RNA therapeutics — The potential treatment for myocardial infarction

Hunghao Chu a, b, c, Daniel S. Kohane a, **, Robert Langer b, c, *

** Corresponding author. Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge, MA 02139, United States.
* Corresponding author. Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge, MA 02139, United States.

Abstract

RNA therapeutics mainly control gene expression at the transcript level. In contrast to conventional gene therapy which solely increases production of a protein, delivered RNAs can enhance, reduce or abolish synthesis of a particular protein, which control its relevant activities in a more diverse fashion. Thus, they hold promise to treat many human diseases including myocardial infarction (MI). MI is a serious health burden that causes substantial morbidity and mortality. An unmet clinical need for treating MI is the recovery of cardiac function, which requires regeneration of the functional tissues including the vasculature, nerves, and myocardium. Several classes of RNA therapeutics have been investigated in preclinical MI models, and the results have demonstrated their benefits and encourage their future development. In this review, we summarize the common RNA therapeutic approaches and highlight their application in MI therapy.

1. Introduction

Regeneration of human tissue after injury is a challenging goal and relies on the careful control of several key factors. First and foremost is the capacity of viable cells to proliferate and repopulate the damaged area. If they fail to fulfill this role, recovery cannot be initiated and the function of the organ often becomes worse over time. This is a common issue preventing recovery after MI. MI, the
leading cause of death in Western countries, is caused by occlusion of a coronary artery [1,2]. Because the blood supply is blocked, MI results in immediate death of cardiomyocytes. Subsequent complications such as oxidative stress and inflammation cause secondary damage that further deteriorates cardiac contractility [3]. Recovery from MI is extremely difficult for two main reasons: (i) human cardiomyocytes have very limited proliferative potential [4]; (ii) the dysfunctional coronary vasculature and inflammation associated with MI create an unfavorable environment for the remaining cardiomyocytes to survive. Dead cardiomyocytes are not replenished, leading to a reduction in cardiac contractility that is not naturally reversible. Apart from heart transplantation, current treatments for MI only restore the blood supply to residual cardiomyocytes, but do not improve function. New therapeutic approaches to repair damaged myocardium more effectively and prevent recurrent MI are of great interest [5].

Presently, the most promising alternative treatments include protein delivery, gene delivery and cell delivery which are each in various stages of clinical development [6,7]. Protein delivery is considered to be the most straightforward approach in which therapeutic proteins such as growth factors are applied to regenerate de novo cardiac tissues [8]. However, their efficacy is often compromised by their short in vivo half-lives. Gene delivery results in a stronger and longer-lasting therapeutic effect compared to protein therapy; nevertheless, unregulated overproduction must be prevented to avoid adverse effects [9]. In comparison to protein and gene therapies, cell therapy can directly replace the dead cells or secrete various factors to promote cardiac regeneration [10]. However, acquiring adequate numbers of cells, on the order of millions per patient, is a significant challenge and their in vivo viability is usually very low. In recent years, RNA-based therapies have been examined in many human disease models including those mimicking human cardiovascular diseases. Distinct from conventional gene therapies that employ DNA to produce a therapeutic protein, RNA-based approaches affect cellular activity in a more diverse fashion as they can increase or decrease the level of a protein. In this review, we summarize the different categories of RNA therapeutics and highlight their potential for treating coronary heart disease.

2. RNA therapeutics

RNA-based approaches to cardiac regeneration are promising and offer several unique benefits because of their ability to modulate production of specific proteins at the transcriptional level [11]. Since a single transcript makes hundreds or thousands of copies of a protein, therapies targeting transcripts may have greater and more widespread effects compared to small molecule drugs or recombinant proteins [12]. Yet the development of RNA therapeutics is still in its infancy. The only RNA drugs approved by the FDA are fomivirsen, an antisense oligonucleotide mitigating cytomegalovirus retinitis, and pegaptanib, an aptamer antagonist of vascular endothelial growth factor used to treat neovascular age-related macular degeneration [13,14]. RNA therapeutic agents including mRNA, small interfering RNA, microRNA and ribozymes are currently in clinical trials. Compared to DNA which is highly stable, RNA is unstable due to several structural differences that can dramatically compromise the efficacy of RNA therapeutics: (i) the hydroxyl group on the C2 position of the ribose can be deprotonated in alkaline conditions and act as a nucleophile to cleave the phosphodiester bond; (ii) RNAs have larger major grooves where nucleases can dock [15]; (iii) exogenous RNAs can activate innate immunity and trigger interferon-mediated responses such as suppression of translation and upregulation of ribonucleases [16]. A common strategy to minimize the degradation and immunogenicity of RNAs is to build them from modified nucleotides. For example, RNAs made from base analogs such as 5-methylcytosine (m5C) and pseudouridine (Ψ) are less likely to be recognized by nucleases and immunoreceptors including Toll-like receptor (TLR) 3, TLR7 and TLR8 [17]. Thus, in vivo half-lives can be prolonged [18].

Another factor that determines the efficacy of a therapeutic RNA agent is its mode of delivery. Naked RNAs delivered systemically or locally are cleared rapidly and thus a targeted delivery mechanism is critical to protect it from degradation and to promote its accumulation at the desired site [19]. In vivo delivery (transfection) is accomplished utilizing viral vectors or synthetic vehicles. Virus-mediated transfection, also called transduction, typically has high efficiency although safety is a major concern [20]. Non-viral synthetic vehicles are cationic lipids or polymers which interact with the phosphate groups of RNA molecules and self-assemble into stable complexes. Additional to cytotoxicity of the vehicle, lower efficiency compared to viral vectors presents an additional design challenge [21]. Therefore, significant efforts have been devoted to improving cell targeting and internalization of RNA agents, two key factors constituting the efficiency of the synthetic vector [22–24].

The route of administration also plays an important role in the therapeutic efficacy. In the common small animal MI models, a left thoracotomy is performed to induce MI followed by local injection of a therapeutic agent. In this fashion, a maximal amount of therapeutic agent can be accumulated in the infarcted region. However, this surgical procedure is highly invasive and is only applied in patients that require advanced procedures such as coronary artery bypass grafting. Administration of RNA therapeutics in patients would largely be done by parenteral routes. Systemic injection requires a delivery vehicle that selectively and efficiently targets an MI-associated biomarker [25]. Local injection avoids rapid clearance of RNA by the circulation but requires a device to reach the myocardium. Catheter-based injection has been employed to deliver genes, proteins and cells, and would be feasible for delivery of RNA therapeutic agents as well [26]. The current progress and future directions in nucleic acid delivery have been discussed in many reviews [27–29].

3. RNA therapeutic agents in cardiac repair

The common classes of therapeutic RNAs and their status of development are summarized in Table 1. The majority of such RNAs, with the exception of mRNA, are non-coding RNAs that regulate cellular functions by various mechanisms [30]. Many other non-coding RNAs are not discussed here as their functions and therapeutic value are not fully understood. Similar with gene- and protein-based therapies for MI, therapeutics RNAs are under investigation for three purposes: (i) induction of neovascularization: MI leads to an ischemic environment that deteriorates the cardiac function over time [31]. Formation of new blood vessels by introducing angiogenic growth factors is considered a promising approach to repair damaged vasculature and restore blood supply. (ii) Reversal of the harsh environment: pathophysiological conditions such as inflammation are responsible for continuing necrosis and apoptosis of cardiomyocytes after MI [32]. A therapeutic agent that corrects the diseased condition is beneficial for preserving remaining cardiomyocytes and cardiac contractility. (iii) Application in stem cell therapy: stem cells have the capability of regenerating cardiac tissues including functional myocardium and vasculature. Therapeutics that effectively control the activities of stem cells including survival and differentiation are of great importance to move this field forward [33]. Here, we describe the
3.1. messenger RNA (mRNA)

Conventional gene therapy delivers DNA into cells to produce a functional protein to replace a deficient or dysfunctional one. This approach still has several drawbacks that limit its therapeutic efficacy. For example, nonviral approaches using synthetic vehicles to deliver DNA have difficulty crossing barriers including the plasma and nuclear membranes to reach the nucleus where transcription occurs. Using viral vectors as carriers has better efficiency, but their immunogenicity raises safety concerns. Delivered DNA also has the potential risk of chromosomal integration, which could lead to gene overexpression and undesired effects such as tumorigenesis. mRNA-based therapy can avoid the above issues because it functions in the cytoplasm, leading to the following advantages: (i) delivery is more efficient to the cytosol than to the nucleus; (ii) mRNA does not integrate into the host genome, obviating several safety concerns; (iii) the response is more rapid than for DNA, as mRNA is directly utilized for protein synthesis [41].

Therapeutic mRNA is prepared by in vitro transcription, in which an RNA polymerase from a bacteriophage drives transcription from a DNA template [42]. Design of the DNA template is critical as it determines the size and stability of the mRNA, which affects its translation efficiency, but their potential risk of chromosomal integration, which could lead to gene overexpression and undesired effects such as tumorigenesis. mRNA-based therapy can avoid the above issues because it functions in the cytoplasm, leading to the following advantages: (i) delivery is more efficient to the cytosol than to the nucleus; (ii)

major classes of RNAs commonly fulfilling these roles and discuss their progress individually.

Table 1

| Function                  | Size (nt) | Status of development |
|---------------------------|-----------|-----------------------|
| messenger RNA (mRNA)      | 400–12,000| Phase 2 trials in cancer immunotherapy [34] |
| antisense oligonucleotide | 15–25     | Fomiviren approved for cytomegalovirus retinitis [35] |
| ribozyme                  | 50–150    | Phase 2 trials in Human Immunodeficiency Virus – 1 infected patients [36] |
| aptamer                   | 70–80     | Pegaptanib approved for neovascular age-related macular degeneration [37] |
| small interfering RNA     | 20–30     | Most in phase 1 and 2 trials; a phase 3 trial terminated early due to the poor result [38] |
| microRNA                  | 20–30     | Phase 1 and 2 trials in cancer treatment [39]; phase 3 trials in cancer diagnosis [40] |
| guide RNA                 | 100       | Experimental studies only thus far |

Fig. 1. Modified mRNA reduces immunogenicity and reveals a better therapeutic outcome than DNA in a MI model. (A) Plasma levels of interferon-γ, interleukin-12 and interferon-α in mice with intravenous injection of unmodified mRNA or modified mRNA, or without treatment. 5′S2U(0.25): modified mRNA in which 50% of uridine was replaced by 2′SU, 5′S2U(0.25): modified mRNA in which 25% of uridine was replaced by 2SU and 25% cytosine was replaced by m5C. The results suggested that modified mRNA resulted in significantly lower levels of cytokine secretion, *p < 0.05. Reprinted from ref. [33] with permission. (B) Production of VEGF-A in cardiac cells transfected with modified mRNA or the plasmid encoding VEGF-A. Transfection of modified mRNA (modRNA) resulted in quick production of VEGF-A and its level decreased to the basal level after 3 days. In contrast, transfection of the plasmid (DNA) had a much longer effect as high levels of VEGF-A were still detected after 6 days. Reprinted from ref. [38] with permission. (C) Macroscopic comparison of vascular density in the infarct area of the murine heart at 7 days post injury. vehicle (control): no treatment; Luc modRNA: modified mRNA encoding the luciferase; VEGF-A modRNA: modified mRNA encoding VEGF-A; VEGF-A DNA: the plasmid encoding VEGF-A. The representative images revealed that the VEGF-A modRNA group had more blood vessels than other groups. Reprinted from ref. [38] with permission.
Therapeutic mRNA can be delivered by ex vivo or in vivo transfection and both approaches have entered clinical trials [47]. Immunotherapy utilizes ex vivo delivery, in which autologous cells (e.g. dendritic cells) are collected and transfected with tumor or viral mRNA, then returned to the patient’s body [48]. The advantage of ex vivo delivery lies in the high degree of control over transfection method and conditions [49]. In contrast, direct injection of mRNA to achieve in vivo transfection is more straightforward and avoids elaborate processes of cell collection. Yet the outcome may be subject to higher patient-to-patient variability due to uncontrollable factors [50].

mRNA could be designed for different therapeutic outcomes when treating MI. For example, mRNA targeted to the proper cell type could produce proteins to: (i) promote vascularization of the myocardium; (ii) reduce inflammation of the damaged myocardium; (iii) produce de novo cardiac tissues. For instance, Elmadbough et al. transfected skeletal myoblasts to produce stromal cell-derived factor-1α and their transplantation in the infarcted myocardium promoted stem cell homing and angiomyogenesis [51]. Zangi et al. compared production of vascular endothelial growth factor-A (VEGF-A) in cardiomyocytes by in vivo transfection of modified mRNA or a plasmid found that expression profiles were quite different (Fig. 1B) [52]. Modified mRNA led to a pulse-like production of VEGF-A as the greatest amount of VEGF-A was detected in the first 8 h post-transfection and was barely detectable after 48 h. In contrast, the plasmid prolonged expression for over one week. Intriguingly, in this mouse MI model, injection of VEGF-A-encoding modified mRNA resulted in a better cardiac function along with functional vasculature, compared to the plasmid-mediated treatment (Fig. 1C). One possible reason could be that transient production of VEGF-A is more favorable to differentiate epicardial progenitor cells into endothelial cells [53].

Reducing inflammation is another promising strategy to treat MI and other cardiovascular diseases [54]. For example, Levy O et al. engineered autologous mesenchymal stem cells with a tissue-targeting ligand and immuno-modulatory capability [55]. Upon re-injection, engineered cells found the inflamed endothelium and effectively reduced inflammation by secreting anti-inflammatory cytokines.

mRNA delivery has also been examined in cell reprogramming. For example, Warren L et al. generated induced pluripotent stem cells (iPSCs) by modified mRNA encoding four reprogramming factors, c-Myc, Klf4, Oct4, and Sox2, which could be later differentiated into any cell types including cardiomyocytes and endothelial cells [56]. An interesting feature of this approach is the high efficiency, as Schlaeger TM et al. found that modified mRNA produced more iPSCs compared to two other non-integrating methods, Sendai viral and episomal vectors. The limitation is that repeated transfection was required to maintain the production of reprogramming factors, which again reflects the short half-life of mRNA. In recent years, direct transfection (transdifferentiation) has drawn more attention as it bypasses long procedures to produce iPSCs [57]. Lee K et al. revealed that efficient delivery of modified mRNA encoding Gata4, MeR2c, and Tbx5 could directly differentiated cardiac fibroblasts into cardiomyocyte-like cells, which express functional markers as endogenous cardiomyocytes [58].

3.2. Small interfering RNA (siRNA)

siRNAs are double-stranded RNA fragments (20–30 nucleotides) that direct a post-transcriptional gene silencing process called RNA interference (RNAi). RNAi was originally observed in the worm Caenorhabditis elegans and was later revealed to be present in mammals against viral infection. It functions by pairing a single-stranded siRNA (ss-siRNA) with the complimentary sequence in a target mRNA followed by endonucleolytic mRNA cleavage. RNAi to target any gene can be introduced by several methods. For example, a plasmid can be transfected to express a short hairpin RNA (shRNA). The shRNA is processed endogenously into the ss-siRNA and is incorporated into the RNAi specificity complex (RISC), which directs degradation of a target mRNA in the cytosol [59]. Another method to bypass transcription is to deliver RNAs (shRNAs, siRNAs or ss-siRNAs) into a cell, which would then be processed and incorporated into RISC [60].

siRNA-induced RNAi has become a powerful tool for deciphering molecular mechanisms of cardiovascular diseases and uncovering new therapeutic targets [61,62]. In terms of therapy, siRNAs that inhibit key proteins overproduced in cardiovascular disorders have shown great promise. For instance, the enzyme proprotein convertase subtilisin/kexin type 9 (PCSK9) increases the level of cholesterol in plasma and PCSK9 inhibition is considered a therapeutic strategy to ameliorate hypercholesterolemia [63]. Frank-Kamenetsky et al. demonstrated that lipidoid nanoparticles delivering the siRNA against PCSK9 effectively lowered cholesterol in monkeys [64]. In a mouse MI model, Majumdar et al. employed an siRNA to silence the chemokine receptor CCR2 and improved MI recovery by reducing infiltration of inflammatory cells into the infarcted area [65]. In addition to inflammation, cardiomyocyte apoptosis also reduces cardiac contractility. Apoptosis post-MI is mediated in part by tyrosine phosphatase Src homology region 2 domain-containing phosphatase 1 (SHP-1) [66]. Kim D et al. reported that RNAi against SHP-1 could rescue cardiomyocytes in a mouse MI model [67]. Off-target binding of siRNAs represents a hurdle in this field as it can lead to non-specific gene silencing [68]. A variety of approaches have been investigated to reduce off-targeting effects including the tough decoy (TuD) RNA which is introduced to inhibit the sense strand of the siRNA duplex (Fig. 2A) [69,70].

3.3. microRNA (miRNA)

Similar to siRNAs, miRNAs are short nucleotides that induce RNAi to repress expression of a gene. Three characteristics distinguish miRNA from siRNA-mediated gene silencing: (i) miRNAs are endogenous non-coding RNAs that play an essential role in controlling cellular processes while siRNAs are artificial constructs introduced to knock down a gene; (ii) a miRNA usually targets the 3’-untranslated region of mRNA while siRNA targets the coding region; (iii) more importantly, miRNA-mediated gene silencing does not require perfect sequence complementarity as does siRNA-mediated gene silencing. Biogenesis of miRNAs follows a series of steps. A long transcript called the primary miRNA (pri-miRNA) is synthesized by RNA polymerase II, which is then processed by the RNase Drosha into a 60- to 100-nt shRNA also called pre-miRNA. After transport into the cytoplasm, the pre-miRNA is further processed by another RNase, Dicer, into the mature miRNA (20–30 nt). Like siRNAs, an exogenous miRNA can be introduced into a cell by a viral vector encoding pre-miRNA or by a synthetic vehicle carrying synthetic pre-miRNA or miRNA [71,72].

miRNA research is a relatively recent development but its importance has been revealed in many human diseases including coronary heart disease (Table 2) [73]. In one example, cardiomyocyte-specific deletion of dgr8, a gene required for miRNA production, led to ventricular malfunction and premature lethality [74]. In another, miRNA-21 (miR-21) was revealed to promote cardiac fibrosis by upregulating the ERK-MAP kinase signaling pathway in cardiac fibroblasts [75]. Suppression of miR-21 activity by a miRNA inhibitor (anti-miR) significantly reversed this outcome and improved cardiac function. Another important
miRNA involved in cardiac fibrosis is miR-101 [76]. Increasing its level was found to mitigate fibrosis by reducing production of collagen. Similarly, a decrease in miR-29 after MI induced production and deposition of collagen fibers [77], miRNAs are also closely associated with other regenerative processes in the heart [78, 79]. Taken together, delivery of miRNA or anti-miR has great potential as a therapy for a variety of diseases [80, 81].

miRNA-based therapeutics in cardiovascular diseases can be used in three different strategies: (i) modulation of microenvironment: Meloni M et al. expressed a decoy against miR-24 to reverse its inhibition on angiogenesis and to improve cardiac function in a mouse MI model (Fig. 2B) [82]; (ii) ex vivo cell modification: Glass C & Singla DK observed that overexpressing miR-1 in embryonic stem cells (ESCs) improved their differentiation into cardiomyocytes and reduced levels of apoptosis following injection in the infarcted heart [83]; Huang L et al. overexpressed miR-126 in mesenchymal stem cells and transplanted them in the infarcted myocardium to improve angiogenesis and overall cardiac function [84]; (iii) in vivo cell reprogramming: Anokye-Danso F et al. showed that lentiviral transduction of murine embryonic fibroblasts to express the miR-302/367 cluster resulted in higher iPSC production efficiency than the standard method using four transcriptional factors [85]; Additionally, Jayawardena TM et al. revealed that treatment with lentiviruses encoding miR-1/133/208/499 enabled direct in vivo conversion of cardiac fibroblasts into cardiomyocyte-like cells in the infarcted heart [86]. The subsequent functional study further demonstrated that these cardiomyocyte-like cells could significantly improve cardiac output [87].

3.4. Antisense oligonucleotide (ASO)

ASOs are synthetic nucleotides (15–25 nucleotides) that bind to target miRNAs via Watson-Crick base paring and inhibit translation of the encoded proteins. The two following mechanisms explain

Table 2

| Implication in coronary heart disease | miRNAs |
|-------------------------------------|--------|
| Atherosclerosis [88]                | miR-21, miR-22, miR-33, miR-122, miR-126, miR-145, miR-155 |
| Angiogenesis [89]                   | Let-7i, miR-15, miR-16, miR-17-92 cluster, miR-126, miR-130a, miR-210, miR-221 |
| Cell proliferation/death [90]       | miR-1, miR-15 family, miR-24, miR-29a, miR-30, miR-195, miR-199a, miR-590 |
| Cardiac remodeling [91]             | miR-1, miR-21, miR-26, miR-29, miR-133, miR-195a, miR-208, miR-408 |
| Cardiac development [92]            | miR-1, miR-15, miR-138, miR-208, miR-218, miR-499 |
the inhibition: (i) steric hindrance prevents normal cellular machinery from performing splicing and translation; (ii) the nuclease RNase H recognizes the complimentary strands and drives degradation of the target mRNA [93]. Because of the size being short, ASOs are made by solid-phase chemical synthesis in which modified nucleotides are incorporated to increase pharmacokineti c properties [94]. Substitution of the 2’ hydroxyl group on the ribose with a methoxyl or fluoro group makes oligonucleotides nuclease-resistant and increases their binding affinity to mRNA targets [95]. Replacing the backbone with a phosphorothioate linkage is another common modification used to prevent nucleolytic cleavage. ASOs have been designed to target proteins whose overproduction is closely related to MI. For example, the heat shock protein 47 (hsp47) drives the production of collagen and induces cardiac remodeling [96]. Hagiwara S et al. employed an ASO to repress hsp47 and revealed improved cardiac function in a rat MI model [97]. ASOs have also been employed to reduce the risk of MI by decreasing the level of apolipoprotein (A) in plasma [98]. The versatility of ASOs suggests promise for their future utility as MI therapeutics.

3.5. Aptamer

Aptamers are nucleic acid-based materials that selectively bind to molecular targets such as proteins and small molecules. The specificity of aptamers for particular proteins are screened by the Systematic Evolution of Ligands by Exponential Enrichment (SELEX) [99]. Like ASOs, aptamers can be made of single-stranded DNA, RNA or their synthetic analogs (e.g. peptide nucleic acid) [100]. Modifications on aptamer nucleotides are also important for higher stability and binding affinity [101]. The advantages of aptamers include that their efficiency in molecular recognition which can be on par with that of antibodies and their production cost is comparatively low [102]. However, the binding affinity of aptamers results from their specific tertiary structures which can be highly variable based on the environment. Selection processes have to be well designed to mimic the in vivo conditions [103]. Aptamers may treat MI by binding to and inactivating proteins that are overproduced. For example, anticoagulant therapy is employed to prevent recurrent MI. In porcine and murine studies, Rusconi CP et al. utilized an aptamer against factor IXa to inhibit coagulation [104]. Its effect was rapidly reversed by introducing a complementary strand that inactivated the aptamer. As a result the potential side effect of excessive bleeding was avoided. Inhibition of von Willebrand factor (VWF), which drives the coagulation cascade, has been evaluated for the same purpose. In a clinical trial with healthy volunteers, Gilbert JC et al. examined an aptamer inhibiting VWF and demonstrated that the antithrombotic effect was both dose and concentration dependent [105]. Their subsequent study involving patients undergoing carotid endarterectomy confirmed its benefit to reduce risk of thromboembolism [106]. Overall, anticoagulation aptamers are considered a promising strategy for many bleeding-associated disorders including stroke and MI [107].

3.6. CRISPR/Cas9

Genome engineering has become a popular field in recent years. It allows site-specific modification of genetic information using a variety of techniques including the Clustered Regularly Interspaced Short Palindromic Repeat (CRISPR) system [108]. Different from protein-based genome editing tools such as zinc-finger nucleases and transcription activator-like effector nucleases (TALENs), CRISPR/Cas9 is a DNA-based system in which a RNA fragment is responsible for specifying the DNA target by complimentary pairing. CRISPR is a segment of DNA identified in bacteria as a component of their adaptive immune system. It contains DNA fragments from invading bacteriophages or plasmids [109]. When the genomically-inserted DNA fragment is transcribed, a long primary transcript (pre-crRNA) is formed. A series of events follow to process the pre-crRNA into short RNAs (crRNAs). The crRNA can complex with the Cas9 nuclease which is also encoded in the CRISPR locus to direct cleavage of a DNA target [110]. This process can be simply manipulated by co-expressing Cas9 and a guide RNA (gRNA) that mimics the crRNA. Compared to protein-based genome editing tools that employ proteins domains to recognize the DNA target, CRISPR/Cas9 has the advantages of simplicity because it avoids elaborate protein engineering processes. It also enables multiplexing as several gRNAs can be introduced to edit multiple genes simultaneously [111]. The main disadvantage of CRISPR/Cas9, as compared to zinc-finger nucleases and TALENs, is the higher frequency of off-target mutagenesis, and new approaches are being developed to reduce this effect [112,113].

CRISPR/Cas9 is commonly introduced into cells by a viral vector or a plasmid encoding the gRNA and Cas9 [114,115]. The synthetic gRNA complexed with the Cas9 recombinant protein can also be transfected into cells using the cationic lipid Lipofectamine [116]. Direct comparison revealed this approach had higher efficiency and specificity than the plasmid-mediated method. Since CRISPR/Cas9 offers effective gene disruption, it has become a tool to reverse cardiovascular diseases associated with genetic mutations. For example, Ann Ran F et al. employed an adenoviral vector to deliver the guide RNA and Cas9 to delete PCSK9 production in the mouse liver. And the result indicated significant reductions in serum Pcsk9 and total cholesterol [117]. Apart from being a therapeutic agent, CRISPR/Cas9 offers additional value of modeling heat diseases and drug screening [118]. In this fashion, cardiomyocytes derived from iPSCs are engineered to recapitulate the genetic mutations in heart diseases followed by selection of an inhibitor from a compound library [119].

4. Future perspective

The versatility of RNAs makes them highly attractive candidate therapeutics for many human diseases. Successful translation to the clinic relies on a deeper understanding of RNA biology including exploring new classes of RNAs that hold therapeutic potential. Long-noncoding RNAs (lncRNAs) represent an example. Distinct from short non-coding RNAs that recognize targets through complimentary base pairing, lncRNAs tend to fold into specific tertiary structures and interact with proteins targets [120]. The biological functions of lncRNAs are not fully understood but their importance in cardiac development and the progression of MI has been recently revealed [121]. Thus, regulating the levels of long-noncoding RNAs in attempt to reverse the diseased state and trigger the endogenous regenerative process could be a promising direction in the future [122,123].

As most diseases result from dysregulation of more than one gene, administration of multiple therapeutic agents is often necessary to bring more benefits [124–126]. Combining RNA therapeutics with protein or small molecule drugs has shown promise. For example, siRNAs and small-molecule anticancer drugs have been demonstrated to reduce drug resistance [127,128]. Combining RNA and protein therapies, Jean SY et al. showed that co-delivery of an siRNA and a transcription factor involved in chondrogenesis achieved higher efficiency in differentiating mesenchymal stem cells into chondrocytes than delivery of either single agent [129]. Delivery of multiple RNAs to target different pathways is another approach in treatment for MI.

Another direction is delivery of RNAs to improve cell-based therapies. As adult cardiomyocytes have limited regenerative
capacity, cell-based therapies are a promising approach to replenish ischemic myocardium. Current protocols allow functional cardiac tissues to be cultivated from iPSCs or ESCs in an efficient fashion [130]. They can later be implanted into a MI patient alone or with a scaffold to recover cardiac function [131,132]. However, this poor cell retention and survival in the infarcted region, and low cardiac tissues to be cultivated from iPSCs or ESCs in an efficient capacity, cell-based therapies are a promising approach to deliver strategies to maximize their benefits.

Incorporating an RNA agent could provide the solution to these challenges. For example, mRNA encoding angiogenic factors, and RNAs reducing inflammation and fibrosis in the host tissue may increase survival of delivered cells [134]. To achieve this goal, a new strategy to effectively deliver cells and RNAs is necessary. Collectively, preclinical studies mostly in small animals have demonstrated the value of RNA therapeutics in treating MI. Future trials in large animals and patients will further explore their efficacy. Development of RNA-based MI therapeutics requires robust design of the RNA agents to avoid adverse effects and optimal delivery strategies to maximize their benefits.

Conflict of interest
All authors declare no conflict of interest.

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