receptors on the cell membrane.

With the development of DNA-editing technology, DNA hydrogels have become a research hotspot in recent years. DNA hydrogels can be formed by covalent interactions between linear or polymeric DNA molecules, which is termed chemical crosslinking. Due to chemical crosslinking, DNA hydrogels tend to be irreversible and highly stable in physiological environments.[103] Moreover, physical crosslinking could also promote DNA gelation through hydrogen bonding, electrostatic interactions, or DNA-metal coordination. Compared to chemical crosslinking, physically crosslinked DNA hydrogels are reversible and demonstrate better degradability, biocompatibility, and biosecurity.[104] In addition, by inducing trigger-responsive

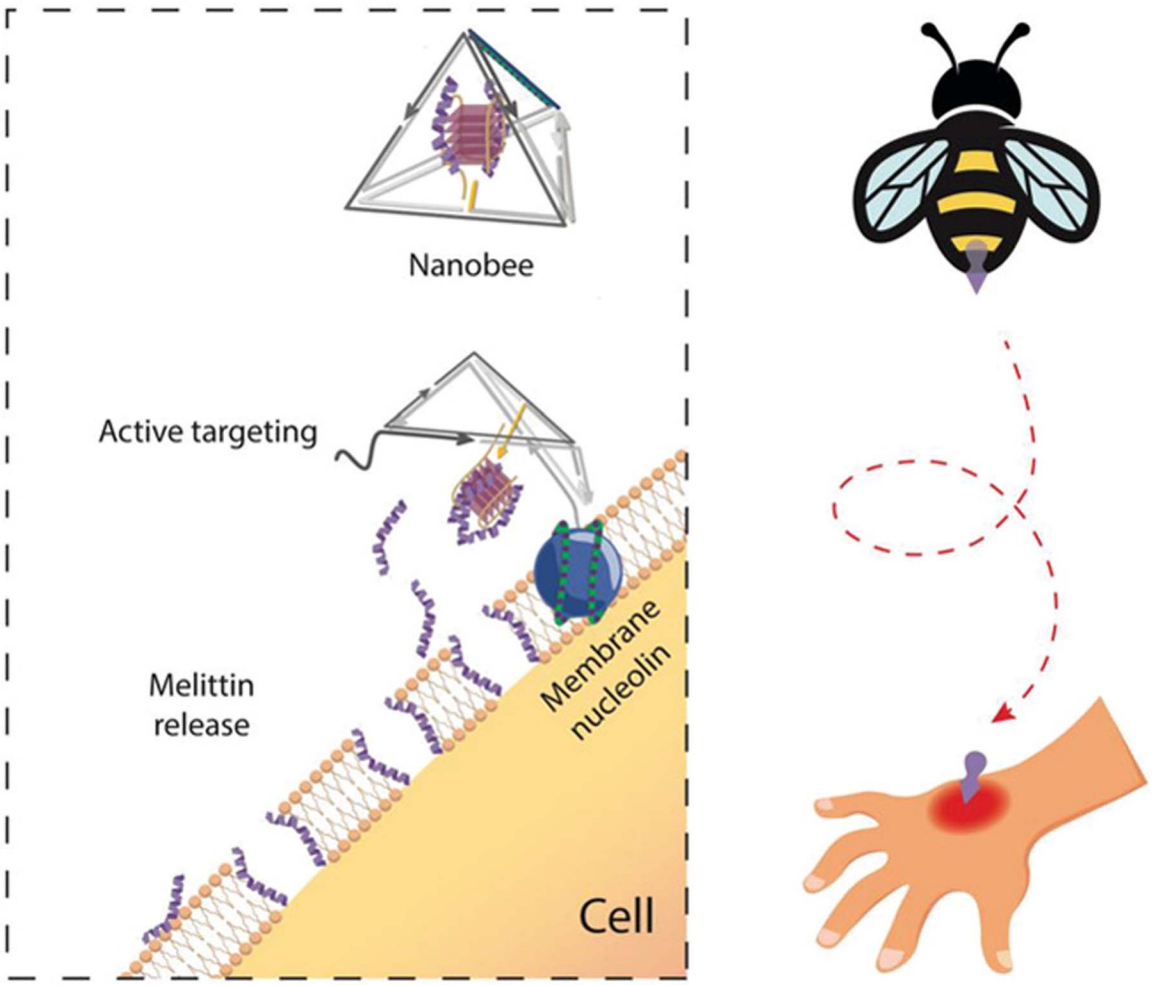

Fig. 3 A nucleolin-triggered dynamic DNA tetrahedron based on AS1411 to reduce the damage to normal tissues caused by chemotherapy drugs. Adapted with permission from ref. 97. Copyright 2021, Wiley-VCH.

units, DNA hydrogels have become smart and show prospects in drug delivery, tissue engineering, and gene editing. Ding *et al.*[105] reported an antitumor DNA hydrogel with embedded siRNA, which displayed self-assembly by grafting DNA strands onto polycaprolactone. These results indicated that this hydrogel structure protected siRNA from RNase degradation and did not disturb the function of RNA interference.

3.2 RNA nanotechnology

Similar to DNA nanotechnology, RNA nanomaterials are attracting the attention of researchers. RNA *origami* involves the folding of a designed single strand into a complex structure with the participation of RNA polymerase. Since the initial framework of RNA *origami* presented by Geary *et al.*,[40] there have been an increasing number of RNA *origami* structures being developed. Wu *et al.*[119] designed an RNA/DNA hybrid *origami* nanoplatform, where target mRNA served as the scaffold and short DNA staples acted as antisense to form a tubular nanostructure. The tailored structure could be cleaved by intracellular RNase H, releasing the short DNA staples which then bind to the target mRNA in the cytoplasm and guide its degradation by RNase H, leading to gene silencing. In Ding *et al.*'s experiments, the tumor-associated PLK1 gene in MCF-7 was effectively silenced. Nucleic acids (NAs) are considered as natural ligands for some pattern recognition receptors (PRRs) in mammalian cells, which can be internalized into endosomes and recognized by Toll-like receptors (TLR) to enhance the mount innate responses. Therefore, Qi *et al.*[120] developed an RNA *origami* structure that could stimulate NK and $CD8^+$ T cell anti-tumor activity and alleviate tumor-mediated immune suppression environment. This structure can self-assemble in PBS solution, without the need for divalent cations to maintain its stability. Their experiments further found that this structure had excellent serum and thermal stability, and demonstrated low toxicity and inflammatory cytokines response.

With the development of programmable RNA technology, there are some kinds of 2D and 3D frameworks of RNA, such as triangles, squares, pentagons, nanoprisms, tetrahedrons, and micelles, *etc.*[85,121–125] Stewart *et al.*[126] designed a *de novo* double crossover (DX) RNA tile that can self-assemble and fold into

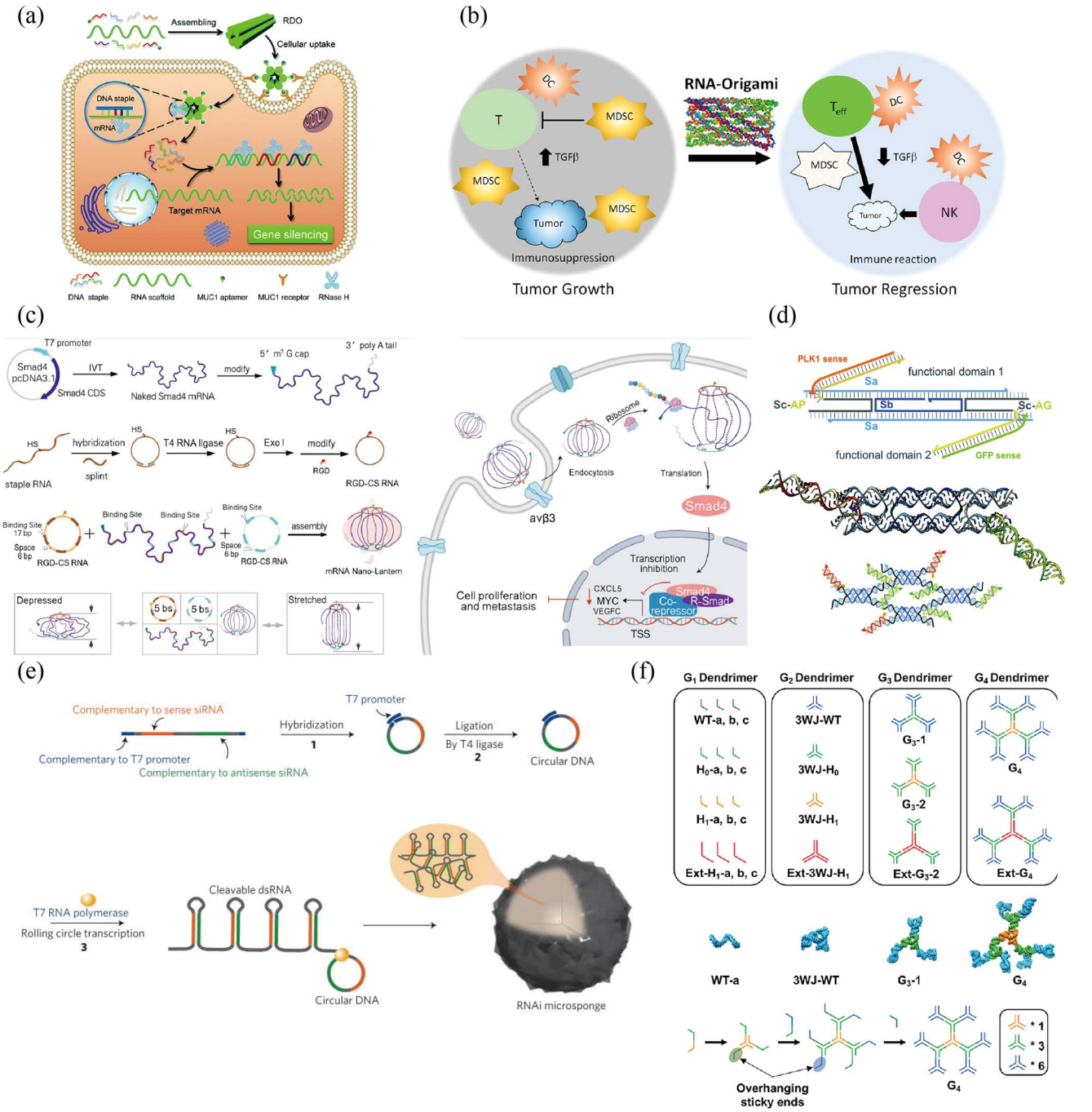

Fig. 4 RNA nanostructure functionalization for tumor therapy. (a) RNA/DNA hybrid *origami* nanostructure demonstrated efficient cellular uptake. Adapted with permission from ref. 119, Copyright 2021, Royal Society of Chemistry. (b) Single-stranded RNA origami technology would not increase the level of interferons in anticancer immunotherapy. Adapted with permission from (ref. 120), Copyright 2020, American Chemical Society. (c) Lantern-shaped flexible RNA *origami* improved the efficiency of RNA delivery and translation. Adapted with permission from (ref. 127) Copyright 2023, Nature Publishing Group. (d) RNA lattices based on DNA tile are more resilient to nuclease degradation, and can be further functionalized with different cargos. Adapted with permission from (ref. 126), Copyright 2023, Royal Society of Chemistry. (e) The synthesis of a single RNAi-microsponge that combines carrier and cargo, provided protection for siRNA form RNase H with a high content of siRNA. Adapted with permission from (ref. 128), Copyright 2012, Nature Publishing Group. (f) Stable RNA dendrimers demonstrated its potential for hydrophobic drug delivery, shielding and controlled release. Adapted with permission from (ref. 133), Copyright 2020, Royal Society of Chemistry.

lattices for siRNA delivery and demonstrated its remarkable stability in micron-scale. In breast cancer cells (MDA-MB-1/GFP), the material can achieve an excellent silencing efficiency for GFP, and showed silencing the similar efficiency for PLK1 in human prostate cell lines (PC1). In addition, more and more attention had been attached to the biological effects of RNA that make up the framework. Hu *et al.*[127] developed a flexible lantern RNA structure that can package Smad4 mRNA and

Table 2 Nucleic acid nanostructure for tumor therapy

Nucleic acid nanostructure	Cargo or junctions	Target cell	Ref.
tFNAs	Doxorubicin	SCC7 cells	134
	Taxol	Not specified	44
	Luteolin, kaempferol and apigenin	Peripheral lymphocytes	135
	Paclitaxel	A549 and A549/T cells	47
	Anti-HER2 aptamer	HER2-positive breast cancer cells	48
	5-Fluorouracil, DNA aptamer and	MCF-7 cell line	49
	miRNA-214-3p	A549 cells	50
	Anti-braf siRNA and DNA aptamer	A375 cells	13
	siRNA, folate molecules	HeLa cells	136
	Antigen and CpG adjuvants	DC and B cell	52
	miRNA-155	Macrophages	53
	Methylene blue	B16F10, SCC7 and MDA-MB231 cells	137
	IR-780	MCF-7 cells	59
	Doxorubicin, aptamer and 17E DNA zyme	SMMC-7721 and HEK293T cell lines	58
DNA *origami*	Doxorubicin	MDA-MB-231 cells, MDA-MB-468 cells, and MCF-7 cells	138
	Doxorubicin	MCF-7 cells	76
	Doxorubicin	MDA-MB-231-GFP cells	75
	CpG	Primary splenic cells	78
	siRNA	DMS53 cell line; H1299 cell line	79
	Doxorubicin; surface-modified gold nanorods and MUC-1 aptamers	MCF-7/ADR cells	81
	Iron transport protein transferrin	KB carcinoma cell line	139
	shRNA, doxorubicin and MUC-1 aptamers	MCF-7R cells	83
	Antisense oligonucleotides and doxorubicin	Hela/adriamycin (ADR) cells	84
	Carbazole-based biscyanine	MCF-7 cells	140
Dynamic DNA nanostructure	Au-Gi, i-motif sequence, ZnPc and doxorubicin	MDA-MB-231 cells	94
	AS1411 aptamer and melittin	L929 cells and A375 cells	33
	DNA hairpin	AS49, MCF-7 and A375 cells	141
	DNA-grafted polycaprolactone and siRNA	U2OS cells	142
RNA *origami*	Antisense	MCF-7 cells	119
	Not specified	RAW 264.7 macrophage cell line	120
	Smad4 mRNA	SW480 and SW620 cells	127
Self-assembled RNA	siRNA	MDA-MB-231 cells	143
	siRNA	T22 cells	128
Self-assembled RNA based on phi29	Paclitaxel, folate	RAW 264.7 cells, human KB cells	133
	siRNA, pRNA	MCF-7 cells and HeLa cells	129

demonstrated its mRNA delivery ability in mouse situ colorectal tumor model. The lantern structure can be recognized by ribosomes in cancer cells and translated into protein, thereby releasing the Smad4 mRNA. Due to its RNA composition, this nanostructure does not affect the functional efficacy of the mRNA compared to DNA/RNA hybrid structures. Lee *et al.*[128] used ligased circular DNA templates, T7 promoter, and T7 RNA polymerase to amplify siRNA and self-assemble it into nucleic acid sponges composed of nanoscale pleated sheets of hairpin RNA to deliver siRNA, and validated the good performance in delivering siRNA in mouse ovarian cancer. Their designed RCT (rolling circle transcription) platform could produce a large amount of siRNA, and without deliver carrier, this structure showed lower biological toxicity Fig. 4.

Originated from bacteriophage phi29 DNA packaging motor, pRNA has the unique ability to form dimers, trimers, hexamers, and other structures through two reengineered interlocking loops, which made it a promising multivalent carrier for delivering siRNA.[129] RNA is considered as a potential material in dendritic polymers due to the ultra-stable phi29 three-way junction (3WJ) motif and its derivatives serving as the central core and repetitive unit.[130–132] Guo *et al.*[133] established dendritic polymers of different generations and sizes using 2′-fluoro (2′-F) modified phosphoramidites, which can covalently bind to drugs (paclitaxel) and release them in a stepwise manner at different temperatures. Li *et al.*[129] used T7 RNA polymerase to construct a pRNA/siRNA dimer and combined folate with pRNA to give the multimer a certain targeting ability. In their experiments, this structure had similar knockdown efficiency to other siRNA delivery vehicles in folate receptor-expressing cells such as MCF-7, but with lower cellular toxicity Table 2.

4. Conclusion

Nucleic acid nanomaterials have become an important field of research for tumor therapy, and various DNA and RNA structures have been considered as potential drug carriers because of their high biocompatibility, excellent editability, and easy internalization. However, the comprehensive application of nucleic acid nanomaterials for tumor treatment as a drug

delivery system still faces monumental challenges, including (1) instability of synthesis, high cost, and high error rate of self-assembly, (2) various unknown biomedical effects, and (3) biosafety concerns arising from the uncertain *in vivo* stability, pharmacokinetic properties, and long-term cytotoxicity. Solving these challenges will greatly enhance the further application of nucleic acid nanomaterials in the field of tumor therapy.

Author contributions

W. T. Lu and T. Y. Chen conceived and wrote the manuscript. D. X. Xiao, X. Qin, Y. Chen and S. R. Shi reviewed and edited the manuscript. All authors contributed to the article and approved the submitted version.

Conflicts of interest

The authors report on declarations of interest.

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