Myocardial infarction (MI) and heart failure (HF) are the leading causes of death in the United States and in most other industrialized nations. MI leads to a massive loss of cardiomyocytes (CMs), which are replaced with non-CM cells, leading to scarring and, in most cases, HF. The adult mammalian heart has a low intrinsic regenerative capacity, mainly because of cell-cycle arrest in CMs. No effective treatment promoting heart regeneration is currently available. Recent efforts to use DNA-based or viral gene therapy approaches to induce cardiac regeneration post-MI or in HF conditions have encountered major challenges, mostly because of the poor and uncontrolled delivery of the introduced genes. Modified mRNA (modRNA) is a safe, non-immunogenic, efficient, transient, local, and controlled nucleic acid delivery system that can overcome the obstacles to DNA-based or viral approaches for cardiac gene delivery. We here review the use of modRNA in cardiac therapy, to induce cardioprotection and vascular or cardiac regeneration after MI. We discuss the current challenges in modRNA-based cardiac treatment, which will need to be overcome for the application of such treatment to ischemic heart disease.

Ischemic Heart Disease

Despite advances in curative and preventive medicine, heart failure (HF) remains the leading cause of mortality and hospitalization worldwide.1,2 Almost 300,000 individuals each year experience recurrent heart attacks in the United States alone,3,4 and the prevalence of ischemic heart disease is projected to rise to about 40.5% of the USA population by 2030.4 Traditional approaches for dealing with end-stage HF are often not feasible, due to the limited number of hearts available for transplantation. Preclinical trials have reported improvements in patient outcomes,5-7 but prognosis remains poor, and there is, therefore, an urgent need for new approaches to the prevention and treatment of HF.

During HF, billions of cardiomyocytes (CMs) are progressively lost, and fibrotic non-functional scar tissue develops, significantly reducing the pumping capacity of the heart muscle. The remaining CMs have a limited intrinsic regenerative capacity and cannot, therefore, replace the lost CMs. Cardiac regeneration studies have shown that dividing CMs are abundant in the fetus, but rapidly lost after birth.8 Cell-based therapies with exogenous cells, such as bone marrow cells, cardiac progenitor cells, and other self-renewing stem cells, have been developed to improve heart function. However, little meaningful improvement has been reported for these treatments, owing to the limited interaction between the various progenitor cells and the myocardium environment during myocardial infarction (MI).

In the last two decades, our understanding of the molecular pathways and genes involved in the disease has improved, and gene therapy has emerged as a possible treatment for HF. Given the limited site specificity of pharmacological inhibitors, gene therapy is an exciting prospect for more precise targeting of the signaling pathways involved in disease progression. The gene therapy approaches currently being developed for HF aim: (1) to increase the proliferation or contractility of endogenous CMs; (2) to reprogram cardiac fibroblasts to develop into beneficial cardiac cell types, such as endothelial cells (ECs) or CMs; and (3) to increase capillary density by activating endogenous ECs or progenitors. Recent studies have reported reactivation of the CM cell cycle following protein delivery to the myocardium, either by direct injection or via patch delivering the protein to the epicardium. CM proliferation has been reported following the delivery of NRG1 protein via intraperitoneal (i.p.) injections,9 intramyocardial (IM) injections of agrin,9,10 or the delivery of follistatin-like 1 to the epicardium.11 Furthermore, the proliferation of adult CMs has been observed following transfection with an adenovirus encoding a dominant-negative p38 mitogen-activated protein kinase (MAPK)11 or an extracellular matrix component, periostin.12 Also, Hajjar and coworkers13,14 have shown that the adeno-associated virus (AAV)-mediated delivery of Serca2a13 or SUMO14 improves cardiac function post-MI and in HF condition via elevation of endogenous CMs contractility.

Another successful avenue of gene therapy for heart repair is the genetic in situ reprogramming of cardiac fibroblasts into CMs. Pioneering work by Srivastava and coworkers15 showed that fibroblasts could undergo cardiac reprogramming to become beating CMs following direct virus-mediated IM delivery and overexpression of the cardiac myocyte transcription factors Gata4, Mef2c, and Tbx5 (GMT). This approach is promising as an alternative to cell-based regeneration
therapies, but the efficiency of in vivo reprogramming remained low, and there is also a potential risk of viral genome insertions associated with the use of viral vectors. Olson and coworkers improved in vivo reprogramming by using Hand2 as an additional reprogramming factor, together with GMT. In addition to its use to stimulate CM proliferation and reprogramming, gene therapy has also been used to induce myocardial repair by enhancing angiogenesis and inducing cardiovascular regeneration. Over the last decade, preclinical studies have reported revascularization in ischemic heart following the direct delivery of vascular endothelial growth factors (VEGFs) by various methods. Widely used vectors provided robust and consistent gene expression leading to neovascularization, but this expression was often accompanied by undesirable effects, such as the induction of edema or angioma due to the prolonged expression of VEGF, the elicitation of an immune response against the vector, or a potential risk of genomic integration. Nevertheless, these studies have provided useful insight and support for the use of gene therapy to repair the injured myocardium, although a number of hurdles remain to be overcome for this therapeutic approach to be considered successful.

Current Approaches in Cardiac Gene Therapy

Gene therapy can be defined as the transplantation of normal genes into cells to replace missing or defective genes, with the aim of correcting genetic disorders or promoting inactive beneficial mechanisms or pathways. With improvements in our understanding of cardiac disease, interest is growing in the use of gene therapies to treat coronary heart disease. The ultimate goal of gene therapies is the expression of protein of interest, and the most feasible way of achieving this goal is to introduce the corresponding protein directly into the myocardium. Direct protein delivery overcomes the difficulties of translation within the cell, thereby offering a potential advantage in terms of higher levels of expression, dose regulation, and control over a viral-based gene therapy approach. However, the short half-life and instability of injected proteins, the lack of use of this approach for intracellular proteins (e.g., transcription factors), and possible immunogenicity due to minor histocompatibility antigens are problems that must be overcome for this approach to be therapeutically successful.

However, approaches based on the insertion of nucleic acids, which can be translated into proteins within the cardiac cells, can circumvent the challenges of protein therapy. In recent decades, considerable advances have been made toward the delivery of nucleic acids into the heart by viral and non-viral vectors. Lentiviral vectors are favored for cardiac gene therapy by many researchers because they can transduce non-dividing CMs. However, their chief advantage—their integration into the host genome, ensuring sustained gene expression—also entails a risk of compromising the genome and tumorigenesis. The exceptional transduction efficiencies of adenoviruses and AAVs have resulted in these vectors being the most widely used for cardiovascular applications. Adenoviruses transfer genes efficiently into the myocardium in large animals, but expression is transient, and these viruses trigger a strong immune response. AAVs have low immunogenicity and are a widely used alternative for gene delivery to the heart. These nonpathogenic vectors ensure cardiac tropism without integration into the host genome and have been used in a recent study in which persistent Yap-associated protein expression resulted in CM proliferation and regeneration post-MI. AAV gene delivery peak levels of expression are reached about 4 weeks after delivery and continue for up to 11 months. Despite the predominance of AAVs over other gene delivery vectors, the production of neutralizing antibodies against the AAV capsid, delayed pharmacokinetics, and limited gene packaging capacity of these vectors restrict their use in cardiac gene therapy.

The delivery of naked plasmid DNA overcomes the risk of immune responses and oncogenesis, because of the absence of the viral vector. Plasmid DNA displays impressive organ specificity, but transfection efficiency is low. The recent elucidation of the role of microRNAs (miRNAs) and long noncoding RNAs in cardiac repair and regeneration has provided new hope for innovative therapy. A recent review by Hermans-Beijnsberger et al. summarized newly found long non-coding RNAs involved in the cellular process during development of cardiovascular disease (CVD). These non-coding RNAs can efficiently suppress the target mRNA post-transcriptionally by promoting mRNA degradation or inhibiting translation. Despite the successful results obtained in vitro, systems for delivering them to the heart in vivo have yet to be optimized. Furthermore, therapeutic miRNAs may have off-target effects, resulting in potential risk of oncogenesis. There is, therefore, an urgent need to explore clinically relevant approaches for enhancing cardiac regeneration and maintaining correct heart function both during and immediately after ischemic injury.

Modified mRNA Therapy

Different gene therapies have proved inefficient due to a short half-life, production of neutralizing antibodies, or a poor transduction capacity. By contrast, mRNA-based therapies are highly promising for the treatment of various human disorders. The delivery of mRNA to the cell has significant advantages over the use of protein or DNA-based delivery systems: (1) the use of mRNA transfection overcomes the need for nuclear localization or for transcription of the gene of interest in the patient’s cells; (2) the introduction of mRNA into cells is safe under physiological conditions, because mRNA does not integrate into the host genome; and (3) the effect of the mRNA is transient, minimizing the risk of mutagenesis after mRNA therapy (Figure 1).

Successful direct mRNA transfer was first reported about three decades ago, when Wolff et al. demonstrated the delivery of mRNA and its translation into protein in mouse skeletal muscle. After a few initial successes with mRNA therapy, research into mRNA structure and delivery methods continued, but the use of mRNA therapy was limited to vaccine development, because of problems of instability and immunogenicity. Within cells, mRNA is prone to cleavage by RNase and can trigger the innate immune system via Toll-like receptors (TLRs) 7 and 8 (which recognize single-stranded RNA) or TLR3 (which recognizes double-stranded RNA), leading to an increase in cytokine levels and associated toxicity. In 2008, pioneering
work by Karikó et al.\textsuperscript{35} addressed these issues and provided a platform for mRNA therapy in genetic, regenerative medicine, immunotherapeutics, and cancer. The study showed that replacing the uridine residues in mRNA with the naturally occurring modified nucleoside pseudouridine (hence the name modified mRNA [modRNA]) enhanced translation, due to changes in the secondary structure of the mRNA limiting its recognition by the TLRs and nucleases.\textsuperscript{35,36} The use of modRNA has since been on the increase in genetic medicine, for protein-replacement therapies and the treatment of genetic diseases. The efficiency of modRNA delivery in vivo has been increased by enhancing the stability of the mRNA and increasing translational efficiency by capping the molecule with the 3’-O-Me-m7G(5’)-ppp(5’)-G Anti Reverse Cap Analog (ARCA) at its 5’ end.\textsuperscript{37,38} The uses of modRNA technology as a model for cardiac repair are listed in Table 1.

Immediately after MI, CMs and other cardiac cells such as ECs are lost due to occlusion of the coronary artery. A chain of events downstream leads to oxidative stress and inflammation, resulting in impaired pump function and, ultimately, HF. The remaining CMs in the heart have a very limited proliferative potential and are therefore unable to replace the lost cells. The damaged coronary vasculature also creates a hostile environment in which it is difficult for the remaining CMs to survive. Various strategies have been developed...
to try to reverse the situation by inducing regeneration of cardiac neovasculature and encouraging CMs to proliferate.

A few independent clinical trials over the last 20 years have assessed the therapeutic potential of a potent angiogenic factor, VEGF-A, after ischemic injury. VEGF-A was delivered by intracoronary, intravenous, or IM injection, in the form of a recombinant adenoviral plasmid,20,22 naked cDNA, or non-viral plasmid.21,23 A moderate improvement in ejection fraction and left hearts, and reported a decrease in infarct size and an improved ventricular (LV) function was reported, but findings differed between trials.39–41 These differences can be explained by the short half-life of VEGF-A in plasma (about 30–45 min in humans), degradation by proteases, off-target effects associated with systemic delivery, and the lack of an efficient delivery platform. Attempts to retain VEGF-A in the infarcted heart for therapeutic purposes have been made, based on the implantation of biodegradable scaffolds including hydrogel,42 collagen,43 or self-assembling peptide nanofibers,44 but the success of these approaches was limited.42–44

Zangi et al.45 introduced a modRNA encoding VEGF-A into mouse hearts, and reported a decrease in infarct size and an improved myocardial outcome with higher survival rates (80% survival with VEGF-A modRNA versus only 20% for the group receiving DNA). They observed that VEGF-A protein secretion levels were much higher following treatment with modRNA than with unmodified mRNA, with no reported apoptosis or increase in the expression of immune response genes, such as retinoic acid-inducible gene (RIG)-1, interferon (IFN)-α, and IFN-β. Both VEGF-A modRNA and VEGF-A DNA increased capillary density and reduced infarct size and apoptotic cell frequency in MI mice, but the prolonged exposure to VEGF-A in DNA-treated hearts increased vessel permeability, a sign of abnormal vessel function. The study also showed that the favorable outcome achieved with pulse-like VEGF-A overexpression was associated with better vessel formation in the peri-infarct area because of the presence of larger numbers of WT1 epicardial progenitor cells activated via the kinase insert domain receptor (KDR) under stress conditions. These activated progenitor cells remain confined to the epicardial layer in the absence of VEGF-A, which induces their mobilization to the myocardial layer. Stimulation of the endogenous epicardial progenitor pool by the right paracrine factor (VEGF-A), time, and place enhances the differentiation of these cells into ECs and, to some extent, into CMs. Therefore, VEGF-A modRNA is an

| No. | Publication | Gene(s) | Role | Cellular Process or Disease | Delivery Material | Animal |
|-----|-------------|---------|------|-----------------------------|-------------------|--------|
| 1   | Zangi et al.45 | VEGFα  | directs the fate of heart progenitor cells and induces vascular regeneration after MI | cellular fate switch post-MI | RNAiMAX | mice |
| 2   | Lui et al.38 | VEGFα  | VEGF-A promotes not only the endothelial specification but also engraftment, proliferation, and survival (reduced apoptosis) of the human Isl1+ progenitors in vivo | VEGFα promotes Isl1+ to endothelial cell fate, proliferation and survival of Isl1+ progenitors | RNAiMAX | mouse |
| 3   | Huang et al.52 | IGF-1   | anti-apoptosis, cardiomyocyte survival, augmented Akt phosphorylation, and decreased caspase-9 activity | anti-apoptosis, cardiomyocyte survival post-MI | polyethyleneimine-based nanoparticle | mouse |
| 4   | Turnbull et al.58 | EGFP | modRNA delivery (direct myocardial or intracoronary administration) into rat and pig heart | modRNA expression in heart | formulated lipidoid nanoparticles (FLNP) | rat and pig |
| 5   | Turnbull et al.59 | EGFP | protocol | lipoid mRNA nanoparticles | formulated lipidoid nanoparticles (FLNP) | rodents |
| 6   | Kondrat et al.57 | EGFP | protocol | optimal modRNA expression | sucrose-citrate buffer | mouse |
| 7   | Sultan et al.51 | modRNA delivery optimization | modRNA delivery optimization, modRNA amount and time optimization | RNAiMAX | mice |
| 8   | Zangi et al.53 | DN-IGF-1R, IGFR | inhibition of adipogenic differentiation post-MI | inhibition of adipogenic differentiation post-MI | RNAiMAX | mice |
| 9   | Carlson et al.47 | VEGFα | increased angiogenesis, improved heart function post-MI, reduced fibrosis | increased angiogenesis, improved heart function post-MI, reduced fibrosis | sucrose-citrate buffer | pig, monkey |
| 10  | Singh et al.57 | EGFP, mCherry, Fluc | modRNA delivery optimization | optimal modRNA expression in heart | alginate, nanomaterial | mice and pig |
| 11  | Magadum et al.48 | mutated FSTL1 | ablation of N180Q, N-glycosylation site of hFSTL1 by modRNA delivery increased CM proliferation, improved cardiac output, and reduced scar size post-MI | CM proliferation, decreased scar size, improved heart function | sucrose-citrate buffer | mice |
excellent clinical approach to repair of the damaged vasculature and can further improve myocardial outcome and survival after injury.

Moreover, VEGF-A modRNA also can promote the engraftment, proliferation, and survival (reduced apoptosis) of transplanted human Isl1-positive cells.46 Carlsson et al.35 recently reported efficient intracardiac transfection and protein expression from a VEGF-A modRNA in a pig model of MI. They reported improvements in % ejection fraction, inotropic function and compliance, border zone capillary and arteriole density, and a decrease in myocardial fibrosis 2 months after the treatment of MI with VEGF-A modRNA. These improvements in cardiac systolic parameters were observed following the delivery of 1 or 10 mg modRNA via intracardiac injections post-MI.

Several attempts have been made to use conventional viral proteins to upregulate cell-cycle promoters or to downregulate the brakes on the cell cycle, with the aim of promoting the re-entry of post-mitotic CMs into the cell cycle. However, the long-term uncontrolled expression of pro-proliferative genes can be detrimental to heart function. For this reason, modRNA technology has been tested in the field of cardiac regeneration. Magadum et al.46 recently investigated the role of hFSTL1 glycosylation in CM proliferation and showed that the myocardial injection of a mutated hFSTL1 modRNA with a single asparagine-to-arginine (N-Q) substitution in the glycosylation site (N180Q) was necessary and sufficient to increase the proliferation of neonatal rat or mouse adult CMs in vitro or after MI, respectively, with no signs of cardiac hypertrophy. This finding can be explained by changes in the glycosylation pattern of hFSTL1 upon N180 site ablation, leading to activation of CM proliferation and regeneration. Interestingly, a single administration of N180Q modRNA in the mouse MI model was sufficient to increase cardiac function significantly, with a decrease in scar size and an increase in capillary density 28 days post-MI, showing modRNA to be an efficient tool for induction of control CM proliferation and cardiac regeneration post-MI. Our studies of the use of modRNA technology have yielded promising results, showing that it is possible to create mutated constructs or proteins for investigations of their role in heart disease and, potentially, to introduce therapeutic constructs for cardiological treatments.

**Cardioprotective Role of modRNA**

modRNA-based gene delivery has several advantages over other intracardiac therapies. Viral vectors and plasmid DNA delivery methods have spatiotemporal shortcomings, whereas modRNA allows rapid, transient, and efficient gene expression to a specific time window after cardiac injury. In this respect, modRNA is an ideal tool for delivering factors targeting the signaling pathways altered in the first few hours of infarction.

A series of events takes place after MI, leading to a massive sudden loss of CMs, beginning within an hour of occlusion. The stress to which CMs are subjected post-MI leads to the induction of pro-inflammatory cytokines and chemokines, and an accumulation of inflammatory cells in the heart.23 This chain of events occurs rapidly, within 2–3 days of ischemia injury. These days thus constitute the time frame in which desirable gene combinations could be delivered to prevent CM apoptosis.50 Sultana et al.51 have shown that luciferase modRNA can be detected in the heart 10 min after injection, and that its expression peaks at 24 hr but remains detectable for up to 10 days. Thus, based on its expression dynamics, modRNA has been used in various studies to deliver genes or gene combinations for cytoprotection and to induce cellular reprogramming in a desired time frame after cardiac injury.

Consistent with this approach, Huang et al.52 delivered insulin growth factor 1 (IGF-1) modRNA to the area of mouse hearts at high risk after injury, and extended the temporal window for the cytoprotection of CMs against apoptosis after hypoxia and MI. The delivery of IGF-1 modRNA, with a polyethyleneimine-based nanoparticle, resulted in efficient transient protein expression within cells. IGF-1 was expressed rapidly, within 2 hr of injection, and its levels peaked 24 hr post-injection, decreasing thereafter to 48 hr, and about 25% of cells in the border zone were transfected. The delivery of IGF-1 modRNA promoted CM survival and decreased cell apoptosis by more than 50% post-hypoxia in vitro and post-MI. The increase in IGF-1 levels was shown to be associated with CM survival and a decrease in the number of TUNEL-positive cells post-hypoxia. The decrease in apoptosis rates was accompanied by higher levels of Akt and Erk phosphorylation and a downregulation of IGF-1-specific miRNAs.

Despite this demonstration of the cardioprotective role of IGF-1, Zangi et al.53 found that the activation of IGF-1 signaling pathways in the heart post-MI also had negative consequences. In addition to its cardioprotective action, IGF-1 expression can lead to the formation of epicardial adipose tissue (EAT) post-MI. EAT is an active tissue located between the myocardium and the visceral pericardium, and contributes to the pathological mechanisms of coronary artery disease. Excessive epicardial fat deposition around the heart may trigger the production of several adipocytokines and chemokines through the activation of various paracrine and vasocrine signaling pathways, resulting in the development of atherosclerotic plaques in the coronary vessels.53 Hence the group evaluated the role of paracrine contributors in the development of EAT under normal and pathological conditions.

The study showed that IGF-1 delivered to post-MI stressed hearts by modRNA contributed to the differentiation of epicardial progenitor cells into adipogenic cells and the formation of EAT.29 It was demonstrated that WT1 expression was essential for epicardium-derived cells (EPDCs) differentiation into adipocytes, by delivering a Cre modRNA by gel application onto the surface of WT1fl/fl;Rosa26Tomate hearts for local WT1 inactivation and EPDC labeling. This study provided unique insight into the modRNA gene delivery method, in which a gene can be delivered locally through the application of a biocompatible gel directly onto the cardiac tissue in situations in which the development of knockout animals is not possible. This mode of gene transfection was also used to deliver dominant-negative IGF-1 receptor antagonists to the injury-exposed epicardial cells shortly after MI,
during the brief time window in which the IGF-1-induced differentiation of progenitor cells into adipocytes appears to occur. The transient inhibition of IGF-1 receptors significantly decreased EAT formation, confirming our hypothesis that IGF-1 receptor signaling is required to stimulate the adipogenic differentiation of EPDCs in the context of MI. This study provides an illustration of the ability of modRNA techniques to deliver a gene transiently at the appropriate time and place to block an undesired signaling pathway in one cell type (EPDCs), but not on another (CMs).

Challenges in the Cardiac Delivery of modRNA

Improvements in our understanding of the pathology of HF over time have led to novel gene therapy targets being identified, although inefficient delivery to the target tissue remains a substantial problem. For efficient gene therapy in the heart, the delivery systems carrying the nucleic acid must ensure: (1) the uptake of the nucleic acid by cardiac cells; (2) escape from the immune response; and (3) efficient translation and biodistribution of the genes in the post-ischemic, peri-infarct, or non-ischemic areas of the myocardium.

Cells typically take up modRNA via endocytosis, a process in which foreign molecules or ligands (in this case, modRNA) are engulfed by an area of plasma membrane, which then buds off intracellularly, leading to the formation of modRNA-containing endosomes. These endosomes later disassemble to deliver the mRNA to the cytoplasm, in which it is immediately translated into protein. However, human TLR8 (hTLR8) and mouse TLR7 (mTLR7), which are expressed only on endosomal membranes, recognize single-stranded RNA [particularly poly(U) and poly(U/G) motifs in the case of hTLR8], and this recognition triggers the innate immune response. TLR3 is also expressed on endosomal membranes and can elicit an innate immune response following its recognition of unmethylated CpG motifs in double-stranded RNA.55 Another obstacle to the translation of the imported mRNA is its degradation by RNase. Over a decade ago, a revolutionary study by Kariko et al.35 demonstrated that the replacement of uridine residues in the mRNA with naturally produced pseudouridine resulted in much lower levels of TLR-mediated immunogenicity and prevented degradation by RNase. Subsequent studies, including studies by our group, have used such modified RNA to achieve high translation efficiencies without immunogenicity in non-cardiac tissues.45,51 In 2015, Andries et al.56 showed that naturally produced 1-mΨU incorporation into mRNA reduced immunogenicity in mammalian cells lines by preventing endosomal TLR3 activation and downstream innate immune signaling. Consistent with these findings, our modRNA, containing 1-mΨU in place of uridine residues, resulted in significantly lower levels of activation for innate immunity genes, such as those encoding IFN-α or IFN-β and RIG, in cardiac cells and tissues than were observed with unmodified mRNA. We were also able to show that modRNA with 1-mΨU was less likely to be degraded by RNase compared with unmodified mRNA (Figure 2). Thus, the choice of an appropriate delivery method is critical for transfection with a large modRNA that cannot simply diffuse into the negatively charged CMs.

Maximum transfection efficiency and modRNA stability in vitro can be ensured by complexing modRNA with transfection reagents, to
The reporter gene detected within 10 min in cardiac muscle. These buffer was translated very effectively, whereas naked modified RNA in sucrose-citrate failed to achieve this. However, despite the efficient delivery of modified RNA to cardiomyocytes in vitro, the use of this agent is associated with higher rates of cell death around the injection site in the myocardium, suggesting it may not be an ideal vehicle for in vivo transfection.

Microencapsulated modified RNA in nanoparticles was recently tested as a way of delivering modified RNA to the heart. Expression of the protein was observed in multiple cell lines and primary CMs, within 2–4 hr of transfection, and persisted for up to 7 days without altering the structural and functional properties of the cells. This study demonstrated the simultaneous delivery of multiple genes to mouse hearts and showed that the reporter gene was efficiently delivered by a alginate gel in the pig MI model. Similarly, Turnbull et al.\(^5\) used formulated lipoid nanoparticles (FLNs) and assessed modified RNA transfer into the heart. They demonstrated that FLNs delivered mRNA much more efficiently, within 20 min, to rat and pig myocardium than saline containing naked modified RNA. In contrast, Sultana et al.\(^5\) found that encapsulating the modified RNA with nanoparticles hindered its effective translation, whereas naked modified RNA in sucrose-citrate buffer was translated very efficiently, with the protein corresponding to the reporter gene detected within 10 min in cardiac muscle. These findings were recently confirmed by Carlsson et al.,\(^5\) who reported efficient intracardiac transfection and protein expression following the delivery of modified RNA in saline citrate buffer. Translation efficiency in mouse heart was highest for 100 μg of naked modified RNA delivered in sucrose-citrate buffer.\(^5\)

Achieving the desired biodistribution of the therapeutic gene in the heart remains one of the largest challenges facing us, but the modified RNA used in this case was expressed in more than 20% of the LV, demonstrating the potential utility of this approach for delivering disease-specific genes to the heart in cases of injury. The use, in most cases, of intracardiac injection to deliver modified RNA to the heart may limit the biodistribution of the modified RNA. A better, systemic, non-invasive cardiac delivery method is therefore required.

With the increasing use of modified RNA in preclinical studies (Table 1), many researchers are now trying to increase its translational capacity of modified RNA in vivo. Conventional mRNA, containing uridine, is associated with low translation rates due to activation of the RNA-dependent protein kinase (PKR), which then phosphorylates translation initiation factor 2-alpha (eIF2α). The phosphorylated form, eIF-2, binds to eIF2B with a higher affinity, preventing the formation of the eIF2•GTP•Met-tRNA, tertiary complex required to deliver mRNA to the ribosome, limiting the translation capacity of the mRNA. However, we have shown that this process can be altered by the complete replacement of uridine with 1-mU, which results in maximal translation and optimal expression kinetics for modified RNA in the heart. The 1-mU modified RNA displayed significantly higher levels of reporter mRNA expression in rat CMs in vitro and in mouse hearts in vivo than the modified RNAs used in previous studies. In a complementary study, Svitkin et al.\(^6\) showed that mRNA with the 1-mU modification resulted in much higher levels of reporter protein production, due to attenuation of the eIF2 phosphorylation-dependent inhibition of translation and an increase in ribosome density on the mRNA.

Given the large amounts of modified RNA needed to transfect large-size heart, such as human heart, and the detrimental nature of the transfection achieved by IM injection, the use of modified RNA as a therapeutic option in cardiac disease patients would require improvements in modified RNA translation and non-invasive transfection methods.

Future Directions in Cardiac modified RNA Therapy

modified RNA is a promising approach for the treatment of cardiovascular disorders because it circumvents the key difficulties presented by conventional protein- and DNA-based gene therapy. Figure 3 summarizes the ideal use of modified RNA in prevention of cardiac remodeling.

Transfection with modified RNA results in transient protein expression and is, therefore, an attractive tool for therapeutic purposes for the correction of cellular processes that do not require long-term protein expression, such as cardiac regeneration, CM proliferation, and reprogramming. However, current modified RNA approaches have no inherent tissue- or cell-type-specific targeting capability in vivo, whereas AAV gene therapy vectors can include tissue-specific promoters.\(^61\)–\(^63\) Improvements in targeting are, therefore, required, because the activation of intracellular genes (e.g., transcription factors) in the wrong cell type can be detrimental. In addition, because the IM injections may be stressful to the tissue, further research is needed in development of non-invasive delivery methods. To ensure targeted and non-invasive delivery of RNA, RNA aptamers, which have high affinity to bind specific cell markers and are widely used in cell-type-specific delivery of other RNA therapeutics like small
interfering RNA (siRNA), can be used in conjunction with modRNA to ensure its target-specific delivery.\textsuperscript{64,65} Moreover, the transient expression of modRNA may have made this tool ideal for approaches targeting regeneration, but it is also the principal obstacle to the replacement of long-term protein therapy by modRNA therapy in the heart. Long-term controlled protein expression, with a method of repeated systemic modRNA delivery, would make it possible to use the modRNA delivery system to promote cardiac function in preclinical or in clinical HF settings.

In our view, as research effects increase safety and scalability, and lead to the development of cost-effective clinical-grade materials, robust delivery methods, and lower treatment costs, modRNA technology will become an excellent therapeutic agent to address experimental and clinical needs to induce cardiac regeneration and promote cardiac function in ischemic heart disease.

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