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Commentary

RNA therapeutics for heart disease

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ABSTRACT

The majority of the human genome encodes non-coding RNAs (ncRNAs), species of RNA without protein-coding potential but with powerful regulatory functions in disease onset and progression. Functional studies demonstrate that both coding and ncRNAs underlie various mechanisms in heart disease and that molecules targeting RNA species show promising efficacy in preclinical development. Accompanying the exciting developments in basic RNA biology, an equally provocative field has flourished for the design of RNA-based strategies to generate innovative types of therapeutics against these new "druggable" targets, going beyond our current repertoire of small chemistry or biologics. Here, we review the (bio)chemical basis of RNA-based drug design, provide examples that show promise as translatable drug products in preclinical studies, give an insight in the current barriers that hamper straight-forward clinical translation and discuss future directions that may overcome these hurdles to expand the current pharmacotherapy for myocardial disorders.

1. RNA avenues for heart disease

Heart diseases are a major cause of morbidity and death in Western societies with little recent progress to reduce their high mortality and are now also steeply on the rise in the developing world [1]. The diseases are preceded by ventricular remodelling and changes in left ventricular mass and volume of the myocardium in response to alterations in loading conditions [2,3]. The molecular events that underlie myocardial remodelling still remain poorly understood. Another major hurdle towards improved therapeutics is that currently cardiovascular disease therapy mainly follows a generic 'one-size-fits-all' approach, ignoring the inter-individual differences caused by underlying genetic susceptibility, age, gender or stages of disease. This calls for better disease stratification and introduction of medical breakthroughs to address the key biological mechanisms that derail in subsets of patients, an approach that will likely signal the end of the big block-buster treatments for vast numbers of patients, introducing the concept of "Precision Medicine". With "Precision Medicine" is meant medical decisions, practices, and interventions tailored to the individual patient who will benefit, sparing expenses and side effects for those who will not.

Heart diseases are most commonly provoked by the acute and chronic loss of cardiomyocyte function secondary to coronary artery disease, hypertension, diabetes or combinations thereof, which places a volume load or a pressure load, respectively, on the surviving portion of the myocardium. Chronic "load" causes quite generic responses in the myocardium, such as hypertrophy of cardiac myocytes, cardiac dilatation and interstitial fibrosis. These structural cardiac changes are preceded by changes in the "genomics" of the heart: changes in transcript abundance of protein-coding or non-coding genes and the re-expression of genes that are normally only observed in the embryonic heart [4]. These complex forms of heart disease are referred to as "acquired heart diseases" [5]. Acquired forms of heart disease are contrasted by monogenetic forms of heart disease caused by mutations in single genes identified using candidate gene studies or linkage analysis, leading to hereditary, familial dilated cardiomyopathy (DCM), hypertrophic cardiomyopathy (HCM) and arrhythmogenic right ventricular cardiomyopathy (ARVC) [5].

Since cardiovascular disease mechanisms are typically genomic in nature (hereditary cardiovascular diseases comprise a very small subset of the patient population), new developments will likely derive from a better understanding of our genome. The results of the Encyclopedia of DNA Elements (ENCODE) project demonstrate that at least 80% of our genome is functional, has a regulatory role and is transcribed into various classes of non-coding, regulatory RNAs. In short, RNA species beyond mRNAs include intronic RNAs, microRNAs (miRNAs), long non-coding RNAs (lncRNAs), circular RNAs (circRNAs) and extracellular RNAs. Collectively, these are known as non-coding RNAs (ncRNAs)

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because they lack clear coding potential, although notable exceptions exist [6]. Here, we will focus on coding and ncRNAs as direct drug targets for heart disease using RNA to silence or mimic the expression of the target RNA molecule. Additionally, we will give examples how the versatility of RNA as drug substance can be employed to function as homing devices or delivery tools to locate other molecules in close proximity of a desired drug target, as is in the case of aptamers or short guide RNAs in CRISPR technology.

2. Modulation of RNA activity: antisense oligonucleotides

Modulation of endogenous ncRNA activity can be efficiently accomplished by the use of antisense oligonucleotide (ASO) technology or modified RNA mimics, such as plasmid or viral vectors carrying RNA sequences to cells and tissues. ASOs were first discovered decades ago and are defined as short, single-stranded chains of nucleotides that hybridize with complementary RNA sequences following Watson-Crick base pairing; they exert their function either by leading to the degradation of the endogenous ncRNA, mediated by RNase H activity, or by sterically blocking the access to its targets [7]. ASOs have already been successfully employed as tools to inhibit ncRNAs and the simple biological principles of their function makes ASO design relatively straightforward. However, since several exo- and endonucleases in organisms rapidly degrade RNA-like structures, premature degradation of ASOs severely hampers their bioavailability. In this context, chemical modifications of ASOs are often employed to alter their pharmacokinetic properties and enhance cellular uptake without concomitant loss of target binding (Fig. 1). Here, we will list the most commonly used chemical nucleotide modifications of ASOs employed for the treatment of heart diseases *in vivo* (Fig. 2).

2.1. ASO: 2′-OMe modifications

Methylation of the hydroxyl group at the 2′ position of the ribose unit is a relatively simple and attractive approach to increase resistance to nuclease attack. The 2′-O-methyl (2′-OMe) ASOs have been widely used in the past decade and have proven *in vivo* efficacy. Besides enhanced resistance to nuclease attack, 2′-OMe ASOs show improved binding affinity to their corresponding RNA sequence when compared to unmodified ASOs. To further enhance resistance to nuclease attack, phosphorothioate moieties in the linking backbone are often introduced: these sulphur analogues of phosphate can be incorporated near the 5′ or the 3′ end, showing, in combination with a 3′-cholesterol unit, enhanced ASO stability in cell cultures. Krützfeldt et al. were the first to explore the potential of 2′-OMe ASO modifications to modulate microRNA activity *in vivo*. Systemic injection of 2′-OMe-modified oligonucleotides with a partial or fully modified phosphorothioate backbone and a 3′ cholesterol conjugation through a hydroxyprolinol linkage, termed “antagomirs”, were able to completely silence endogenous miR-122 levels in the liver through microRNA degradation; moreover, high sequence specificity was demonstrated by introducing position specific mismatches that abolished ASOs activity. The authors reported that the administration of a miR-122-targeting antagomir at a dose of 80 mg/kg for 3 consecutive days effectively reduced miR-122

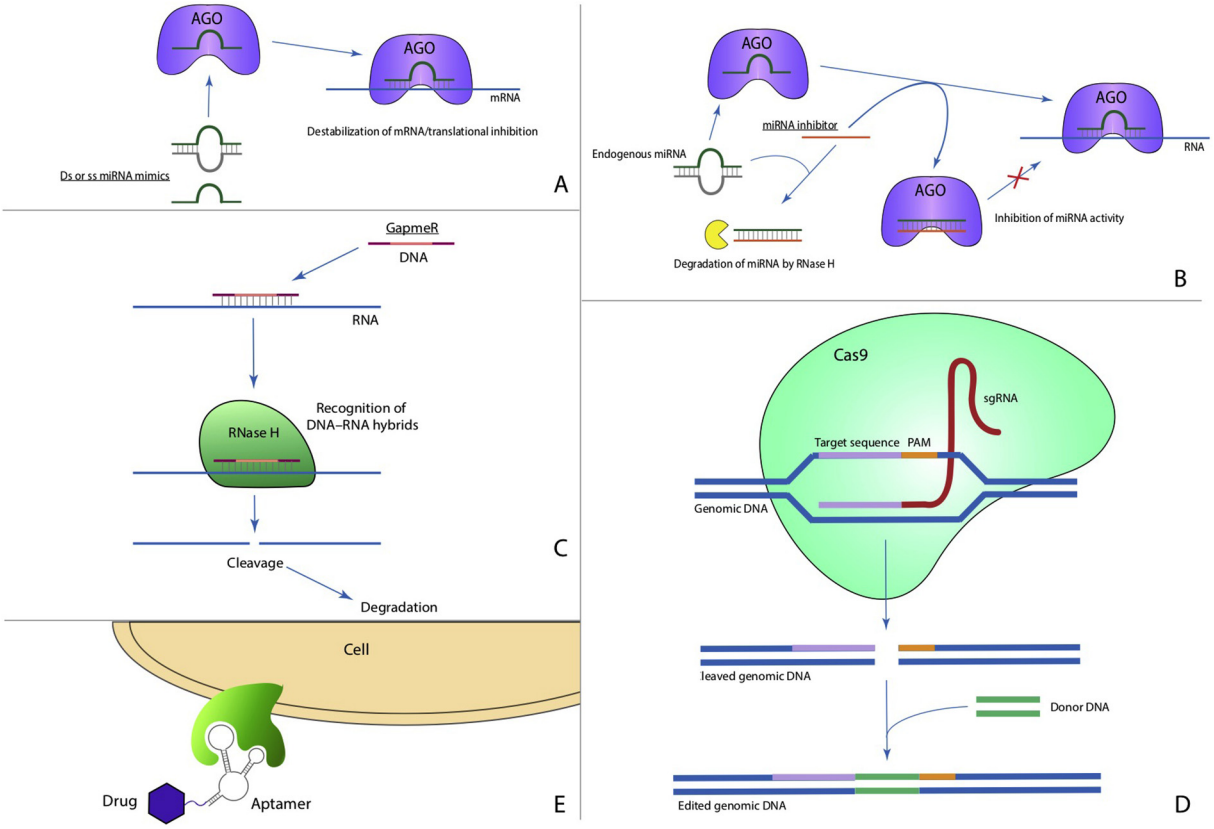

Fig. 1. Strategies for the regulation of RNA levels with antisense oligonucleotides, ncRNA mimics and CRISPR/Cas9. A) Transcription regulation by miRNA mimics. Double-stranded (ds) or single-stranded (ss) miRNA mimics reduce transcription of target mRNAs by destabilization or inhibition of the translation of the transcript they couple with. B) Regulation by miRNA inhibitors. MiRNA inhibitors can form DNA-RNA duplexes with their miRNA targets that are then cleaved by RNase H. Alternatively, miRNA inhibitors can act by binding their miRNA targets when loaded in the Argonaute protein (AGO), impairing their activity. C) Reduction of mRNAs by antisense oligonucleotides GapmeRs. RNase H recognizes the duplex formed by the GapmeR and its mRNA target and degrades it. D) Genome editing strategy using CRISPR/Cas9 tool. SgRNA molecule coupled with Cas9 enzyme binds to the target DNA region, Cas9 introduces a DSB upstream its PAM sequence and if a donor DNA molecule is present it can be used for homology-directed repair (HDR) of the DSB. E) Aptamers function as nucleotide analogues of antibodies for the tissue or cell-specific delivery of drugs.