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Stable RNA nanoparticles as potential new generation drugs for cancer therapy ☆

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ABSTRACT

Human genome sequencing revealed that only ~1.5% of the DNA sequence coded for proteins. More and more evidence has uncovered that a substantial part of the 98.5% so-called "junk" DNAs actually code for noncoding RNAs. Two milestones, chemical drugs and protein drugs, have already appeared in the history of drug development, and it is expected that the third milestone in drug development will be RNA drugs or drugs that target RNA. This review focuses on the development of RNA therapeutics for potential cancer treatment by applying RNA nanotechnology. A therapeutic RNA nanoparticle is unique in that its scaffold, ligand, and therapeutic component can all be composed of RNA. The special physicochemical properties lend to the delivery of siRNA, miRNA, ribozymes, or riboswitches; imaging using fluogenenic RNA; and targeting using RNA aptamers. With recent advances in solving the chemical, enzymatic, and thermodynamic stability issues, RNA nanoparticles have been found to be advantageous for *in vivo* applications due to their uniform nano-scale size, precise stoichiometry, polyvalent nature, low immunogenicity, low toxicity, and target specificity. *In vivo* animal studies have revealed that RNA nanoparticles can specifically target tumors with favorable pharmacokinetic and pharmacodynamic parameters without unwanted accumulation in normal organs. This review summarizes the key studies that have led to the detailed understanding of RNA nanoparticle formation as well as chemical and thermodynamic stability issue. The methods for RNA nanoparticle construction, and the current challenges in the clinical application of RNA nanotechnology, such as endosome trapping and production costs, are also discussed.

Contents

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1. Introduction

Nanotechnology refers to the creation and application of materials using either a top-down approach or bottom-up assembly at the nanometer scale. In nature, a wide variety of macromolecules that form patterned arrays and highly-ordered structures in nano-scale have inspired several biomimetic strategies. Macromolecules, such as DNA, RNA, and proteins have intrinsically defined features with the potential to serve as versatile building blocks for bottom-up assembly of nano-structures and nano-devices.

More and more evidence has revealed that a substantial part of ~98.5% of the human genome, so-called "junk" DNA [1], codes for noncoding RNAs. These noncoding RNAs play major roles in gene expression [2–4], gene regulation [5,6], cellular catalytic reaction [7], and so on [8]. The malfunction of some noncoding RNAs will end up as abnormal cellular activity closely related to cancers, for example, microRNAs (miRNAs) have been shown to function as oncogenes or tumor suppressors [9–13]; and snoRNAs (SNORD33, SNORD66, and SNORD76) were identified as biomarkers for non-small cell lung cancer [14]. Many other diseases, such as dilated cardiomyopathy and heart failure [15], were found to be related to RNA functionality. This has led to treatment strategies that use RNA as therapeutic targets [16,17]. In other aspects, the discoveries of small interfering RNAs (siRNAs) [18,19], ribozymes [20,21], riboswitches [22,23], and miRNAs [24,25] have induced a heightened interest in using RNAs as therapeutics for disease treatment.

Natural RNA possesses versatile sequences, secondary structures, and tertiary/quaternary interactions [26–28]. Several assembly mechanisms of naturally occurring RNA complexes have been applied to construct RNA nanoparticles with defined structure and stoichiometry *via* intra- and/or inter-molecular interactions. Through this innovative approach based on RNA nanotechnology [29,30], varieties of therapeutic RNA nanoparticles harboring multiple therapeutic modules, such as siRNA, aptamer, or miRNA, have been constructed. Each incorporated siRNA, aptamer, miRNA, or other functionalities within the nanoparticle fold into its respective, authentic structure and retain its independent function for specific cell binding, cell entry, gene silencing, catalytic function, in both *in vitro* and animal trials [31–33]. Following the two milestones of chemical and protein drugs, respectively, in medical history, we speculate that the third milestone in drug development will be RNA drugs or drugs that target RNA, thus, RNA nanoparticles have the potential to be a new generation of drugs. This review will discuss the application of the achievements in modern RNA nanotechnology for cancer therapy, especially focusing on well-constructed pRNA-based RNA nano-delivery systems.

2. Definition of RNA nanotechnology

RNA nanotechnology is a unique field that studies the design, fabrication, and application of RNA nanoparticles with architectures primarily made up of RNA *via* bottom-up self-assembly [29,30,34,35] (Fig. 1). This concept contrasts with other widely studied drug delivery nano-systems that conjugate functional RNA modules to polymers, lipids, dendrimers, gold, or other nanomaterial-based particles.

3. Proof-of-concept of RNA nanotechnology in 1998

Compared to classical RNA studies, RNA nanotechnology is a relatively new field [36–41]. The first evidence showing that RNA

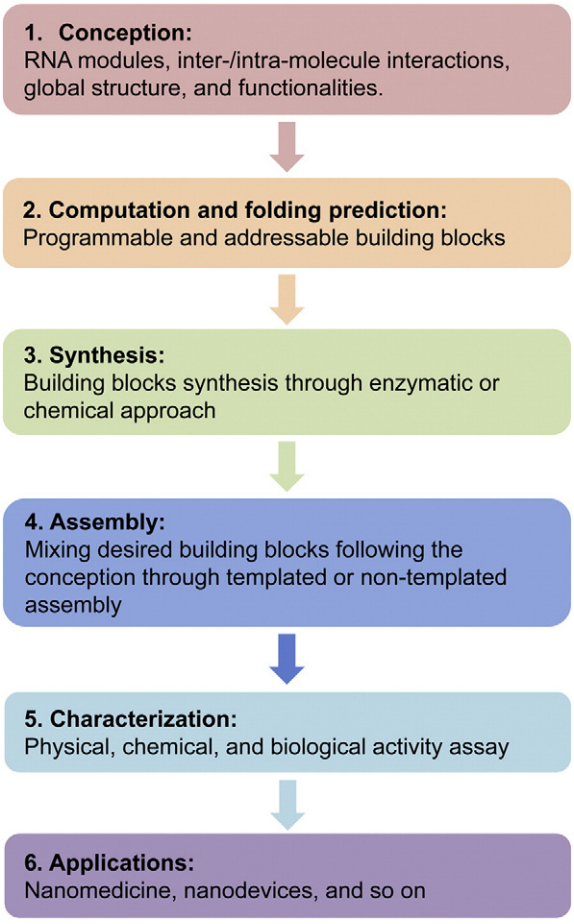

Fig. 1. Approaches to RNA nanotechnology. The construction of RNA nanoparticles starts from a conception design to define the desired properties of the nanoparticles. The RNA structure and folding of building blocks are then computed. After monomeric RNA building block synthesis, the RNA nanoparticles can be assembled following the designed conception. The resulting RNA nanoparticles can be characterized by gel electrophoresis, atomic force microscopy and electron microscopy. After thorough evaluation, the RNA nanoparticles can be used for various applications *in vitro* and *in vivo* [29]. This figure was adapted and modified from ref. [29] with permission.