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Transforming Drug Delivery: The Impact of RNA Nanotechnology on Modern Medicine

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A B S T R A C T

Over the past decade, RNA nanotechnology has emerged as a transformative field, leveraging RNA's unique structural and functional attributes for innovative applications in nanomedicine. RNA's propensity for bottom-up self-assembly, diverse structural patterns, and favorable in vivo characteristics position it as a highly desirable biomaterial for nanoparticle drug delivery. This review explores the multifaceted nature of RNA nanotechnology, emphasizing the design and construction of RNA nanoparticles through canonical and noncanonical base pairings, base stacking, and complex tertiary interactions.The historical milestone set by Peixuan Guo's discovery of pRNA (packaging RNA) dimers, trimers, and hexamers in 1998 marked the inception of RNA nanotechnology, showcasing the potential of RNA as a central scaffold for therapeutic modules, regulatory moieties, and targeted ligands. This review delineates the advancements in RNA nanoparticle synthesis, highlighting innovative techniques such as hand-to-hand and foot-to-foot interactions, utilization of stable natural RNA motifs, and computational design.Moreover, the integration of RNA's structural versatility with chemical modifications enhances its stability and functionality in vivo, overcoming challenges like nuclease degradation and limited serum stability. The strategic design of RNA nanoparticles, such as the phi29 pRNA-3WJ motif, allows precise control over their assembly, size, and shape, crucial for therapeutic applications.RNA nanotechnology's potential to revolutionize drug delivery systems, particularly in targeting cancer and other chronic diseases, is immense. The incorporation of functional RNA modules, such as siRNA, ribozymes, and aptamers, into RNA nanoparticles demonstrates significant promise in reducing off-target effects and improving therapeutic efficacy. However, the field faces challenges, including large-scale production, endosomal escape, and cost-effective synthesis, which need to be addressed for clinical translation.

Keywords: RNA nanotechnology, Nanoparticle drug delivery, Noncanonical base pairings, RNA modularity, RNA therapeutic potential.

1. Introduction

The last ten years have seen a significant advancement in the field of RNA nanotechnology .The adaptability of RNA's structure and function, as well as its tendency for bottom-up self-assembly, Its distinct size and structure, advantageous in vivo characteristics, and significant therapeutic potential make it a desirable option for a biomaterial used in nanoparticle drug delivery. The diverse range of natural structural patterns seen in RNA can be attributed to its capacity to adopt complex quaternary structures, base stack, and generate canonical Watson-Crick (A-T, G-C) and noncanonical (G-U wobble, sheared G-A pair, G-A imino pair, A-U reverse Hoogsteen) base pairings [1].These characteristics are used by RNA nanotechnology to create nanoparticles that can be applied in bionanotechnology and nanomedicine. A variety of RNA secondary and tertiary structural motifs, such as bulges, stems, hairpins, loops, and junctions, can be readily browsed by the RNA designer (Figure 1). These patterns serve as the building blocks for creating nanoparticles with a variety of sizes and shapes through the manipulation of engineering variables including sequence length and motif angle. Other factors, such as the kind of nucleotide, can change the characteristics of nanoparticles[2]. For example, base or backbone changes can be used to improve chemical stability, and fluorescent dyes can be added to enable tracking of nanoparticles. RNA nanoparticles' physiochemical characteristics are easily adjustable, allowing for customized in vitro and in vivo applications. An updated review is required due to the RNA nanotechnology field's rapid expansion. The most current developments in RNA nanotechnology will be discussed in this article, along with the methods used to create stable RNA nanoparticles with a variety of shapes and functions. Lastly, we will talk about RNA's possible applications in nanotechnology and medicine[3].

RNA Nanotechnology

The study of RNA-based major-frame RNA constructions at the nanoscale is known as RNA nanotechnology. The central scaffold,RNA can be the only material used to make therapeutic modules, regulatory moieties, and targeted ligands. Proteins' structural flexibility and functional diversity are combined with the straightforward nature of DNA canonical base pairing in RNA nanoparticles[4]. In addition to improving thermodynamic stability, noncanonical base pairing, base stacking, and complex networks of tertiary contacts expand the diversity of RNA structures. RNA nanotechnology, in contrast to conventional RNA biology research, is concerned with using RNA's characteristics to create architectures that have uses in nanomedicine[5]. While RNA

nanotechnology concentrates on inter-RNA interactions and quaternary interactions of RNA motifs, classical studies on RNA structure and function focus on intra-RNA interactions and 2D/3D structure−function correlations. But the information within the domains of RNA research is not limited [6]. A significant portion of the body of previous research on RNA biology is applied in RNA nanotechnology. For instance, a number of RNA nanoparticles employ functional RNAs that were previously identified by conventional RNA biology, such as ribozymes, riboswitches, and miRNAs. Furthermore, RNA structural biologists' discovery of RNA motifs is exploited by RNA nanoparticles[7].

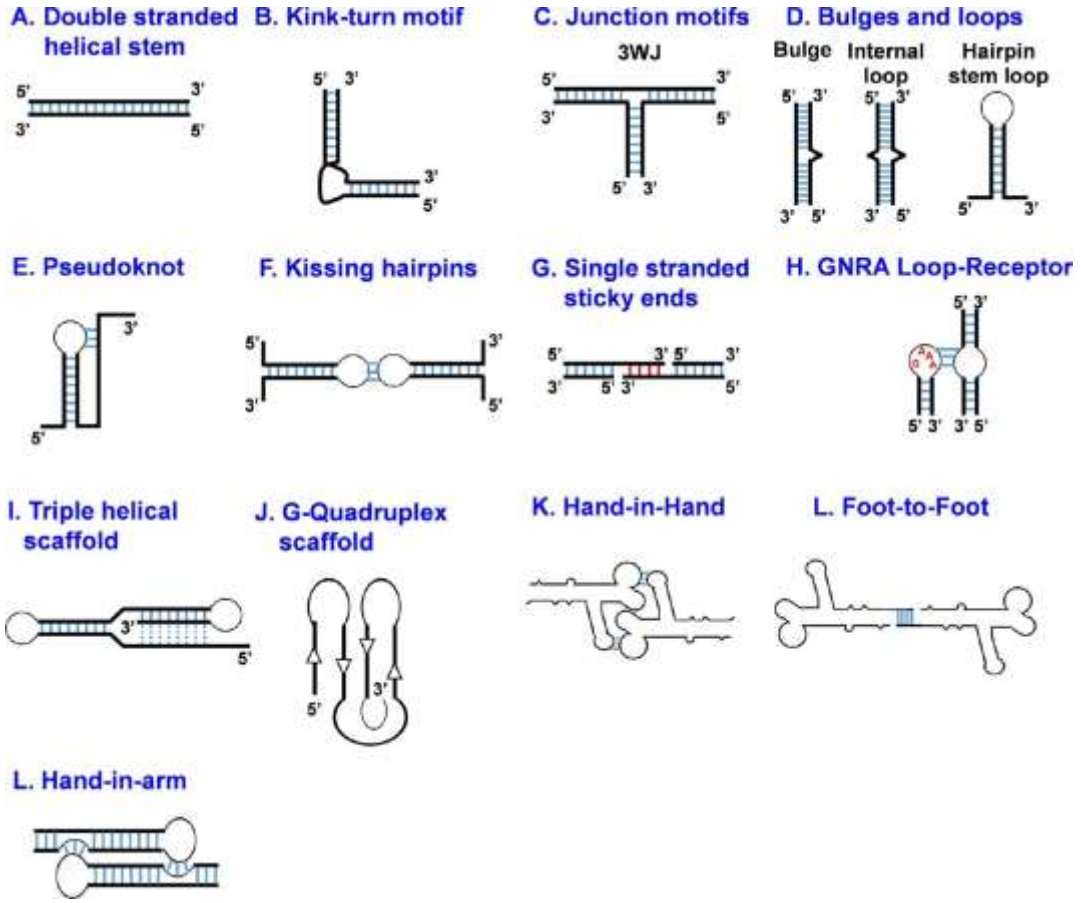

Fig.1. RNA nanoparticle construction motif sets. To create RNA nanoparticles, a wide variety of RNA motifs can be used. After thorough structural investigation, RNA motifs that are retrieved from biological RNAs can be utilized to create higher-order structures. For a variety of uses in nanobiotechnology, the resultant RNA nanoparticles can be functionalized with targeting, imaging, and therapeutic modules.

The field of RNA nanotechnology is emerging.

The development of the science of RNA nanotechnology is not solely attributable to the efforts of one individual, but rather the result of the combined efforts of numerous perceptive people. When Peixuan submitted a manuscript to Cell in 1998, he reported on his discovery of pRNA (packaging RNA) dimers, trimers, and hexamers assembled from re-engineered RNA fragments[8]. Vivian Siegel, the associate editor of Cell, and Benjamin Lewin, the founding editor, were immediately interested in this discovery. They realized that this significant discovery would raise awareness of their just launched journal, Molecular Cell. As a result, this important finding was featured in a mini-review in Cell and published in Molecular Cell . Roger Hendrix was selected by the Cell editors as the expert in the field to assess this discovery, based on Guo's recommendation.This Molecular Cell study demonstrated the idea of RNA nanotechnology by demonstrating how to precisely edit RNA to create succinct RNA architecture, such as dimers, trimers, and hexamers, via bottom-up self-assembly[9].

A team under the direction of Eric Westh of postulated in the early 2000s that the RNA kissing loop would encourage the creation of unique RNA structures. In RNA nanotechnology, the first idea of "TectoR NA" or RNA "Tetonics" has produced empirical findings.To further popularize the idea of RNA nanotechnology, the editor and reporters of MSNBC produced a ground-breaking news article titled "Scientists build tiny structures out of RNA" in 2004, the same year that another empirical paper on the subject was published in Nano Letters[10]. Later, after realizing the significance of RNA nanotechnology, the editors of Science released Luc Jaeger's work on tectoRNA32 together with Hao Yan's commentary. More importantly, the NCI Alliance in Cancer Nanotechnology, headed by Piotr Grodzinski, acknowledged the potential of RNA nanotechnology in cancer treatment after three papers demonstrating its application were published.Bruce Shapiro, a pioneer in computational RNA nanotechnology, held a Workshop on RNA and Disease as part of the NCI's efforts to advance the subject. An invited review by Nature Nanotechnology1 and a follow-up paper reporting the discovery of a stable phi29 pRNA three-way junction used as an in vivo delivery system are credited with providing a significant boost to the RNA nanotechnology research.The editors of ACS Nano and Nano Today are among those who support the field of RNA nanotechnology[11].

Third Milestone in Drug Development Is Shown by the Amount of RNA in Cells.

In the pharmaceutical sciences, there have been two significant moments thus far: Drugs classified as (1) chemical and (2) protein, including hormones, enzymes, and antibodies or substances that target proteins[12]. However, the sequencing of the human genome showed that just 2% of the genome codes for proteins, with the other 98% of the genome being regarded as "junk DNA." It was discovered via further research that some of the so-called "junk DNA" really codes for long and small noncoding RNAs[13].It is now well acknowledged that noncoding RNAs are important for many biological functions, including DNA replication, RNA splicing, translation control, and so on[14]. Consequently, it is anticipated that RNA, along with RNA nanoparticles, tiny therapeutic RNAs, and compounds targeting RNAs, will represent the third major turning point in the drug development process[15].

RNA's favorable structure and chemistry for the creation of nanoparticles.

Nanobiotechnology is the branch of nanotechnology that deals with the manipulation of biological materials. It encompasses the study of nanotechnology using nucleic acids. When DNA self-assembly was initially proposed more than 30 years ago,40 it was used to create nanoparticles by utilizing the base-pairing mechanism (A-T, G-C). Since then, researchers have built DNA nanostructures for use in medication delivery, such as nanocapsules and other nanocarriers.The art of creating enormous 2D and 3D constructions, such as tetrahedrons, nanorobots, helix bundles, and tensegrity-based geometries, has been facilitated by the development of 43–45 DNA origami[16].DNA cannot compare to RNA, its nucleic acid counterpart, in terms of thermostability and structural and functional variety, despite the fact that DNA nanostructures have demonstrated the power of the base-pairing process for structure creation. RNA is a chain-like biopolymer made up of nucleotide subunits connected by phosphodiester bonds, just like DNA. A phosphate group, a nitrogenous base, and a ribose sugar make up each nucleotide. The most prevalent bases are uracil (U), adenine (A), guanine (G), and cytosine (C). Similar to canonical base pairing in DNA, hydrogen bonding between complementary nucleotides, G-C and A-U, is the basis of RNA secondary structure. Base stacking is just as crucial for nucleic acid stability as base paring is for the secondary structure of RNA. Moreover, the characteristics of RNA are dramatically altered by the presence of the 2′ -OH. RNA forms A-type helices (11 bp/turn) due to the C3′ -endo sugar structure, which has a better thermostability than the B-type helix seen in DNA[17]. RNA was initially identified as the connection between genomic DNA and protein, delivering the protein's genetic code to the cell's translation machinery. With the discovery of ribozymes, an examination of RNA structure demonstrated the catalytic nature of RNA[18].The discovery of catalytic RNA, which was previously believed to be exclusive to proteins, changed our understanding of RNA's role. The functional repertoire of RNA has been further extended by particular base-pairing interactions mediated by single-stranded sections of unstructured RNA, in addition to functionalities produced by intricately organized RNA molecules. After 52 years of study, it was discovered that RNA has a wide range of biological activities, including gene control, protein synthesis, and catalysis.After 52 years of study, it was discovered that RNA has a wide range of biological activities, including gene control, protein synthesis, and catalysis. After 52 years of study, it was discovered that RNA is involved in many different biological processes, including gene control, protein synthesis, and catalysis [19].

RNA nanoparticles' negative charge reduces toxicity and prevents non-specific cell entry.

A lot of nanoparticle systems, such the increased permeability and retention (EPR) effects, only use passive targeting.Taking aim at the Positively charged nanoparticles penetrate negatively charged cell membranes through charge interactions and fusion. Nevertheless, a large number of positively charged nanoparticles are hazardous and have unwanted off-target effects[20].RNA nanoparticles are extremely anionic due to the negatively charged phosphate backbone of RNA, which prevents nonspecific targeting. Moreover, this polyanionic charge density limits the formation of a protein corona that may impact targeted delivery and causes significant hydration. RNA nanoparticles use receptor-mediated endocytosis to enter cells because they are functionalized with targeting molecules like chemical ligands or RNA aptamers[21].

RNA Nanoparticles Can Have Several Uses While Preserving Their Original Folding.

RNA-based functional modules, including siRNA, ribozymes, riboswitches, RNA aptamers, and a number of noncoding RNAs, are accessible and easily incorporated into RNA nanoparticles. The RNA scaffold's core sequences are merely joined to these motifs. Each part of the multifunctional structures self-assembles since RNA nanoparticles are modular by nature. Additionally, distinct subunits can be used to embellish each helical branch of the RNA motif. The pRNA-3WJ scaffold, for instance, is able to support three functional modules. The proper folding of RNA functional modules is ensured by the driving force from the thermodynamically stable 3WJ scaffold, maintaining their functionality.Additionally, well-established chemical conjugation techniques make it simple to add chemical ligands to a single RNA strand, such as fluorescent dyes, chemotherapeutic medications, or biotin.It is therefore possible to integrate several functional units for tracking, therapy, and targeting into a single nanoparticle. Alternatively, for improved or synergistic therapeutic effects, numerous units of the same function, such as distinct or identical siRNAs targeting different genes, can be combined on a single nanoparticle[22].

It is critical that the payload be accurately characterized in medical applications. The construction of RNA nanoparticles is done from the bottom up, with the creator having exact control over every stage. Every characteristic of the nanoparticle, including size, shape, oligomer choice, functional units, and tracking molecules, is customized for a particular use. Because RNA therapies have a unique mechanism, the features of RNA nanoparticles have the ability to both generate novel therapeutic avenues and improve upon existing delivery techniques. Entire RNA Nanoparticles Shows Positive Pharmacological Profiles. Aside from therapeutic efficacy, the pharmacokinetic and pharmacodynamic (PK/PD) profiles of nanoparticles are arguably the most significant aspects of them[23]. PK/PD profiles are influenced by several elements, such as surface charge, size, and shape. Narrow size and shape dispersion and consistent assembly are the outcomes of controlled RNA nanoparticle manufacturing. Consequently, a collection of PK/PD parameters that can be repeated is obtained, enabling a methodical investigation of the impact of size and shape on PK/PD as well as some predictability of PK/PD profiles. When they get to the clinic, the uniform RNA nanoparticle assembly will hasten FDA approval. RNA nanoparticles' thermodynamic

stability is a key component for in vivo applications. RNA nanoparticles usually form by means of intermolecular interactions, necessitating metal ions in the tens of millimolar concentrations. Lately, this restriction was overcome by pRNA-3WJ, which assembles in the absence of metal ions, resists urea denaturation, and withstands extremely low concentrations.Additionally, RNA nanoparticles have a size that is beneficial for in vivo uses. They avoid being engulfed by lung/liver/spleen macrophages and liver Kupffer cells because they are tiny enough to enter cells via receptor-mediated endocytosis yet larger than the 10 nm cutoff for fast renal elimination. Autoimmunity might arise if RNA nanoparticles were ingested by macrophages. Furthermore, interactions with negatively charged macrophage membranes are further reduced by negatively charged RNAs. In contrast to naked/unformulated siRNA, which has a half-life of 0.25–0.75 hours in vivo, RNA nanoparticles usually have a longer half-life of approximately 5 – 12 hours, depending on the percentage of chemically modified nucleotides in the sequence, such as 2′ – F.58 Most notably, pRNA-3WJ nanoparticles may specifically target cancer cells with little to no accumulation in healthy essential organs and tissues after being injected systemically into orthotopic xenografts, subcutaneous xenografts, or metastatic tumor-bearing mice[24].

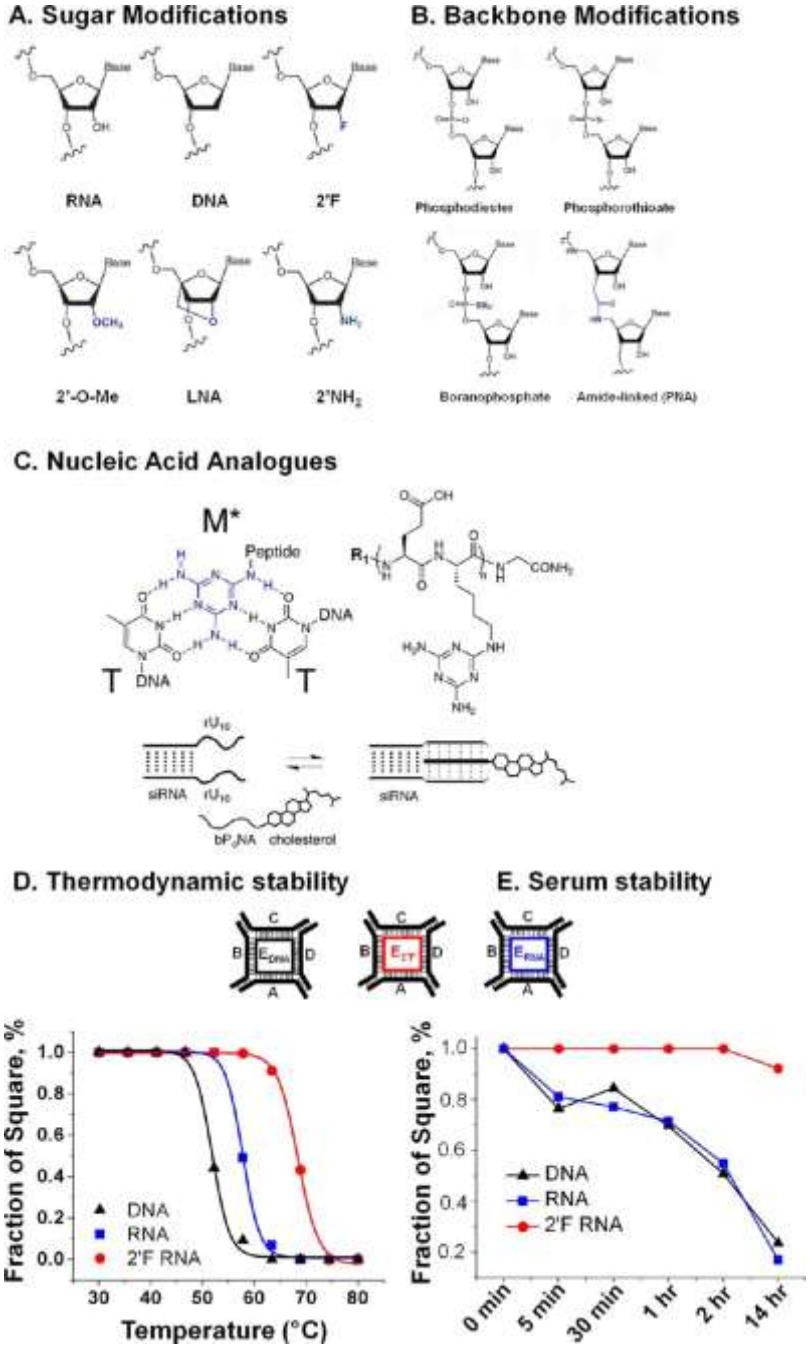

Fig.2. RNA alterations that affect RNA nanoparticle stability. (A) Changes to the sugar moiety of RNA. Reprinted from ref 72 with permission, 2008. (B) Changes to the backbone. Reprinted from ref 71 with permission.(C) The replacement of the native duplex with a synthetic RNA triplex that maintains original functionality. Reprinted with permission from ref 68. 2015 American Chemical Society All rights reserved. (D) 2′ modification caused RNA nanoparticle Tm alterations. reproduced from reference 22. 2014 American Chemical Society All Rights Reserved.

(E) Various serum stability levels were the consequence of the 2′ change. reproduced from reference 22. 2014 American Chemical Society All Rights Reserved.

Size and Shape of RNA Nanoparticles Modify Their Immunogenic Potential for Use in Cancer Immunotherapy and Increased Medication Efficiency.

Immune responses are often the body's defensive strategy. Because cancer cells evolve quickly, they can become resistant to protein treatments if they are treated repeatedly. management. Chemoresistant cancers arise from traditional chemotherapy because it targets only one pathway of tumor development. This problem can be solved with repeated administration and cocktail medicines. RNA nanoparticles avoid antibody induction, such as that brought on by protein treatments, even though their circulation length is prolonged[25]. Thus, RNA nanoparticles may be used to treat chronic diseases repeatedly. RNA sequence, chemical changes, size, and shape all have a significant impact on the immunological response that RNA nanoparticles generate. pRNA nanoparticles serve as an example of this since they are wholly nonimmunogenic on their own[26].Interferon or cytokine induction was not detectable when pRNA nanoparticles were either unmodified or 2′ -F modified. RNA nanoparticles can also be made to be highly immunogenic by adding CpG DNA, an immunological adjuvant that has FDA approval.The amount of CpG per polygon and the geometry of the RNA polygons determined the induction of TNF-α and IL-6. The findings imply that CpG coupled to RNA polygons of various forms has significant immunostimulatory effects and may be applied to improve the efficacy of cancer immunotherapy[27].Toll-like receptors (TLR) expressed on cell surfaces (TLR3), endosomes (TLR3/7/8), and cytoplasmic immunoreceptors, including protein kinase R (PKR) and helicases (RIG-1 and MDA5), are generally the main mechanisms via which RNA nanoparticles are detected. Although the exact mechanism of immune activation via various immunoreceptors is unknown, it is known to rely on the type of cells—such as immune cells—and the design of the nanoparticles as well as the delivery system.RNA from various receptors can have its immunostimulatory qualities greatly reduced by various chemical changes. For example, compared to their unmodified counterparts, siRNA constructs containing 2′ -F and/or 2′ -O-Me induced negligible amounts of interferon or cytokines. Surprisingly, 2′ -F- or 2′ -O-Me-modified U nucleotide RNA constructs were enough to remove immunological off-target effects, including pathways that are TLR-dependent and TLR-independent.Using LNA to modify the siRNA terminal ends can similarly effectively prevent the immunostimulatory effects of interferon-α while maintaining strong silencing activity.Similar to this, base alterations involving 2-thiouracil or pseudouracil can eliminate immunological activation mediated by RIG-1 since 5′ -triphosphate is present.[28]Therefore, chemical changes offer a potent tool for both eliciting gene knockdown without inducing immune system stimulation and eliciting immunoresponse for the treatment of chronic viral infections and malignancies.

2.Chemical alterations in RNA: consequences for serum stability and beyond

The use of RNA as a building material was previously hampered by its inherent instability. While the size and structure of RNA nanoparticles can offer some nuclease resistance, it is not adequate for in vivo application. Changes made to the inherent structure of RNA can make RNA therapies less vulnerable to endonucleases and exonucleases in serum. Among the most widely used tactics are base modification, alterations to the ribose sugar of the bases, and changes to the connection between bases (backbone modification). Chemical alterations can provide RNA structure more chemical and thermal durability, enabling the application of RNA therapies and nanoparticles in vivo[29].

Sugar Modifications.

Due to the fact that most changes increase thermal and enzymatic stability, the sugar moiety of RNA is the portion of the molecule that is most frequently modified. however it has no effect on folding into the RNA helix in the A-form (Figure 2A). The 2′ -OH is not necessary for the effective silencing of RNAi, but the A-form helix is. Therefore, siRNA maintains its silencing activity even after modification[30]. Compared to 2′ -OH natural RNA, 2′ -Fluorine-(2′ -F), 2′ -O-methyl-(2′ -O-Me), and 2′ -amine-modified (2′ -NH2) RNA are smaller in size, which permits modified duplexes to maintain their folding. Bulkier substitutions have been used more frequently as termini modifiers because internal alterations have an impact on RNA folding, such as 2′ -O-methoxyethyl (2′ -O-MOE). LNA is a sugar alteration in which a methylene bridge connecting the 4′ -carbon and the 2′ -oxygen structurally locks the ribose sugar into the A-form helix (3′ -endo).Folding will be impacted by a large percentage of LNA modification to the RNA duplex, but usually only a few modifications are required.

Base Modifications

RNA is more resistant to nucleases when the base-to-base connection is altered (Figure 2B). Modification of phosphorothioate, replacement of a Sulfur is frequently utilized to provide a nonbridging oxygen on the phosphodiester backbone[32].When phosphorothioate modification occurs at the proper locations on the siRNA sequence, it improves thermostability and siRNA efficiency. A library of DNA aptamers known as X-aptamers has also been created by phosphorus othioate modification and is utilized for cell-specific targeting. Boranophosphate modification, which results in a considerable increase in serum stability over native RNA, and enhanced siRNA efficacy by replacing oxygen on the phosphate backbone with borane.Peptide nucleic acids (PNA), which are amide-linked bases, are used in a more substantial alteration.The neutrally charged amide bond replaces the negatively charged phosphate, increasing the affinity between PNA and its corresponding RNA or DNA. Furthermore, PNA is more resistant to degradation because its foreign structure avoids being recognized by proteases and nucleases alike. Numerous analogues of nucleic acids are being actively investigated; among the most promising ones that have recently been created are synthetic nucleic acid mimics with an α peptide backbone and a triaminotriazine base shown at different residues (Figure 2C). This family of macromolecules is known as "bifacial peptide nucleic acid" (bPNA) because the triazine (melamine) base can interact with two Watson-Crick faces of thymine or uracil to form an obligatory triplex structure with two T/U-rich strands of DNA/RA. Allosteric control of aptamers and duplexes is made possible by these bPNA hybrid stems, which have the ability to structurally substitute native duplexes. These designs can be introduced into HEK293 and MCF7 cells either via ligand-driven entrance or passive nanoparticle uptake. By using this technique,

up to 40% luciferase silencing was achieved by delivering a bPoNA and cholesterol-modified siRNA duplex targeting firefly luciferase into HeLa-Luc cells, which express both firefly and renilla luciferase[33].

Effects on Structure and Function.

The principal objective of the previously stated chemical alterations of RNA was to mitigate the vulnerability of RNA therapies to nucleases. and prolong the duration of in vivo circulation for therapeutic benefit in the process. More and more beneficial structural and functional ramifications of these chemical alterations have emerged over the last ten years; a few of these are listed below.

Modulation of Thermal Stability.

One benefit of using RNA nanoparticles as a delivery mechanism is the ability to regulate the duration of in vivo circulation. For instance, replacing an RNA with DNA strand in the synthesis of RNA nanoparticles reduces thermal stability, whereas unmodified RNA substituted with 2′ -F increases thermal stability (Figure 2D)[34].When employing nucleic acid nanoparticles, it will be possible to adjust the delivery vector's circulation period, which will be very beneficial in various therapeutic situations. Furthermore, an excellent heat stability in an RNA nanoparticle can be advantageous for various applications. Numerous studies showed that thermodynamic stability complies with the following pattern: 1/76-79,91-93 LNA/LNA > LNA/2′ -F-RNA > 2′ -F-RNA/2′ -F-RNA > 2′ -F-RNA/RNA > RNA/RNA > RNA/DNA > DNA/DNA It is possible to include medication release and delivery methods into an RNA nanoparticle system by using different thermodynamic parameters. A recent publication demonstrating the inclusion of an 8 nt LNA-labeled RNA fragment into RNA nanoparticles that is complementary to the "seed region" of miRNA,60,94, serves as an example of this. The 8 nt LNA thermodynamically competed with the 8 nt seed in the miRNA upon delivery to the cytosol, binding to it and preventing its activity[34].

Changes in Serum Stability.

RNA as a scaffold for nanoparticles is a problematic material due to its low stability in blood serum. When changes are made to the By using the chemical structure of RNA, it is feasible to increase the stability of RNA in serum. The pRNA monomer structure's 2′ -F alteration, for instance, enhanced serum stability from less than 10 min to more than 36 h.[35].Furthermore, altering RNA's backbone connection increases its stability.In a deteriorating serum environment, circulation time is significantly increased by substituting amide-linked (PNA) connectivity, phosphor othioate, or boranophosphate for the conventional phosphodiester bond[Therefore, by using specific oligonucleotide ratios, the stability of nanoparticles in serum can be adjusted. This was demonstrated by the replacement of 2′ -F oligonucleotide for DNA during the synthesis of RNA nanosquares, which, respectively, stabilized and destabilized the nanosquares in serum (Figure 2E).

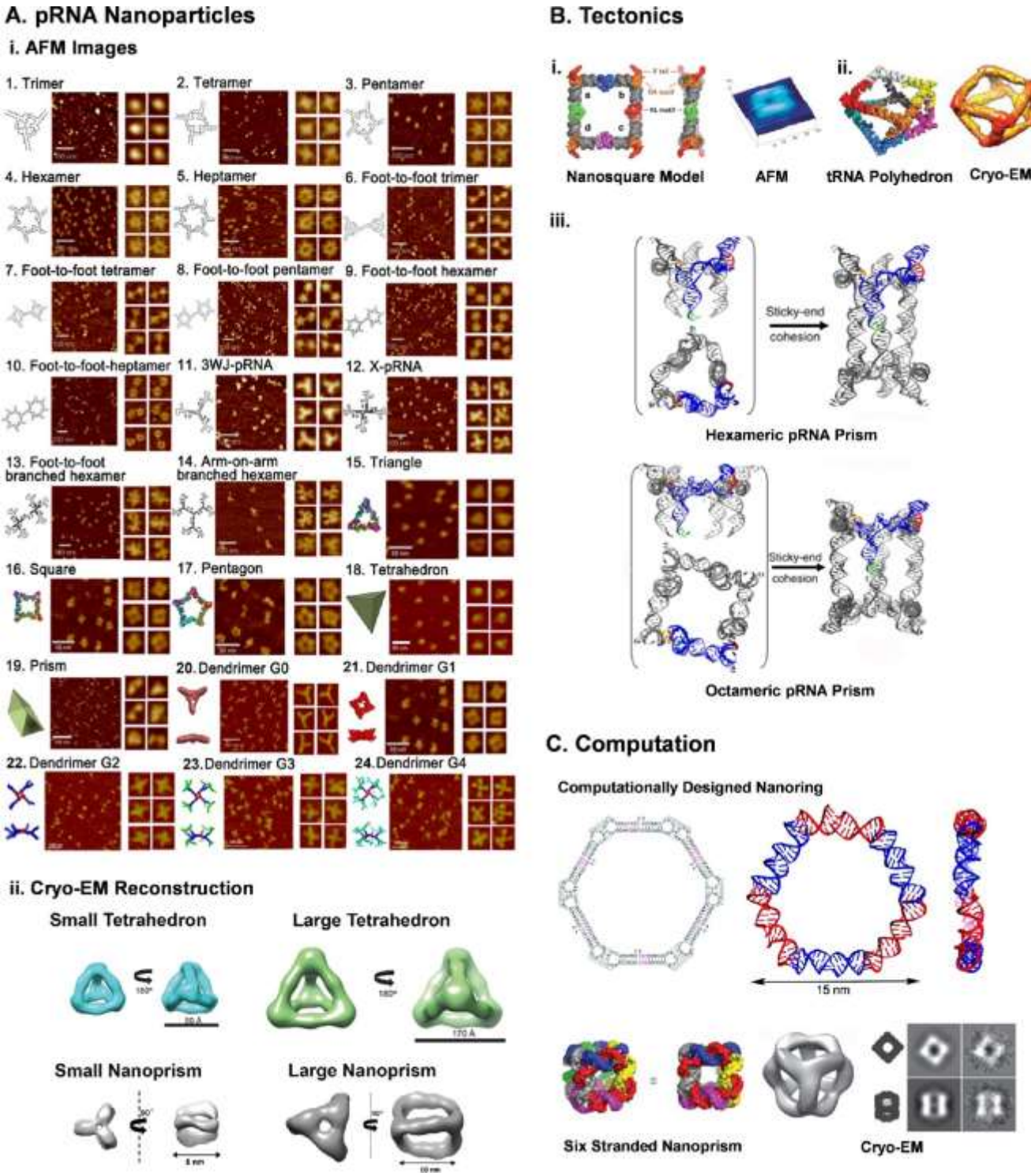

Fig.3. Techniques for making RNA nanoparticles. (A–i) RNA nanoparticles made using bacteriophage phi29's pRNA-3WJ. (A–ii) RNA nanoprisms and RNA tetrahedron nanoparticles reconstructed using cryo-EM based on the pRNA-3WJ motif. Wiley Publishing. All rights reserved. (B) Using tectonics, RNA nanosquares, tRNA polyhedrons, and phi29 pRNA nanoprisms are created.(C) Using computational methods to accelerate the production and optimization of RNA nanoparticles

3.Techniques for RNA Nanoparticle Construction

The goal of this paper is to outline the most recent techniques for creating RNA nanoparticles and the areas of RNA nanotechnology that are expanding the fastest. We recommend that readers look at the following reviews for comprehensive details on the ensuing building techniques:

1. Construction of RNA Nanoparticle via Hand− Hand Interactions.

The structural characteristics of packing RNA (pRNA), which is obtained from the bacteriophage phi2997 to form dimers, trimers, tetramers, and other structures, provide as examples of the design principles. Interlocking loops allow pentamers, hexamers, and heptamers to interact "hand-in-hand" .

2. Construction of RNA Nanoparticle via Foot-to-Foot Interactions.

In order to facilitate self-assembly through "foot-to-foot" interactions, single-stranded palindrome sequences are used to connect pRNA molecules[36].

3. Rational Design Utilizing Stable Natural RNA Motifs.

Motifs including kissing loops, dovetails, pseudoknots, kink twists, and multiway junctions can be used to create RNA nanoparticles.

4. Construction of RNA Nanoparticle via Extension of Robust pRNA-3WJ Motif.

The folding of pRNA modules linked to each vertex of the 3WJ is driven by the robust 3WJ (Figure 3A-i:11–14).

5. Construction of RNA Nanoparticle via Tectonics.

Growing understanding of RNA folding and the accessibility of databases like the NAD (Nucleic Acid Database) and Because there are so many motifs that could be employed as possible tectoRNAs, the RNAjunction database10 has expanded the power of tectonics. The tecto RNA cubes and squares made from tRNA (Figure 3B-i) and the nanoprisms made from pRNA (Figure 3B ii).

6. Construction of RNA Nanoparticles via Computational Design.

Over time, a number of software applications have been created, including RNA2D3D, INFO-RNA, NUPACK, NanoTiler, Assemble RNA2D3D,and beneficial for creating new RNA nanoparticles. Two such are an RNA nanocube made with Nanotiler and a six-membered RNA nanoring (Figure 3C).It is possible to computationally design and empirically create long sequences and single monomer units with internal structures in a one-pot process, including co-transcriptional assembly[37].

4.Latest Progress and Innovations in RNA Nanoparticle Synthesis Methods.

A thorough review of the literature on the creation of RNA nanoparticles was done, as was mentioned in the previous section. Those who read are urged, should they be interested, to consult these reviews. Here, we primarily analyze the most recent advancements, benefits, and discoveries in RNA nanoparticle technologies that haven't been thoroughly discussed in other assessments[38].

The creation of RNA nanoparticles with precise sizes using phi29 pRNA-3WJ.

One of the most difficult things to manage during the creation of nanoparticles is precise size control, which is crucial for therapeutic situations. It is commonly recognized that altering a nanoparticle's size or molecular weight may significantly impact PK/PD in an in vivo system. It is common to observe the random, and consequently frequently irreproducible, assembly of nanoparticles. The creator has precise control over how RNA nanoparticles are assembled. Each nucleotide in the nanoparticle is deliberately selected to fit together according to a clear design scheme, much like a jigsaw piece fitting in a precise place. Accurate assembly procedures guarantee a high yield and reliable RNA nanoparticle production. RNA nanoparticles can be divided into component bases, or "puzzle pieces," in any shape or size. phi29 pRNA-3WJ nanoparticles were used in the development of a recent technique for the exact regulation of RNA nanoparticle size. This 3WJ can function as a sturdy scaffold for the multistrand assembly method used in the design and fabrication of RNA polygons[39].The equilateral triangles were built with the native 3WJ inner angle of 60°.Square geometry can be created by simply lengthening the inner strand and adding one extra external strand to raise the inner angle of the 3WJ to 90°.22 Pentagon geometry can be created using the same process. The great thermal stability of the RNA polygons is not affected by the angle stretching of the 3WJ. RNA squares were created and built at 5, 10, and 20 nm along each edge using the same design procedure. Narrow size distributions were seen in their diameters as determined by dynamic light scattering and atomic force microscopy. It was simple to alter the RNA squares' dimensions by changing the amount of base pairs joining each 3WJ at the squares' corners[40].

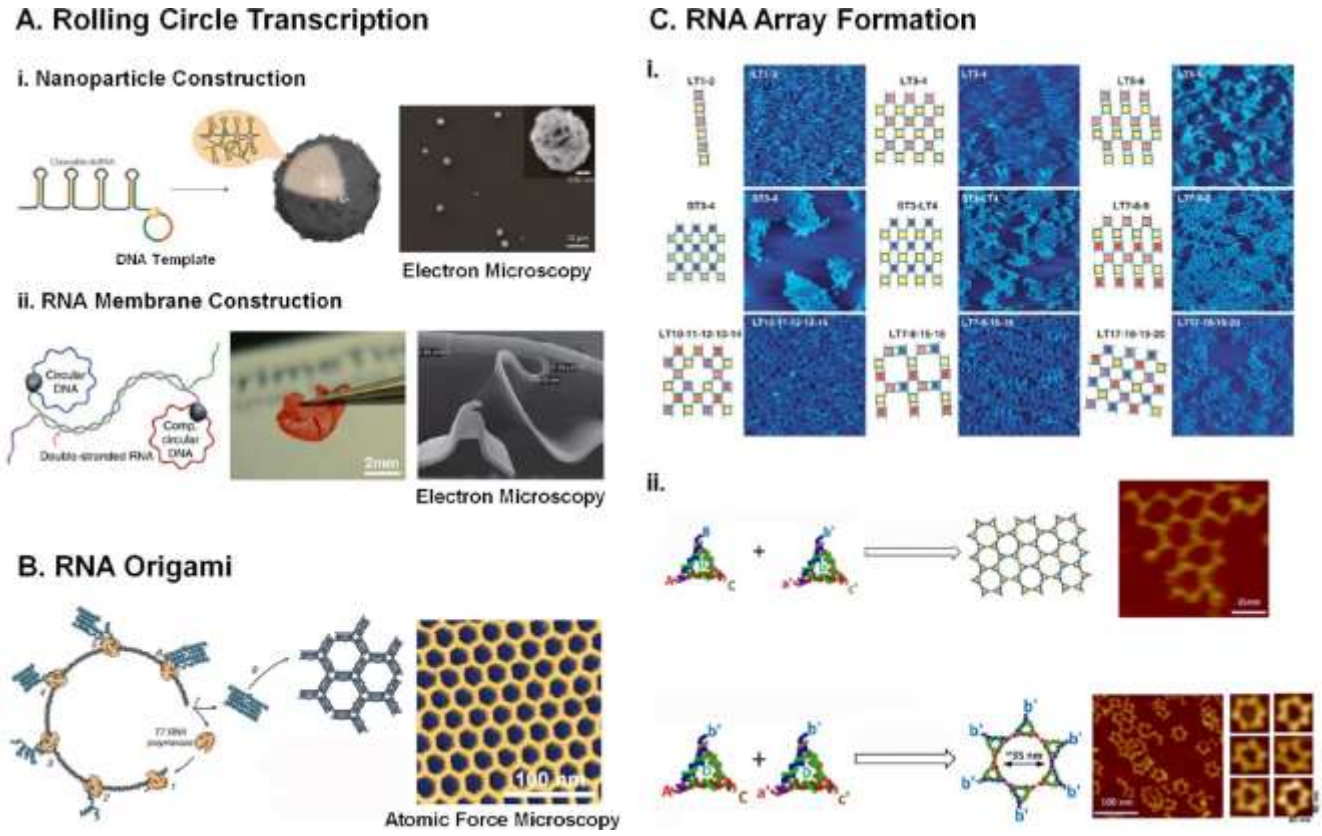

Fig.4. techniques for making RNA nanoparticles. (A) RNA structures and membranes are constructed during rolling circle transcription.(B) Using co-transcriptional folding, RNA origami is used to create massive RNA nanostructures, like hexameric arrays.

phi29 pRNA 3WJ is used to construct 3D RNA architectures and containers.

3D RNA nanoparticles with tetrahedral geometry—four triangular faces and six edges—have been created more recently using 2D architectures (Figure 3A-i:18).The upper panel of Figure 4A-ii).RNA tetrahedrons are expected to find use in a wide range of nanomedicine and nanomaterial applications due to their great mechanical stiffness and structural stability. Moreover, the EFGR aptamer fused to the tetrahedron structure demonstrated efficient targeting of triple-negative breast cancer by the RNA tetrahedrons. The encapsulation of a model small molecule medicine inside a nanoprism made of two pRNA triangles demonstrated additional uses for 3D nanostructures (Figures 3A i:19 and Figure 4A-ii bottom panel). Two distinct nanoprism sizes were created, whereby the smaller prism served as protection for a delicate RNA aptamer that contained a fluorogenic malachite green molecule. Fluorescence sharply increases as the malachite green molecule binds to its aptamer. By preventing the entry of breaking proteins with steric hindrance, the tiny nanoprism nearly doubles the luminous half-life of malachite green dye.By shielding small molecule medications from leaking cancer drugs, which can occasionally be hazardous to healthy cells, and preventing them from reaching cancerous cells, this encapsulation technique will contribute to the advancement of the field of RNA nanotechnology[41].

RNA Dendrimer Structure Construction Using phi29 pRNA-3WJ.

Highly branched RNA dendrimer structures were also created using the 3WJ-driven design approach (Figure 3A-i:20–24).Utilizing the pRNA nanosquare.

A symmetrical core that produces dendrimers of RNA. The square form lessens the potential for steric hindrance to occur during the construction of higher-ordered structures. Then, using intramolecular interactions between pRNA-3WJ motifs, a stepwise iterative assembly method was used to create highly branched generation-4 (G4) RNA dendrimers. Particles that were produced showed exact control over their size (about 65 nm), shape (3D globular), and stoichiometry (32 terminal units). RNA dendrimers exhibit a high loading capacity and can be readily functionalized through the use of chemical ligands, RNAi modules, RNA aptamers, and chemotherapeutics in large quantities. Furthermore, high resolution MRI or SPECT/PET imaging of targeted cells or tumors in vivo will be possible with dense loading of imaging agents[42].

Co-transcriptionally and intracellularly produced RNA nanoparticles using phi29 pRNA-3WJ.

Co-transcriptional formation of RNA nanoparticles, or folding of the nanoparticle during transcription, is one of its capabilities. In vitro.The phi29 pRNA-3WJ coupled with RNA functions, such as streptavidin aptamer, survivin siRNA, HBV ribozyme, spinach fluorogenic aptamer, and malachite green f fluorogenic aptamer, was utilized to demonstrate this.Each functional modality in the several multifunctional nanoparticles maintained its original function while co-transcriptionally and intracellularly assembling. Large-scale RNA nanoparticle production will be possible through intracellular fabrication since plasmids encoding for the components of the nanoparticles are easily cloned and expressed inside bacterial cells. In the future, fermentation of bacteria encoding for RNA nanoparticles will enable the large-scale manufacturing of RNA nanoparticles. To summarize, rational design

necessitates prior understanding of both the folding of the functional modules that will be integrated into the scaffold and the 3D folding of individual motifs that will be utilized as scaffolds for RNA nanoparticles. Any changes to the nucleic acid sequences must be carefully considered since they may have an impact on the overall folding of RNA molecules. To aid in the prediction of RNA structure or folding for nanoparticle assembly, a number of RNA folding algorithms are available. This method uses modular building blocks, so each component strand may be produced chemically with a high yield and then self-assembles in one pot or in steps. The approach can be applied to a broad spectrum of functional compounds due to its overall simplicity[43].

Rolling Circle Transcription: Creating RNA Nanoparticles.

One issue that might prevent the usage of RNA nanoparticles is getting significant dosages of therapeutic RNAi into the cell. RCA rolling circle amplification (RCA) is similar to using T7 in vitro transcription and isothermal DNA amplification, rolling circle transcription (RCT)generates substantial amounts of concatemeric RNA sequences from a circular DNA template. After ligating a ssDNA oligomer antisense to the target region, a short splint ssDNA bearing the T7 RNA polymerase promoter sequence is annealed to the circular DNA, enabling the start of RNA polymerase. In vitro transcription proceeds continuously following ligation. As an illustration, consider the creation of a circular DNA containing the siRNA gene, which is activated by the T7 promoter and lacks any terminators.T7 RNA polymerase gradually moves thousands of times along the circular DNA during in vitro transcription to produce several repetitions of siRNA. The RNA strand first took on the shape of a fiber as it lengthened, then it formed into a lamellar sheet, and lastly it became spherical sponge-like RNA nanoparticles. The characteristics of the microsponges prevented serum's breakdown of the unmodified RNA. Dicer processed the multimeric transcript successfully, producing a significant amount of siRNAs that effectively knocked down the target gene. Additionally, membranes made entirely of RNA were created via RCT (Figure 4A-ii)[44] was possible to create several circular templates with complementary sequences inside of them. Long ssRNA oligomers form massive complexes during the RCT reaction, and uniform RNA sheets are formed after evaporation-induced self-assembly. The final dimensions, as determined by electron microscopy, were a few millimeters wide and roughly 2.5 μm thick. It is conceivable that RNA sheets may be employed to transport a sizable payload of intercalating small molecules, such the anticancer drug doxorubicin, or to load vast numbers of therapeutic RNAs. Many of the characteristics of RNA nanoparticles produced via RCT can be manipulated, despite being less accurate than tectonics or computer-aided designs. Because metal ions generated during transcription cause aggregative formation, RCT-based RNA nanoparticles have a spherical morphology. By varying the amount of polymerase used in the RCT reaction, the size of the RNA nanoparticles can be adjusted. RNA nanoparticles can be made further smaller by employing artificial polycations, like polyethylenimine, to make them easier to enter cells.The obvious benefit is that an RNA nanoparticle can contain several siRNA targeting distinct genes or the same gene locus. On the other hand, introducing an excessive amount of siRNA into cells might have harmful effects, thus it's important to carefully examine how much RNA is supplied to each cell[45].

RNA Nanoparticle Construction using Origami.

Due to the success of DNA origami46, RNA scientists are attempting to use the same techniques for RNA nanoparticles. building. Despite being more difficult to construct, RNA origami has been accomplished because of RNA's more intricate folding mechanism. By co-transcriptionally folding a single RNA strand into premade tiles,single-stranded RNA tiles were created (Figure 4B). Then, through interactions between kissing loops, the tiles took on intricate shapes. By adjusting the angle of the kissing loop utilized for tile association, the origami's shape was adjusted. With this method, the time-consuming annealing processes and expensive chemical synthesis involved in DNA origami are eliminated.

The true benefit, however, is their enormous potential for cloning and large-scale in vivo expression for possible uses in synthetic biology[46].

RNA Nanoparticle Array Construction.

Enzymatic pathways are grouped physically onto scaffolds or separated into specifically made compartments in a cellular context. like organelles. By placing proteins and their substrates in close proximity, spatial organization promotes faster enzymatic reactions while reducing the possibility of enzyme cross-talk. The spatial organization of biomolecules has been the main focus of DNA nanotechnology; nevertheless, stability concerns have restricted its usage to in vitro settings. The in vivo stability of RNA nanoparticles enables more fundamental cellular regulation. The isothermal assembly of RNA molecules into predetermined discrete 1D and 2D structures with different protein docking sites that serve as scaffolds for the construction of an enzyme pathway that produces hydrogen is a well-known example of this.4 RNA nanotechnology, as opposed to protein-based methods, allows the construction of intricate multidimensional structures with nanoscale accuracy to design biological pathways through spatial limitations[47].

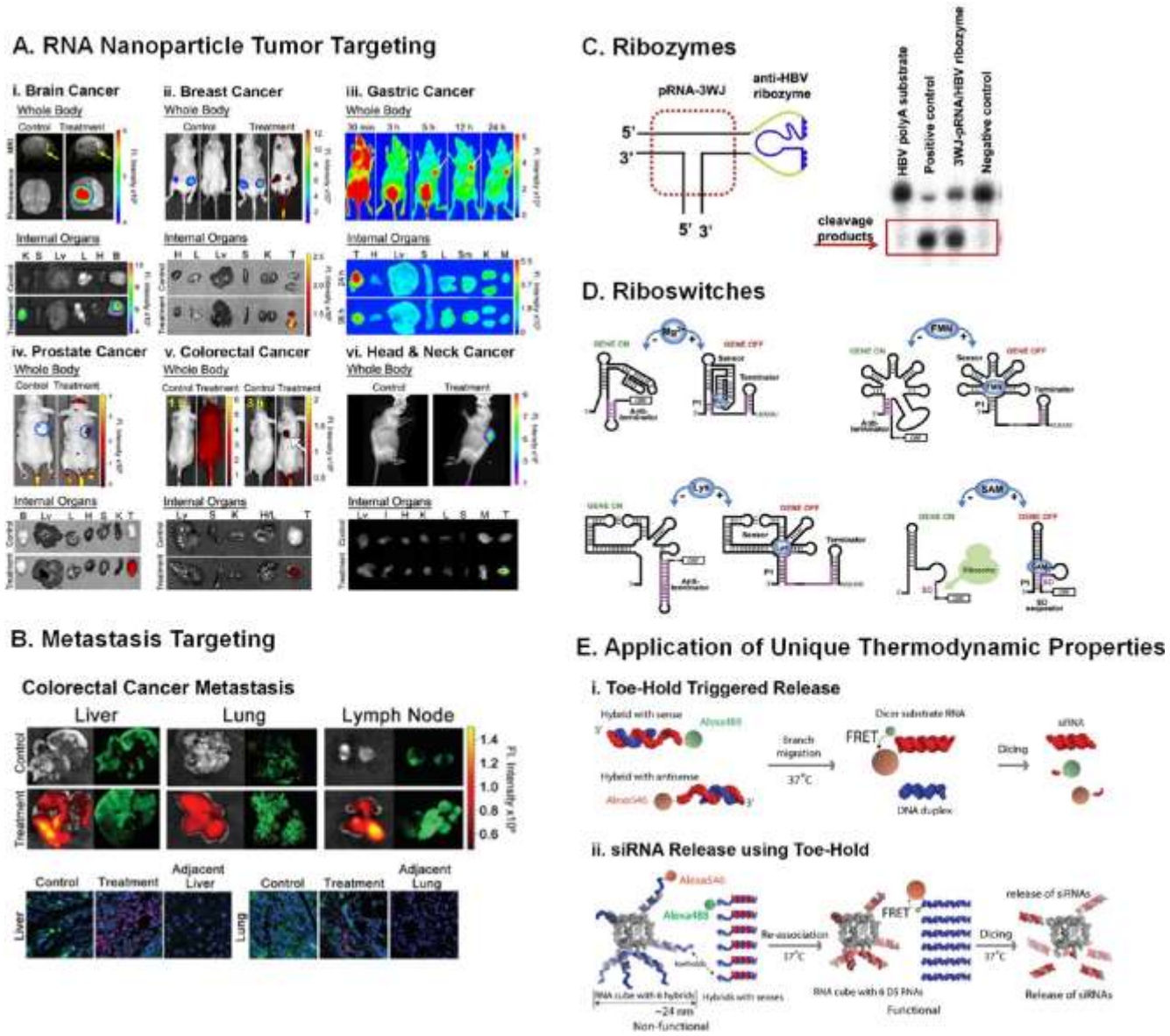

Fig.5. RNA nanoparticles for medicinal use. (A) Utilizing chemical ligands and RNA aptamers, cancers in vivo that are particularly targeted to avoid accumulating in healthy organs are targeted, including glioblastoma, breast cancer, gastric cancer, prostate cancer, colorectal cancer, head and neck cancer (B) Using RNA nanoparticles to target colorectal cancer metastases. (C) Ribozymes that cleave particular RNA substrates that resemble RNAi (D) The control of gene expression using riboswitches.(E) Nucleic acid thermodynamic characteristics that are utilized for reagent release and activation.

5.Nanomedical and nanobiotechnical applications

Numerous platforms for nanoparticles have been developed throughout the years due to advancements in nanotechnology, such as liposomes, polymers,viral nanoparticles,Inorganic nanoparticles and dendrimers.These sub-micron-sized platforms have the potential to enhance medicinal approaches' efficacy.However, a significant obstacle continues to be the non-specific accumulation of therapeutic nanoparticles in healthy essential organs such as the liver, lungs, kidneys, and spleen. Low specificity increases toxicity and adverse consequences while lowering the percentage of nanoparticles that reach sick cells. Particle heterogeneity, aggregation, dissociation, negative PK/PD profiles, and difficulty piercing biological barriers around sick cells, such as the tumor microenvironment, are frequently linked to these detrimental consequences.Furthermore, clinical translation is hindered by high production costs, unstable chemical and thermodynamic properties, and a lack of controlled-release methods.Regulatory licensing problems for these technologies may arise from the complex compositions of nanocarriers with a variety of functional modules (inorganic/organic nano scaffolds, RNAi/protein targeting antibodies, chemical drugs/antibodies).Recent research has demonstrated that RNA nanoparticles can address a number of these problems to get beyond significant obstacles in the field of nanomedicine for cancer therapy.Viral infections,ocular diseases[48].

6.RNA Nanotechnology's Use of RNA Biochemical Properties.

Applying RNA Aptamers for Targeted Therapeutic Delivery or as Potent Inhibitors.

In general, off-target toxicity and nanoparticle accumulation in healthy organs are significantly decreased by targeted delivery of nanoparticles. connected to passive systems that only use EPR effects. Based on RNA aptamers, a new class of targeted medicinal compounds has been created. These molecules consist of single-stranded RNA sequences that fold into particular three-dimensional configurations and bind to the extracellular domains of cell surface receptors with great selectivity and affinity. Binding is mediated by hydrogen bonds, base stacking, van der Waals forces, and electrostatic interactions. When used as targeting reagents, aptamers have several benefits over protein antibodies, including as affordability, a quicker selection and optimization process, ease of synthesis and modification with excellent batch fidelity, low immunogenicity, quick tissue penetration, and long-term stability. Aptamer creation for several proteins and peptides has been made feasible by the establishment of the systematic evolution of ligands through an exponential enrichment (SELEX) approach. It is theoretically possible to select an aptamer for any target due to SELEX's broad applicability.Tens of RNA aptamers are currently available for targeting specific cell surface receptors, which then lead to the internalization of RNA nanoparticles: EGFRvIII in glioblastoma; EGFR, HER2, HER3, in breast cancer; PSMA in prostate cancer; E-s electin in ovarian cancer; EpCAM in colon cancer; 166 and CD19 in lymphoma; and[49] in prostate cancer (Figure 5A-iv).Cells in addition to those infected with viruses like HIV (like CD4).Cell internalizing RNA aptamers are integrated into RNA nanoparticles through sequence fusion to enable the targeted delivery of therapeutic drugs, including chemotherapeutics, siRNA, and miRNA, into the cytosol. The attachment of small molecule ligands to RNA nanoparticles—such as folic acid, which binds to cell surface receptors—is one of the additional tactics for cell-specific targeting.Folate receptor overexpression is a common feature of epithelial cancer cells, which makes it possible for folic acid attached nanoparticles to target these cells more frequently than they do normal cells.pRNA-3WJ-folate nanoparticles have been used to target many cancer types, including glioblastoma (Figure 5A-i), gastric cancer (Figure 5A-iii), colorectal cancer (Figure 5A-v), and head and neck cancer (Figure 5A vi).An further area where RNA nanoparticles have demonstrated significant potential is in the targeting of cancer metastases. Since malignant cells can migrate to distant organs and lymph nodes, many metastatic tumors are difficult to treat. RNA nanoparticles were able to concurrently target colon cancer cells in the liver, lymph nodes, and lungs—the three main sites of metastasis—by using folic acid as a targeting agent (Figure 5B).In order to modify downstream signaling, aptamer-based treatments rely on the direct binding of an aptamer to a therapeutic target. Prominent instances encompass cancer therapy agents that target receptor tyrosine kinases (like anti-EGFR inhibitors), cell adherence factors (like E-selectin), immune system modulators (like anti-CTLA-4 inhibitors), and cell growth (like α-fetoprotein).Numerous aptamers are undergoing preclinical and clinical trials. Additionally,Aptamers have been created to treat neurological conditions such as multiple sclerosis (IL-17), Alzheimer's disease (β-scretase1), Parkinson's disease (AMPA), stroke (Factor IX a), and Parkinson's disease (β-scretase1).RNA nanotechnology provides a means of incorporating strong scaffolding into the in vitro selection process, hence improving the thermodynamic stability and usefulness of therapeutic aptamers[50].

Utilizing Ribozymes in Focused Treatment.

Treatment approaches based on ribozymes have been increasingly effective in the fight against viral infections and cancer.RNA motifs with catalytic qualities akin to those of proteins. In animal models, ribozymes targeting mRNA have demonstrated promise for gene therapy of hepatocellular carcinoma and breast cancer because they are equally successful as RNA interference (RNAi) with fewer side effects.Anti-retroviral medicines are frequently associated with toxicity and the evolution of resistant phenotypes; ribozyme-based therapies are thought to be feasible substitutes.But creating nonviral vectors to deliver ribozymes to certain cells in vivo is a difficulty. To deliver ribozymes precisely, ribozyme sequences are fused with RNA scaffolds to create a targeting platform. Hepatitis B virus (HBV) hammerhead ribozyme, which preserves its enzymatic activity to cleave the poly(A) signal on HBV mRNA and hence suppress HBV replication, is an example of this in the form of pRNA nanoparticles (Figure 5C)[51].

Using Riboswitches as Antimicrobial Targets and to Modulate Gene Expression.

Through their ability to control gene expression, riboswitches provide therapeutic options for treating a variety of illnesses. They are common in many evolutionary distant bacteria that have analogs in algae, fungus, plants, and archaea. The two domains that make up riboswitches are an expression platform that can adopt two mutually exclusive conformations dependent on ligand binding to control gene expression (without the need for protein cofactors) and a sensor component (a natural aptamer) for ligand recognition (Figure 5D)[52].Metabolite-binding riboswitches are antimicrobial targets that may be able to address multiple drug resistance. Examples include the lysine riboswitch, which regulates the biosynthesis and transport of lysine; the FMN riboswitch, which regulates the biosynthesis of riboflavin; the guanine riboswitch, which regulates the expression of genes involved in purine biosynthesis and transport; and the TPP riboswitch, which is involved in the biosynthesis and transport of thiamine in bacteria.TPP riboswitches control gene expression in eukaryotes by means of alternative splicing, which results in mRNAs with internal stop codons that either result in premature translation or the translation of aberrant peptides. As riboswitches regulate essential metabolic and virulence genes in pathogenic organisms, they offer an appealing approach for therapeutic intervention that doesn't harm the eukaryotic host.By introducing artificial ligands into human cells, riboswitches can also be employed to build synthetic genetic circuits. Relying on straightforward design concepts, RNA nanotechnology can be utilized to rationally create synthetic riboswitches that can either activate or repress gene expression in a ligand-dependent manner, in addition to delivering RNA switches in vivo[53].

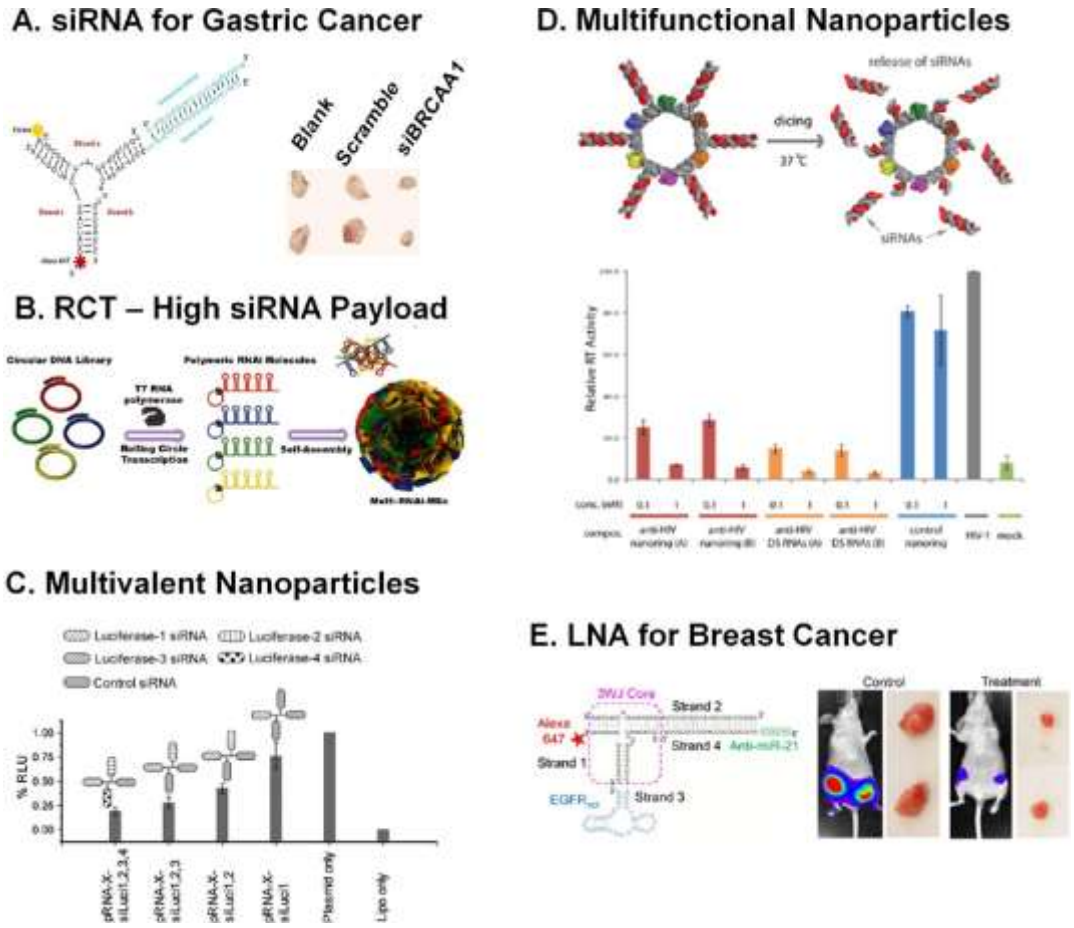

Fig.6. RNAi with RNA nanoparticles. To deliver BRCAA1 siRNA in vivo and stop the growth of stomach cancers, use (A) pRNA-3WJ (B) RCT was utilized to create kilobase concatemeric RNA oligomers, which allowed numerous siRNA to be delivered with a high payload.(C) Multiple siRNAs having a cooperative effect on gene silencing. The amount of light units drastically decreases when luciferase siRNA is copied. (D) RNA nanorings that carry six siRNAs to knock down distinct HIV targets through gene expression. (E) pRNA-3WJ to deliver antimi RNA, LNA, to help triple negative breast cancer patients decrease the growth of their tumors.

Using Thermodynamic Properties to Release Functional Agents Under Control.

It is possible to program RNA nanoparticles to exhibit a thermodynamic property that will cause various nucleic acid types to release or activate functional modules. As was previously mentioned, nucleotide selection offers adjustable stability for nanoparticles, allowing for controlled release. This is demonstrated by pRNA-based nanosquares, which are tuneable through the replacement of DNA and 2′ -F RNA22 for their "core" strands (Figure 2D,E). In contrast to DNA substitution, which has the exact opposite effect, 2′ -F RNA as the core strand raises the melting temperature and boosts the material's resilience to serum breakdown. Regardless of whether the medications are conjugated to the vertices of the particles or intercalated, strategies leveraging variations in nucleic acid thermodynamic stability can be employed to adjust the release of the drug from the particle[54].

Triggers for dicer substrate RNA's conditional release have also been designed using strand displacement and RNA/DNA hybrid duplex.By attaching to its target mRNA, an RNA nanoparticle having a partial RNA/DNA duplex region that is complementary to the target mRNA can conditionally release the Dicer substrate, preventing the RNAi's off-target harm. Moreover, functionality can be divided across several helices, and their functions are activated upon toe-hold interaction. Each component of the split-functionality is labeled with a FRET pair molecule to track the release; as a result, the FRET signal sharply increases upon functionality connection (Figure 5E)[55].

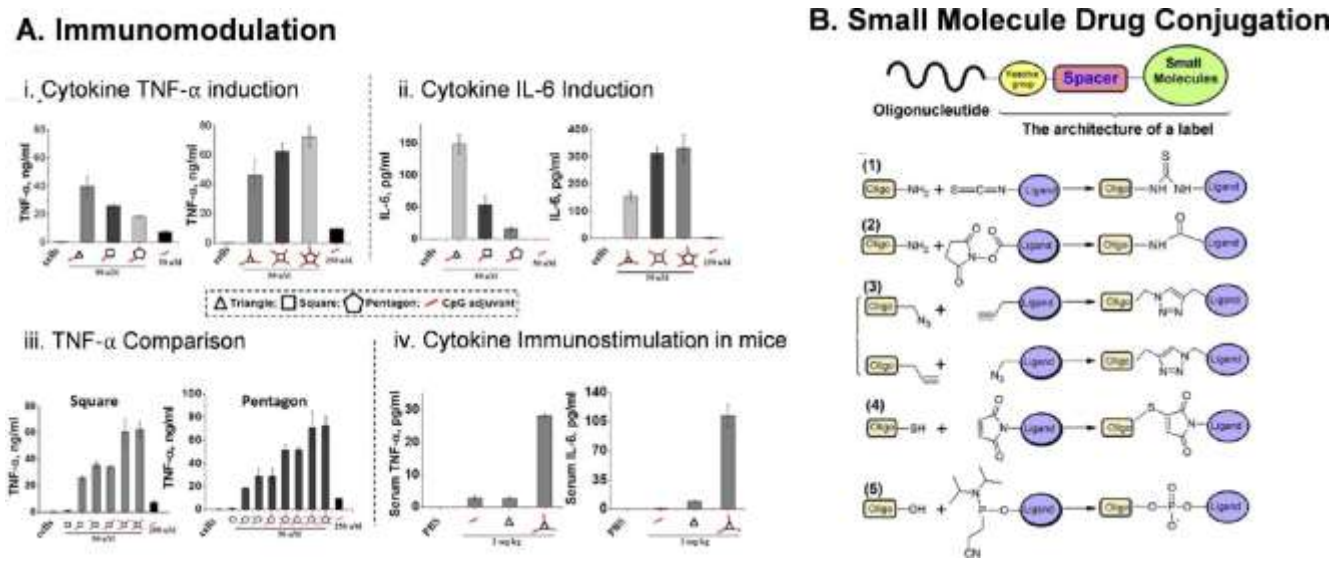

Fig.7. RNA nanoparticles for administration of chemotherapy and immunotherapy. (A) Under normal circumstances, RNA nanoparticles show little to no immunological response; however, the addition of immunostimulatory CpG sequences causes a marked increase in the immune response and cytokine production. Reprinted from ref. 21 with permission. Oxford University Press 2014 All rights reserved. (B) Chemical conjugation techniques and medications to nanoparticles of RNA.

Implementing RNA-Interference Treatment:

LNA, mirai, and siRNA. Many diseases now have therapy options thanks to the 1998183 discovery of the RNA interference (RNAi) pathway. such as cancer. RNA interference (RNAi) is the mechanism by which short double-stranded RNA sequences, usually ranging from 21 to 23 nucleotides in length, control a gene's expression. RNAi is made up of two main molecules: siRNA and miRNA.While siRNAs are generally synthetic sequences (although some endogenous siRNAs do exist in cells) intended to target a particular mRNA, miRNAs are indigenous to cells and target numerous genes. Theoretically, RNA interference (RNAi) therapy can target any known mRNA sequence using siRNAs and miRNAs, therefore rendering previously incurable diseases accessible. The siRNA and miRNA encoding dsRNA sequences are accessible to endonuclease Dicer through the design of RNA nanoparticles. Dicer then cleaves the dsRNA sequences to produce functional siRNA and miRNA, which are subsequently loaded into the RNA-induced silencing complex (RISC) to selectively target mRNAs and regulate gene expression.pRNA-3WJ nanoparticles are a prime example of siRNA delivery in vivo.In one investigation, the therapeutic module was BRCAA1 siRNA and the targeted ligand was folate-carrying pRNA-3WJ[56]. Demonstrated potential for treatment of stomach cancer (Figure 6A).61% Systemic injection of the RNA nanoparticles into subcutaneous gastric tumor xenograft-bearing mice resulted in their internalization into the tumors by folate-receptor-mediated endocytosis, and the siRNA effectively repressed BRCAA1 expression. Regressing the growth of the tumors, BRCAA1 knockdown up-regulated the expression of pro-apoptotic Rb and Bax genes and down-regulated the expression of the antiapoptotic Bcl-2 gene.According to a different study, intracranial glioma xenograft-bearing mice can effectively suppress the luciferase gene's expression following systemic injection of pRNA-3WJ nanoparticles containing folate and luciferase siRNA .As part of a different tactic, RCT produced tandem repetitions of luciferase siRNA, or RNAi microsponges, were effectively cleaved by Dicer to convert stable hairpin RNA to siRNA only after cellular absorption (Figure 6B). In mice carrying subcutaneous ovarian cancer xenografts, luciferase expression was successfully suppressed.This technique was further improved such that it could target several genes with different siRNAs[57].By proving that intravaginal administration of RNA nanoparticles, consisting of CD4 aptamer and siRNAs targeting HIV coreceptor CCR5, gag, and vif, protected humanized mice against sexual transmission of HIV, another work illustrated the possibility for viral treatment[58].

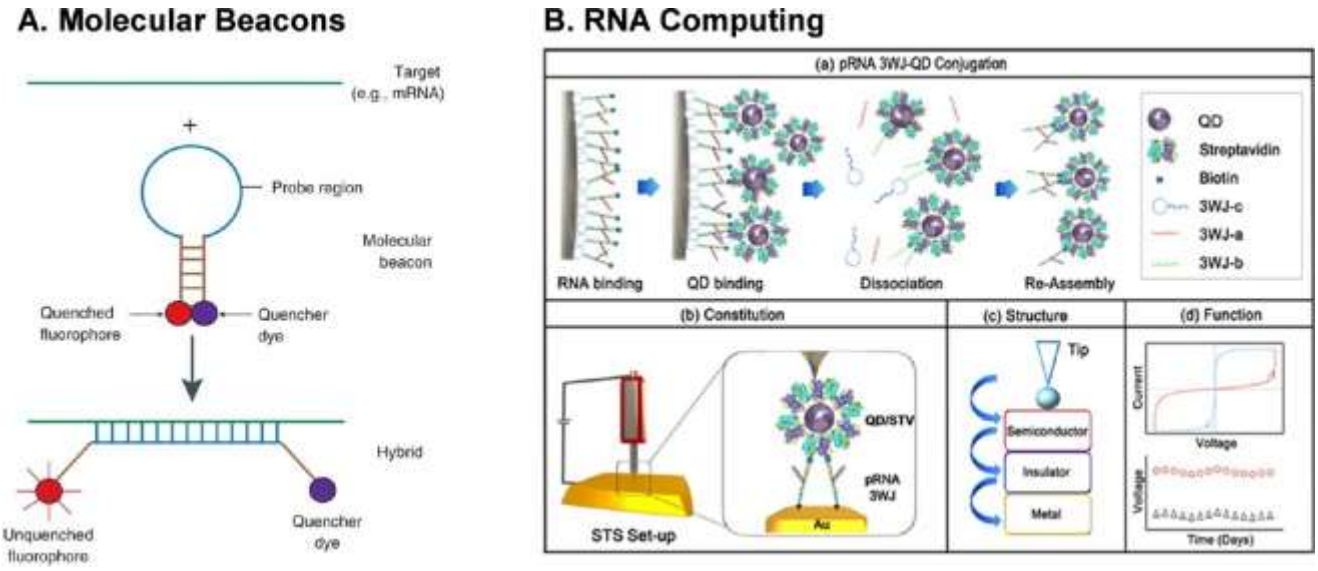

Fig.8. RNA nanotechnology application in resistive biomemory (B) and beacons (A). Reprinted from reference 27 with permission for Panel A.

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7.Difficulties and goals

Recently, there has been a lot of interest in RNA nanotechnology for drug delivery because of its enormous potential to treat a wide range of illnesses, including cancer. Undoubtedly, advancements are essential to get this cutting-edge platform closer to clinical trials and commercialization. We go over a few of the issues and potential fixes below[59].

Restricted payload of small molecules.

The ability of RNA nanoparticles to load tiny molecules with drugs is currently restricted. The terminal ends might bear labels for individual medications.

RNA strands with complex drug release systems. The release of the pharmaceuticals can be compromised by whole chain labeling techniques, which can also boost the chemical payload but cause steric hindrance and RNA nanoparticle misfolding.Finding sites for drug delivery without interfering with the nanoparticle's ability to fold can be aided by computational methods[60]. Any structural flaws in the scaffold can also be reduced by making sure the medications are oriented away from the RNA nanostructure. Drug release patterns must be carefully examined because premature drug release can result in nonspecific side effects. Intercalation is another practical method to boost drug loading capacity. The majority of systemically administered pRNA-3WJ nanoparticles localize in the tumor site within 1-4 hours, according to extensive biodistribution studies. Therefore, if the pRNA-3WJ nanoparticle is used as a delivery vector to target solid tumors, the projected half-life of the intercalated drug release should be more than 4 hours. RNA dendrimers have recently been created up to generation[61].With their branched architecture, repeating concentric layers, hollow cavities that can accommodate drug loading, and peripheral/terminal units that can house numerous medicines and other targeting modules, dendrimers may be able to overcome the loading difficulties. Hundreds of small molecule medications may be contained in 3D RNA polygons, which have recently been designed as RNA cages or containers[62].In addition, the RNA cages are biocompatible and biodegradable in comparison to synthetic nanocarriers and can assemble in an organic or aqueous solvent without altering the fundamental properties of the RNA[63].Many believe that RNA interference (RNAi) holds the potential to "drug the undruggable" and is the next big thing in cancer therapy.The quantity of RNAi molecules that may be given to cells by RNA nanoparticles is constrained, just like in chemical drug conjugation. Since there are only roughly[64].Dicer molecules per cell, research suggest that this restriction might not be a problem. Additionally, oversaturation of therapeutic RNAs can result in nonspecific binding and off-target gene silencing[65].Cytotoxic effects can result from delivering too much shRNA to cells.RNA nanoparticles' smaller payload may therefore eventually prove to be advantageous, as demonstrated by animal experiments demonstrating their low toxicity. Consequently, it's likely that having too much of a good thing is actually bad. High payloads of siRNAs can be delivered using RNA microsponges, and if the particle size can be lowered to prevent organ accumulation, their usefulness in more extensive preclinical research will increase[66].

Endosome Escape to Reap RNAi's Promise.

RNA nanoparticles enter cells by receptor-mediated endocytosis, just like the majority of nanoparticle platforms. Consequently, the trafficking of intracellular RNA nanoparticles becomes the next a test. The initial location of RNA nanoparticles is early endosomal vesicles. RNA nanoparticles are sorted and then moved to late endosomes and lysosomes, where they become stuck before reaching their destination. Although cancer regression following siRNA delivery via receptor-mediated endocytosis is relatively low, the effectiveness of endosomal escape of siRNA in RNA nanoparticles is still unknown. Fortunately, endosomal escape using small 8 nt anti-miRNA LNA fragments in RNA nanoparticle delivery was successful and efficient[67].The many internalization or cellular endocytosis processes that control intracellular processing and endosomal escape of RNA nanoparticles are still mostly unknown. However, there are well-studied methods for improving endosome escape, such as chemical functional groups like sulfonamide and amino

esters, or other acid protonating groups like hydrazone, maleic amides, and acetal, linkers that can be broken down by acid[68].The "proton-sponge" effect is caused by the pH-sensitive materials being highly protonated, which causes an inflow of ions into the endosome and causes osmosis and endosome rupture. As was previously mentioned, RNA nanoparticles can include endosome-disrupting molecules into them in a variety of ways using chemical conjugation techniques[69].

Production and Purification of RNA Nanoparticles on a Large Scale.

The large-scale manufacture, large-scale purification, and expense of RNA nanoparticle production are key obstacles to future clinical uses.RNA nanoparticles are generally engineered to be modular, consisting of several short strands that fall well within the parameters of chemical synthesis, with a maximum length of 80 nt. Due to significant advancements in the efficiency of chemical synthesis based on 2 protecting groups, such as ether, acetal, orthoester, ester, O-acetalester, and pivaloyloxymethyl (PivOM), the cost of RNA oligosynthesis has gradually dropped over time[70].Commercial suppliers have created production facilities with GMP-grade production capabilities that can generate tens of grams of RNA every synthesis cycle. Large-scale purification is still difficult, though. With relatively low yields, HPLC and gel electrophoresis have restricted limitations. Because of the unique properties of RNA nanotechnology, the assembled nanoparticle's size differs greatly from that of its constituent parts. As a result, preparative ultracentrifugation has been used recently and appears to be a promising method for the high-yield purification of fully assembled RNA nanoparticles[71].

Reasonable Integration of Chemically Alternated Nucleotides.

Although chemical changes have numerous benefits in terms of immunogenicity, serum stability, and thermodynamic stability, they can occasionally have an impact on the folding of RNA patterns. For instance, 2′ -F alteration causes misfolding of the malachite green (MG) f luorogenic RNA aptamer[72], rendering it nonfunctional. Reasonable modification is a case-by-case matter that depends on the structure and RNA sequence. Certain chemical modification patterns may be effective in one system but not in another. The difficult part is coming up with general chemical modification techniques that can be used to any sequence. Clinical translation of a wide range of RNA-based therapeutics still requires general consideration of improvements in RNA nanoparticle designs related to chemical modification[73].

RNA Nanoparticle Assembly: A Computational Guide.

To guarantee the genuine functionality of multifunctional RNA nanoparticles, it is crucial to comprehend the proper global folding of RNA constructs. RNA molecules are dynamic and capable of switching between several 3D conformations with marginally varying ΔG values. More limitations are placed on the kinetic and thermodynamic characteristics of RNA folding in larger assemblies. While many easily navigable web-based tools have been created, such as Mfold, RNA Designer, Sfold, 235, NUPACK, 115, Nanofolder,[74]and Hyperfold, precise RNA structure and folding prediction for nanoparticle assembly is still a significant problem. The development of prediction tools that can anticipate inter-RNA interactions and analyze 3D and 4D structures for nanoparticle designs is required by RNA nanotechnology. Although the field is still in its infancy, some progress has been achieved in RNA 3D computing from the conventional intramolecular interactions to intermolecular interactions [75].

Conclusions

The past decade has witnessed remarkable advancements in the field of RNA nanotechnology, establishing it as a pivotal area in the realm of nanomedicine. The unique structural and functional characteristics of RNA, such as its ability to self-assemble and form intricate quaternary structures, have made it an attractive biomaterial for nanoparticle drug delivery. The flexibility of RNA to engage in both canonical and noncanonical base pairings enhances its structural diversity and stability, essential for creating robust therapeutic nanoparticles.The journey of RNA nanotechnology has been driven by the collaborative efforts of many researchers, with significant milestones such as the discovery of pRNA dimers, trimers, and hexamers laying the foundation for this field. The understanding of RNA's role beyond mere genetic information carriers has ushered in a new era in pharmaceutical sciences, where RNA and its nanoparticles are poised to become the third major milestone in drug development, following chemical and protein-based drugs.The favorable properties of RNA, including its negative charge and modularity, make it ideal for constructing multifunctional nanoparticles that can be used for targeted delivery and cancer immunotherapy, among other applications. Advances in chemical modifications have significantly improved RNA's stability and functionality, paving the way for its in vivo application. Techniques such as rolling circle transcription and RNA origami have further expanded the toolkit for RNA nanoparticle construction, enabling precise control over nanoparticle size and structure.Despite the immense potential, challenges such as payload limitations, endosomal escape, large-scale production, and computational prediction of RNA folding remain. However, with ongoing research and innovation, RNA nanotechnology is well on its way to overcoming these hurdles, bringing us closer to realizing its full therapeutic potential. As we continue to explore and harness the biochemical properties of RNA, the future of RNA nanotechnology in nanomedicine looks promising, offering new solutions for the treatment of a wide array of diseases.

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References

(1) Guo, P. The Emerging Field of RNA Nanotechnology. Nat. Nanotechnol. 2010, 5, 833−842.

(2) Guo, P.; Zhang, C.; Chen, C.; Trottier, M.; Garver, K. Inter-RNA Interaction of Phage Phi29 PRNA to Form a Hexameric Complex for Viral DNA Transportation. Mol. Cell 1998, 2, 149−155.

(3) Guo, S.; Tschammer, N.; Mohammed, S.; Guo, P. Specific Delivery of Therapeutic RNAs to Cancer Cells Via the Dimerization Mechanism of Phi29 Motor PRNA. Hum. Gene Ther. 2005, 16, 1097− 1109.

(4) Delebecque, C. J.; Lindner, A. B.; Silver, P. A.; Aldaye, F. A. Organization of Intracellular Reactions With Rationally Designed RNA Assemblies. Science 2011, 333, 470−474.

(5) Lee, J. B.; Hong, J.; Bonner, D. K.; Poon, Z.; Hammond, P. T. Self-Assembled RNA Interference Microsponges for Efficient SiRNA Delivery. Nat. Mater. 2012, 11, 316−322.

(6) Zuo, H.; Wu, S.; Li, M.; Li, Y.; Jiang, W.; Mao, C. A Case Study of the Likes and Dislikes of DNA and RNA in Self-Assembly. Angew. Chem., Int. Ed. 2015, 54, 15118−15121.

(7) Laurenti, E.; Barde, I.; Verp, S.; Offner, S.; Wilson, A.; Quenneville, S.; Wiznerowicz, M.; MacDonald, H. R.; Trono, D.; Trumpp, A. Inducible Gene and ShRNA Expression in Resident Hematopoietic Stem Cells in Vivo. Stem Cells 2010, 28, 1390−1398.

(8) Hoeprich, S.; ZHou, Q.; Guo, S.; Qi, G.; Wang, Y.; Guo, P. Bacterial Virus Phi29 PRNA As a Hammerhead Ribozyme Escort to Destroy Hepatitis B Virus. Gene Ther. 2003, 10, 1258−1267.

(9) Chang, K. Y.; Tinoco, I., Jr. Characterization of a ″ Kissing″ Hairpin Complex Derived From the Human Immunodeficiency Virus Genome. Proc. Natl. Acad. Sci. U. S. A. 1994, 91 (18), 8705−8709.

(10) Bindewald, E.; Hayes, R.; Yingling, Y. G.; Kasprzak, W.; Shapiro, B. A. RNAJunction: a Database of RNA Junctions and Kissing Loops for Three-Dimensional Structural Analysis and Nanodesign. Nucleic Acids Res. 2008, 36, D392−D397.

(11) Wagner, C.; Ehresmann, C.; Ehresmann, B.; Brunel, C. Mechanism of Dimerization of Bicoid MRNA: Initiation and Stabilization. J. Biol. Chem. 2004, 279, 4560−4569.

(12) Sugimoto, N.; Nakano, S.; Katoh, M.; Matsumura, A.; Nakamuta, H.; Ohmichi, T.; Yoneyama, M.; Sasaki, M. Thermodynamic Parameters to Predict Stability of RNA/DNA Hybrid Duplexes. Biochemistry 1995, 34, 11211−11216.

(13) Searle, M. S.; Williams, D. H. On the Stability of Nucleic Acid Structures in Solution: Enthalpy-Entropy Compensations, Internal Rotations and Reversibility. Nucleic Acids Res. 1993, 21, 2051−2056.

(14) Lemieux, S.; Major, F. RNA Canonical and Non-Canonical Base Pairing Types: a Recognition Method and Complete Repertoire. Nucleic Acids Res. 2002, 30, 4250−4263.

(15) Leontis, N. B.; Westhof, E. Geometric Nomenclature and Classification of RNA Base Pairs. RNA 2001, 7, 499−512.

(16) Leontis, N. B.; Lescoute, A.; Westhof, E. The Building Blocks and Motifs of RNA Architecture. Curr. Opin. Struct. Biol. 2006, 16, 279−287.

(17) Leontis, N. B.; Westhof, E. Analysis of RNA Motifs. Curr. Opin. Struct. Biol. 2003, 13, 300−308.

(18) Khisamutdinov, E. F.; Jasinski, D. L.; Li, H.; Zhang, K.; Chiu, W.; Guo, P. Fabrication of RNA 3D Nanoprism for Loading and Protection of Small RNAs and Model Drugs. Adv. Mater. 2016, 28, 10079−10087.

(19) Khisamutdinov, E. F.; Bui, M. N.; Jasinski, D.; Zhao, Z.; Cui, Z.; Guo, P. Simple Method for Constructing RNA Triangle, Square, Pentagon by Tuning Interior RNA 3WJ Angle From 60 Degrees to 90 Degrees or 108 Degrees. Methods Mol. Biol. 2015, 1316, 181−193.

(20) Khisamutdinov, E. F.; Jasinski, D. L.; Guo, P. RNA As a BoilingResistant Anionic Polymer Material to Build Robust Structures With Defined Shape and Stoichiometry. ACS Nano 2014, 8, 4771−4781.

(21) Khisamutdinov, E.; Li, H.; Jasinski, D.; Chen, J.; Fu, J.; Guo, P. Enhancing Immunomodulation on Innate Immunity by Shape Transition Among RNA Triangle, Square, and Pentagon Nanovehicles. Nucleic Acids Res. 2014, 42, 9996−10004.

(22) Jasinski, D.; Khisamutdinov, E. F.; Lyubchenko, Y. L.; Guo, P. Physicochemically Tunable Poly-Functionalized RNA Square Architecture With Fluorogenic and Ribozymatic Properties. ACS Nano 2014, 8, 7620−7629.

(23) Scott, W. G. Ribozymes. Curr. Opin. Struct. Biol. 2007, 17, 280− 286.

(24) Breaker, R. R. Riboswitches and the RNA World. Cold Spring Harbor Perspect. Biol. 2012, 4, a003566.

(25) Ha, M.; Kim, V. N. Regulation of MicroRNA Biogenesis. Nat. Rev. Mol. Cell Biol. 2014, 15, 509−524.

(26) Hendrix, R. W. Bacteriophage DNA Packaging: RNA Gears in a DNA Transport Machine (Minireview). Cell 1998, 94, 147−150.

(27) Leontis, N. B.; Stombaugh, J.; Westhof, E. The Non-WatsonCrick Base Pairs and Their Associated Isostericity Matrices. Nucleic Acids Res. 2002, 30, 3497−3531.

(28) Jaeger, L.; Westhof, E.; Leontis, N. B. TectoRNA: Modular Assembly Units for the Construction of RNA Nano-Objects. Nucleic Acids Res. 2001, 29, 455−463.

(29) Ishikawa, J.; Furuta, H.; Ikawa, Y. RNA Tectonics (TectoRNA) for RNA Nanostructure Design and Its Application in Synthetic Biology. Wiley. Interdiscip. Rev. RNA 2013, 4, 651−664.

(30) Jaeger, L.; Chworos, A. The Architectonics of Programmable RNA and DNA Nanostructures. Curr. Opin. Struct. Biol. 2006, 16, 531−543.

(31) Westhof, E.; Masquida, B.; Jaeger, L. RNA Tectonics: Towards RNA Design. Folding Des. 1996, 1, R78−R88.

(32) Chworos, A.; Severcan, I.; Koyfman, A. Y.; Weinkam, P.; Oroudjev, E.; Hansma, H. G.; Jaeger, L. Building Programmable Jigsaw Puzzles With RNA. Science 2004, 306, 2068−2072.

(33) Yan, H. Nucleic Acid Nanotechnology. Science 2004, 306, 2048−2049.

(34) Abdelmawla, S.; Guo, S.; Zhang, L.; Pulukuri, S.; Patankar, P.; Conley, P.; Trebley, J.; Guo, P.; Li, Q. X. Pharmacological Characterization of Chemically Synthesized Monomeric PRNA Nanoparticles for Systemic Delivery. Mol. Ther. 2011, 19, 1312−1322. (35) Shu, D.; Shu, Y.; Haque, F.; Abdelmawla, S.; Guo, P. Thermodynamically Stable RNA Three-Way Junctions for Constructing Multifuntional Nanoparticles for Delivery of Therapeutics. Nat. Nanotechnol. 2011, 6, 658−667.

(36) Haque, F.; Shu, D.; Shu, Y.; Shlyakhtenko, L.; Rychahou, P.; Evers, M.; Guo, P. Ultrastable Synergistic Tetravalent RNA Nanoparticles for Targeting to Cancers. Nano Today 2012, 7, 245−257.

(37) Lander, E. S.; Linton, L. M.; Birren, B.; Nusbaum, C.; Zody, M. C.; Baldwin, J.; Devon, K.; Dewar, K.; Doyle, M.; FitzHugh, W.;

Funke, R.; Gage, D.; Harris, K.; Heaford, A.; Howland, J.; Kann, L.; Lehoczky, J.; Levine, R.; McEwan, P.; McKernan, K.; et al. Initial Sequencing and Analysis of the Human Genome. Nature 2001, 409, 860−921.

(38) Claverie, J. M. Fewer Genes, More Noncoding RNA. Science 2005, 309, 1529−1530.

(39) Lieberman, J.; Slack, F.; Pandolfi, P. P.; Chinnaiyan, A.; Agami, R.; Mendell, J. T. Noncoding RNAs and Cancer. Cell 2013, 153, 9−10. (40) Seeman, N. C. Nucleic Acid Junctions and Lattices. J. Theor. Biol. 1982, 99, 237−247.

(41) Jones, M. R.; Seeman, N. C.; Mirkin, C. A. Nanomaterials. Programmable Materials and the Nature of the DNA Bond. Science 2015, 347, 1260901.

(42) Seeman, N. C. Nanomaterials Based on DNA. Annu. Rev. Biochem. 2010, 79, 65−87.

(43) Andersen, E. S.; Dong, M.; Nielsen, M. M.; Jahn, K.; Subramani, R.; Mamdouh, W.; Golas, M. M.; Sander, B.; Stark, H.; Oliveira, C. L.; Pedersen, J. S.; Birkedal, V.; Besenbacher, F.; Gothelf, K. V.; Kjems, J. Self-Assembly of a Nanoscale DNA Box With a Controllable Lid. Nature 2009, 459, 73−76.

(44) Goodman, R. P.; Heilemann, M.; Doose, S.; Erben, C. M.; Kapanidis, A. N.; Turberfield, A. J. Reconfigurable, Braced, ThreeDimensional DNA Nanostructures. Nat. Nanotechnol. 2008, 3, 93−96.

(45) Yang, Y. R.; Liu, Y.; Yan, H. DNA Nanostructures As Programmable Biomolecular Scaffolds. Bioconjugate Chem. 2015, 26, 1381.

(46) Rothemund, P. W. K. Folding DNA to Create Nanoscale Shapes and Patterns. Nature 2006, 440, 297−302.

(47) Ke, Y.; Sharma, J.; Liu, M.; Jahn, K.; Liu, Y.; Yan, H. Scaffolded DNA Origami of a DNA Tetrahedron Molecular Container. Nano Lett. 2009, 9, 2445−2447.

(48) Douglas, S. M.; Bachelet, I.; Church, G. M. A Logic-Gated Nanorobot for Targeted Transport of Molecular Payloads. Science 2012, 335, 831−834.

(49) Dietz, H.; Douglas, S. M.; Shih, W. M. Folding DNA into Twisted and Curved Nanoscale Shapes. Science 2009, 325, 725−730.

(50) Liedl, T.; Hogberg, B.; Tytell, J.; Ingber, D. E.; Shih, W. M. SelfAssembly of Three-Dimensional Prestressed Tensegrity Structures From DNA. Nat. Nanotechnol. 2010, 5, 520−524.

(51) Zaug, A. J.; Grabowski, P. J.; Cech, T. R. Autocatalytic Cyclization of an Excised Intervening Sequence RNA Is a CleavageLigation Reaction. Nature 1983, 301, 578−583.

(52) Jady, B. E.; Kiss, T. A Small Nucleolar Guide RNA Functions Both in 2'-O-Ribose Methylation and Pseudouridylation of the U5 Spliceosomal RNA. EMBO J. 2001, 20, 541−551.

(53) Matsumura, Y.; Maeda, H. A New Concept for Macromolecular Therapeutics in Cancer Chemotherapy: Mechanism of Tumoritropic Accumulation of Proteins and the Antitumor Agent Smancs. Cancer Res. 1986, 46, 6387−6392.

(54) Prabhakar, U.; Maeda, H.; Jain, R. K.; Sevick-Muraca, E. M.; Zamboni, W.; Farokhzad, O. C.; Barry, S. T.; Gabizon, A.; Grodzinski, P.; Blakey, D. C. Challenges and Key Considerations of the Enhanced Permeability and Retention Effect for Nanomedicine Drug Delivery in Oncology. Cancer Res. 2013, 73, 2412−2417.

(55) Lammers, T.; Kiessling, F.; Hennink, W. E.; Storm, G. Drug Targeting to Tumors: Principles, Pitfalls and (Pre-) Clinical Progress. J. Controlled Release 2012, 161, 175−187.

(56) Shu, D.; Khisamutdinov, E.; Zhang, L.; Guo, P. Programmable Folding of Fusion RNA Complex Driven by the 3WJ Motif of Phi29 Motor PRNA. Nucleic Acids Res. 2014, 42, e10.

(57) Paredes, E.; Evans, M.; Das, S. R. RNA Labeling, Conjugation and Ligation. Methods 2011, 54, 251−259.

(58) Behlke, M. A. Progress Towards in Vivo Use of SiRNAs. Mol. Ther. 2006, 13, 644−670.

(59) Shu, Y.; Haque, F.; Shu, D.; Li, W.; Zhu, Z.; Kotb, M.; Lyubchenko, Y.; Guo, P. Fabrication of 14 Different RNA Nanoparticles for Specific Tumor Targeting Without Accumulation in Normal Organs. RNA 2013, 19, 767−777.

(60) Shu, D.; Li, H.; Shu, Y.; Xiong, G.; Carson, W. E.; Haque, F.; Xu, R.; Guo, P. Systemic Delivery of Anti-MiRNA for Suppression of Triple Negative Breast Cancer Utilizing RNA Nanotechnology. ACS Nano 2015, 9, 9731−9740.

(61) Cui, D.; Zhang, C.; Liu, B.; Shu, Y.; Du, T.; Shu, D.; Wang, K.;

Dai, F.; Liu, Y.; Li, C.; Pan, F.; Yang, Y.; Ni, J.; Li, H.; Brand-Saberi, B.; Guo, P. Regression of Gastric Cancer by Systemic Injection of RNA Nanoparticles Carrying Both Ligand and SiRNA. Sci. Rep. 2015, 5, 10726.

(62) Rychahou, P.; Haque, F.; Shu, Y.; Zaytseva, Y.; Weiss, H. L.; Lee, E. Y.; Mustain, W.; Valentino, J.; Guo, P.; Evers, B. M. Delivery of RNA Nanoparticles into Colorectal Cancer Metastases Following Systemic Administration. ACS Nano 2015, 9, 1108−1116.

(63) Lee, T. J.; Haque, F.; Shu, D.; Yoo, J. Y.; Li, H.; Yokel, R. A.; Horbinski, C.; Kim, T. H.; Kim, S.-H.; Nakano, I.; Kaur, B.; Croce, C. M.; Guo, P. RNA Nanoparticles As a Vector for Targeted SiRNA Delivery into Glioblastoma Mouse Model. Oncotarget 2015, 6, 14766− 14776.

(64) Chiu, H. C.; Koh, K.; Evich, M.; Lesiak, A.; Germann, M. W.; Bongiorno, A.; Riedo, E.; Storici, F. RNA Intrusions Change DNA Elastic Properties and Structure. Nanoscale 2014, 6, 10009−10017.

(65) Krieg, A. M. CpG Motifs in Bacterial DNA and Their Immune Effects. Annu. Rev. Immunol. 2002, 20, 709−760.

(66) Krieg, A. M. Therapeutic Potential of Toll-Like Receptor 9 Activation. Nat. Rev. Drug Discovery 2006, 5, 471−484.

(67) Vollmer, J.; Krieg, A. M. Immunotherapeutic Applications of CpG Oligodeoxynucleotide TLR9 Agonists. Adv. Drug Delivery Rev. 2009, 61, 195−204.

(68) Vanneman, M.; Dranoff, G. Combining Immunotherapy and Targeted Therapies in Cancer Treatment. Nat. Rev. Cancer 2012, 12, 237−251.

(69) Whitehead, K. A.; Dahlman, J. E.; Langer, R. S.; Anderson, D. G. Silencing or Stimulation? SiRNA Delivery and the Immune System. Annu. Rev. Chem. Biomol. Eng. 2011, 2, 77−96.

(70) Judge, A. D.; Sood, V.; Shaw, J. R.; Fang, D.; McClintock, K.; MacLachlan, I. Sequence-Dependent Stimulation of the Mammalian Innate Immune Response by Synthetic SiRNA. Nat. Biotechnol. 2005, 23, 457−462.

(71) Judge, A. D.; Bola, G.; Lee, A. C. H.; Maclachlan, I. Design of Noninflammatory Synthetic SiRNA Mediating Potent Gene Silencing in Vivo. Mol. Ther. 2006, 13, 494−505.

(72) Morrissey, D. V.; Lockridge, J. A.; Shaw, L.; Blanchard, K.; Jensen, K.; Breen, W.; Hartsough, K.; Machemer, L.; Radka, S.; Jadhav, V.; Vaish, N.; Zinnen, S.; Vargeese, C.; Bowman, K.; Shaffer, C. S.; Jeffs, L. B.; Judge, A.; Maclachlan, I.; Polisky, B. Potent and Persistent in Vivo Anti-HBV Activity of Chemically Modified SiRNAs. Nat. Biotechnol. 2005, 23, 1002−1007.

(73) Hornung, V.; Ellegast, J.; Kim, S.; Brzozka, K.; Jung, A.; Kato, H.; Poeck, H.; Akira, S.; Conzelmann, K. K.; Schlee, M.; Endres, S.; Hartmann, G. 5′-Triphosphate RNA Is the Ligand for RIG-I. Science 2006, 314, 994−997.

(74) Cekaite, L.; Furset, G.; Hovig, E.; Sioud, M. Gene Expression Analysis in Blood Cells in Response to Unmodified and 2'-Modified SiRNAs Reveals TLR-Dependent and Independent Effects. J. Mol. Biol. 2007, 365, 90−108.

(75) Hornung, V.; Guenthner-Biller, M.; Bourquin, C.; Ablasser, A.; Schlee, M.; Uematsu, S.; Noronha, A.; Manoharan, M.; Akira, S.; de Fougerolles, A.; Endres, S.; Hartmann, G. Sequence-Specific Potent Induction of IFN-[Alpha] by Short Interfering RNA in Plasmacytoid Dendritic Cells Through TLR7. Nat. Med. 2005, 11, 263−270.