The importance of being ncRNAs: from bit players as "junk DNA" to rising stars on the stage of the pharmaceutical industry

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Regulatory networks that orchestrate heart development and adaptation have always been under profound investigation, mainly because the heart is the first organ to form and function in order to sustain the entire life of the organism (1). Consequently, alterations is the molecular pathways controlling heart development as well as adaptation to physiological and environmental stress might result in cardiac pathological conditions, which represent the leading causes of death worldwide (2).

For many years it has been assumed that only functional proteins, encoded by genomic sequences containing open reading frames (ORFs), were accountable in playing crucial roles in almost all biological processes, including cardiac physiology and pathologies (3). However, only 2% of the eukaryotic genome codes for proteins, which is why the formerly called "junk DNA" has become an expanding area of interest. Indeed, the latest advances in sequencing technologies and initiatives such as ENCODE (*Encyclopedia of DNA Elements*) have highlighted that the mammalian genome is actively transcribed into a myriad of non-coding transcripts, collectively defined as ncRNAs (4). In the context of the cardiovascular system, only recently it has become evident how these ncRNAs may play a crucial role in the gene regulatory networks that control physiological and pathological development (5).

Here, we discuss the review of Li *et al*. (6) which summarizes the role and function of microRNAs (miRNAs) and long noncoding RNAs (lncRNAs) in cardiac disease and how they interact with each other to finely regulate the molecular events in ischemic heart diseases. We then underline recent advances involving the targeting of both miRNAs and lncRNAs and the consequent challenges for their exploitation in the pharmaceutical industry (7).

Based on size, ncRNAs can be classified into two categories: (I) small ncRNAs that comprise miRNAs, PIWI-interacting RNA (piRNAs) and endogenous short interfering RNAs (siRNAs); and (II) lncRNAs.

MiRNAs are 19–24 nucleotides long, and mainly function as negative regulators of gene expression, since they can influence the stability and the subsequent translation of nascent mRNA transcripts (8-10) . Indeed, miRNAs inhibit mRNA translation and/or mRNA stability through base-paring with the three untranslated regions within transcripts (11). Ultimately, several studies have also demonstrated the presence of mature miRNAs in the nuclear compartment, where they can act on the stability of nuclear transcripts, silence or activate transcription at specific gene promoters through epigenetic modifications and modulate co-transcriptional alternative splicing events (12).

Since the first discovery in *C. elegans* in 1993 (13), there was a remarkable gain in knowledge regarding regulation, expression and functionality of miRNAs in many human diseases, including cardiovascular pathologies (14). Van Rooij *et al*. found that some

miRNAs (miR-23a, miR-23b; miR-24, miR-195 and miR-214) were able to induce a hypertrophic response in cardiomyocytes (15). Successive studies revealed that other miRNAs can affect the development of hypertrophy. This is the case of miR-133, that was demonstrated to be inversely related to cardiac hypertrophy (16). Carè *et al.* showed for the first time, that *in vitro* overexpression of miR-133 blocks the up-regulation of different hallmarks of hypertrophy. On the contrary, even in absence of stress stimuli, the *in vivo* suppression of endogenous miR-133 with a decoy sequence was responsible of a massive cardiac hypertrophy response. Moreover, the cardio-protective effects of this miRNA was also demonstrated by Castaldi *et al.*, who described that through its action on different effectors of the β_1-Adrenergic cascade, miR-133 preserved cardiac performance and attenuated pathological remodeling in a mouse model of induced cardiac hypertrophy (17).

Alteration in miRNAs expression was also found to be associated in other models of cardiac diseases such as myocardial infarction (MI), a condition caused by coronary artery occlusion that in turn results in death of cardiomyocytes and fibrosis. Among the dysregulated miRNAs, miR-21, which is preferentially expressed in cardiac-fibroblasts, was demonstrated to increase the development of fibrosis (18).

This rapidly expanding interest in miRNAs led to the development of increasingly sophisticated techniques of RNA sequencing that in turn allowed the discovery of the second class of relevant ncRNAs: lncRNAs. lncRNAs are defined as RNA molecules of more than 200 nucleotides in length, lacking a significant ORF, and classified according to their genomic positioning as sense, antisense, intronic, divergent, or intergenic. Contrary to miRNAs, lncRNAs do not interact only with transcripts, but depending on their site/subcellular location, can exert several different functions. Some lncRNAs elicit their function in the cytosol mainly by regulating protein localization, mRNAs translation and stability (19). However, the majority of lncRNAs are located in the nucleus, where they control gene expression by interaction with transcription factors or histone-modifying enzymes. In the heart, one of the most well-studied lncRNAs is the myosin heavy chain-associated RNA transcript *Mhrt* (20). *Mhrt* was demonstrated to inhibit cardiac hypertrophy by antagonizing the function of Brg1, a chromatin-remodelling factor responsible for expression of many hypertrophic genes.

Despite a large number of studies have revealed that miRNAs and lncRNAs equally contribute in the onset of cardiac diseases, recently it has been suggested that miRNAs and lncRNAs can react with each other, pointing out a novel level of complexity in the already well-orchestrated regulatory network of ncRNAs. As discussed by Li *et al.*, the interactions between miRNAs and lncRNAs can be mechanistically divided into: (I) lncRNAs acting as a sponge/decoy of miRNAs; (II) lncRNAs as precursor of miRNAs; (III) miRNA-triggering lncRNAs decay; and (IV) competition for mRNA targets. Among these, the most characterized mechanism in the ischemic heart is the absorption of miRNAs by lncRNAs. Nonetheless, more detailed studies are required to further dissect the full mechanistic network of cross-talk between miRNAs and lncRNAs.

The strong association between aberrant expression of ncRNAs and pathological conditions has corroborated the concept that manipulation of miRNAs or lncRNAs could represent a new frontier for innovative therapeutic strategies. Indeed, though most of the work in this field has been done in cancer therapy, the application of ncRNA-based therapeutic approaches for the treatment of cardiac diseases has been facing a rapid spreading towards pre-clinical and clinical phases (5). With respect to miRNA-based therapies, the knowledge of their mechanism of action and the frequent abnormal up-regulation in some pathologies has prompted the development of three main approaches: (I) expression vectors (miRNA sponges); (II) small-molecule inhibitors and (III) antisense oligonucleotides (ASOs) (21). The first technology was described by Ebert *et al.* and it was conceived as reporter vector containing multiple miRNA-binding sites. When delivered into cells, the binding sites would serve as decoys for the targeted miRNA, thereby reversing the suppression of endogenous target genes (22). The second strategy is based on low molecular-weight compounds, which interfere with miRNA function, targeting different steps of miRNA pathways. These small molecules can interfere with the transcript of primary miRNAs or inhibit miRNA processing by interfering with Dicer activity or even with their loading into Argonaute 2 (AGO2) to form an active RNA-induced silencing complex (RISC). In this field a pioneering study was the work of Gumireddy *et al.* who demonstrated that azobenzene was able to directly inhibit the action of miR-21 (23). The third technology was certainly the most studied and currently used. It is based on chemically modified miRNA-targeting ASOs (anti-miRs) designed to target specific miRNA and bind with

high affinity. So far, various chemical modifications (i.e., 2'-OME; cholesterol and locked nucleic acid) are available in order to facilitate the cellular uptake and the specificity of these anti-miRs (16,24-26).

In addition to the silencing of miRNAs, their up-regulation to physiological levels has become a goal for research institutes as well as for pharmaceutical industries. However, the approaches to optimize miRNA overexpressing tools have been rather inadequate so far (27). The synthetic RNA-duplexes known as "mimics" are largely used to emulate miRNA function. However, since they must function as a miRNA and become recognized by the RISC complex, the necessary chemical modifications have been so far limited (28). Thus, deeper studies in developing chemical stabilization and/or delivery modifications are required to increase the biological effects. This represents the current limiting step for this technology to become an acceptable avenue for future clinical studies.

In light of the chemical tools that have already been developed for miRNA manipulation, analogue approaches are now applied for new therapeutic strategies towards the use of lncRNA-based tools. However, despite lncRNA modulators (i.e., gapmers) has started to be used for different *in vitro* and *in vivo* studies (29,30), more efforts are still required for overcoming some obstacles for their exploitation in translational medicine. Additionally, the poor rate of lncRNA conservation between species makes the designed compounds difficult to be translated towards pre-clinical and clinical studies. Moreover, side-effects have to be carefully evaluated because of the more complicated mechanism of action of lncRNAs (31). In addition, further limitations exist as a result of their targeting efficiency, which is related to the nuclear compartmentalization of the majority of lncRNAs.

Besides the chemistry used for down- or up-modulation of the altered ncRNAs, a further difficulty is the successful and safe delivery of the therapeutic compound to target tissues. To overcome this obstacle, a new frontier has been recently examined by the world of nanomaterials. In fact, nanostructured biomaterials with their physiochemical properties such us small and controllable size, biocompatibility and biodegradable chemical composition, high reactivity and functional structure, might successfully face the drug delivery limitations of traditional approaches (32). Additionally, the non-immunogenicity of some biomaterials and the incorporation of the therapeutic molecules loaded within nanocarriers facilitate the therapeutic preservation against potential immunogenicity reactions and enzymatic degradation, respectively (33). Furthermore, functionalization of nanocarriers with cell-specific targeting moieties (i.e., aptamers or peptides) might enable a more specific targeting thus avoiding side effects due to uncontrolled biodistributions of the drug in other organs.

In conclusion, the field of ncRNA therapeutics is still in its infancy and yet already there are promising data. However, before we might use these new therapeutic tools regularly, more detailed functional and structural investigations are necessary to better characterize the biology of ncRNAs and the proper approach for their modulation. It is reasonable to think that the more we learn about the contribution of ncRNAs in diseases, the higher the chances for achieving a better diagnosis and prognosis will be. Thus, the scientific community and pharmaceutical companies must band together to pursue this goal in order to obtain more rigorous tools that will eventually be acceptable for clinical trials and subsequently be used as therapeutic tools for a wide range of diseases in the coming years.

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Footnote

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