Send Orders for Reprints to reprints@benthamscience.net

Current Stem Cell Research & Therapy, 2020, *15*, 000-000

REVIEW ARTICLE

The Noncoding Side of Cardiac Differentiation and Regeneration

Francesca Pagano[1,*], Alessandro Calicchio[2], Vittorio Picchio[1] and Monica Ballarino[2,*]

[1]*Department of Medico-Surgical Sciences and Biotechnology, Sapienza University of Rome, C.so della Repubblica 79, 04100 Latina, Italy;* [2]*Department of Biology and Biotechnology "Charles Darwin", Sapienza University of Rome, P.le A. Moro 5, 00185 Rome, Italy*

ARTICLE HISTORY

Received: October 08, 2019
Revised: November 18, 2019
Accepted: December 06, 2019

DOI:
10.2174/1574888X15666200123120249

Abstract: Large scale projects such as FANTOM and ENCODE led to a revolution in our comprehension of the mammalian transcriptomes by revealing that ~53% of the produced RNAs do not encode for proteins. These transcripts, defined as noncoding RNAs (ncRNAs), constitute a heterogeneous group of molecules which can be categorized in two main classes, namely small and long, according to their length. In animals, the first-class includes Piwi-interacting RNAs (piRNAs), small interfering RNAs (siRNAs) and microRNAs (miRNAs). Among them, the best-characterized subgroup is represented by miRNAs, which are known to regulate gene expression largely at the post-transcriptional level. In contrast, long noncoding RNAs (lncRNAs) represent a more heterogeneous group of > 200 nucleotides long transcripts, that act through a variety of mechanisms at both transcriptional and post-transcriptional level.

Here, we discuss how miRNAs and lncRNAs are emerging as pivotal regulators of cardiac muscle development and how the alteration of ncRNA expression was seen to disturb the physiology of all the different cell types forming the cardiac tissue. Particular emphasis is given to those species that are expressed and are known to regulate the capacity of cardiac progenitor cells (CPCs), currently used in regenerative medicine protocols, to proliferate and differentiate. Understanding how the ncRNA-mediated circuitries regulate heart homeostasis is one of the research areas expected to have a high impact, improving the therapeutic efficacy of stem/progenitor-cells treatments for translation into clinical applications.

Keywords: ncRNA, miRNA, lncRNA, circRNA, cardiogenesis, cardiac regeneration.

1. INTRODUCTION

Recent advances in sequencing technologies have led to an expanded knowledge of the transcriptional output of mammalian genomes and revealed that biological complexity cannot be directly ascribed to the number of protein-coding genes, rather to increasing content of noncoding DNA (ncDNA) [1, 2]. ncDNA can be broadly classified into *cis*-regulatory regions and DNA elements producing non-protein coding RNAs (ncRNAs). The interest towards ncRNAs has increased in recent times, since they have been shown to participate in multiple physiological and pathological processes. However, the knowledge on the involvement of ncRNAs in the development of the cardiac muscle and in the differentiation of all the cell types that constitute the heart is still limited and requires attention [3]. In fact, besides their physiological role, dysregulation in ncRNA expression has been reported to contribute to the initiation, progression and severity of several cardiovascular diseases (CVDs) including hypertrophy, myocardial infarction (MI) and heart failure (HF). Therefore, there is an urgent need to disentangle ncRNA-mediated mechanisms of action to gain insight into more successful diagnosis and therapeutics of such pathologies.

ncRNAs constitute an abundant class of transcripts that can be grouped into different categories based on their functions and sizes. Short RNAs are < 200 nucleotides in length and include small interfering RNAs (siRNAs), piwi-interacting RNAs (piRNAs) and microRNAs (miRNAs). miRNAs are highly versatile molecules that regulate gene expression at a post-transcriptional level. Their biogenesis starts from a longer capped and polyadenylated RNA polymerase II transcript (pri-miRNA), which is processed in the nucleus by the Drosha/DGCR8 complex and then in the cytoplasm by the DICER complex, releasing the mature 21 nucleotide long miRNA, in its active form [4]. Each miRNA can bind many messenger RNAs (mRNAs) at specific sites on their 3'-UTR to cause gene silencing through the block of

*Address correspondence to this author at the Department of Biology and Biotechnology "Charles Darwin", Sapienza University of Rome, P.le A. Moro 5, 00185 Rome, Italy; Tel: 39-06-49912201;
E-mail: monica.ballarino@uniroma1.it
Department of Medico-Surgical Sciences and Biotechnology, Sapienza University of Rome, C.so della Repubblica 79, 04100 Latina, Italy; Tel: 39-0773-1757234; E-mail: francesca.pagano@uniroma1.it

1574-888X/20 $65.00+.00

translation and/or mRNA degradation [5]. In humans, miRNAs regulate the activity of ~50% of functional genes and play crucial roles in many biological processes, including embryogenesis, cell proliferation, apoptosis, differentiation as well as neoplastic transformation in all tissues [3, 6, 7]. Specifically, in the heart, several miRNAs are expressed and regulated cardiomyogenesis, heart development, cardiac homeostasis and regeneration [8]. LncRNAs represent a novel class of RNA polymerase II transcripts longer than 200 nucleotides, which exhibit mRNA-like features such as being 5'-capped, spliced and polyadenylated [9, 10]. LncRNAs are thought to possess remarkable regulatory potential, which acquires different facets depending on their binding partners and their subcellular (nuclear or cytoplasmic) localization [11]. In addition to miRNAs and lncRNAs [12], the recently recognized circular RNAs (circRNAs) emerge as a new class of evolutionarily conserved regulatory molecules [13, 14] where alteration is associated with different forms of CVDs [15]. Importantly, their exceptional intracellular stability combined with the tissue specificity, suggests that circRNAs might be used as promising therapeutic targets and biomarkers for CVDs [16]. Finally, a multifaceted interplay among miRNAs/lncRNAs/circRNAs and mRNAs has also reported to influence the initiation and progression of CVDs [17, 18], revealing new paradigms to transcriptional and post-transcriptional regulation of gene expression (Fig. **1**).

Here, we summarize the data reported in the recent literature on the role of ncRNAs in cardiac cells, and discuss how the alteration of their expression was seen to disturb the physiology of all the different cell types forming the cardiac tissue (i.e. cardiomyocytes, fibroblasts, vascular smooth muscle cells and others) leading to aberrant proliferation, apoptosis, autophagy and fibrosis. The strong impact of noncoding RNAs on the cardiovascular system makes them good candidates for therapeutic strategies. For this reason, we also discuss what has been recently described about ncRNA expression and function in cardiac progenitor cells, used in regenerative medicine protocols. Regenerative medicine is one of the most promising options for long term treatment of CVDs; many cellular sources and strategies have been used for such an approach involving the use of both differentiated as well as progenitor cells, surgically delivered to the damaged heart [19]. The main mechanisms whereby cardiac regeneration can be achieved through cell therapy are: i) the direct differentiation of either transplanted or resident cardioblasts into functional cardiomyocytes or ii) the modulation of cardiac environment through the release of soluble factors, which can sustain angiogenesis and limit cardiac muscle scarring and fibrosis [20]. The cell sources which have been tested clinically in CVD cell therapy applications can be divided into noncardiac stem cells, such as mesenchymal stem cells isolated from the bone marrow, and cardiac-committed cells, including c-Kit$^+$ cardiac stem cells, cardiosphere-derived cells, and cardiovascular progenitor cells derived from embryonic stem cells. Ultimately, also iPSCs are under investigation in pre-clinical phase [21]. The results obtained with the introduction of these strategies seemed promising, showing that these treatments can provide some benefits to the patients, such as improvements in heart functionality, limited scar formation after ischemic insult and/or some degree of regeneration [22]. However, the information obtained by later research and early clinical trials are controversial and this raised doubts on the feasibility of these therapies [23]. One of the main issues regarding regenerative medicine strategies involving stem and progenitor cells is the lack of knowledge on the mechanism by which the implanted cells exert their beneficial effects [24]. At first, researchers thought that these pools of stem cells could differentiate into functional cardiomyocytes, but this process is unlikely to happen at an appropriate rate to give any clinical benefit, also given the not well defined and possibly low proliferative and differentiation potential of the transplanted cells. At present, the most accepted is the paracrine hypothesis, whereby the beneficial effects of the injected cells seem to be mainly due to the secretion of diffusible molecules (including ncRNAs) that stimulate tissue regeneration [25]. Further understanding of the molecular machinery and pathways that govern the life of stem cells is essential to improve these therapies. To this purpose, several research groups directed their studies on the genetic regulation of stem cells [26], many of them focusing on the activity of noncoding RNAs in cardiac progenitor cells [27]. In the last years, a growing body of research has also hinted at the possibility to achieve cardiac regeneration by miRNA targeting. This opportunity has been increased by the recent discovery of lncRNAs and circRNAs, which functional impact on cardiac physiology can provide the foundation for new therapeutic strategies to repair the injured heart. Therefore, the studies on their functions in both cardiac development and disease as well as in the CPCs regenerative capacities, become crucial for the development of future clinical applications in CVD treatment [28].

2. SMALL NONCODING RNAs

Transcriptomic, as well as functional analyses, revealed that miRNAs have control cardiomyocytes proliferation in both embryonic and post-natal development, as well as cardiac stem cell proliferation and function (Table **1**). The role of miRNAs in cardiac muscle development and cardiac progenitors fate choices has been initially investigated through the generation of knock out mice carrying DICER conditional alleles. Cardiac specific genetic ablation of DICER in embryos led to severe cardiac development impairment of the ventricular chamber and sept [29]. When deletion was induced in animals after birth, it caused a dilated cardiomyopathy phenotype with early lethality [30, 31], and a deranged expression of the fetal genes program observed in cardiomyocytes [32].

3. FUNCTIONAL miRNAs IN DEVELOPMENTAL PROLIFERATION CONTROL

miR-1 is the most expressed miRNA in cardiomyocytes (CMs), and during the early stages of heart development, it works synergistically with miR-133 to promote mesoderm differentiation from embryonic stem cells (ESCs) and to suppress ESCs differentiation into endodermal or ectodermal (non-muscle) lineages [33]. At a later developmental stage, miR-1 and miR-133 display opposite roles. Indeed, while miR-1 promotes the differentiation of mesoderm into cardiomyocytes through the translational inhibition of Hand2 and Irx5 transcription factors, miR-133 enhances proliferation by

Table 1. Functional miRNAs in cardiogenesis, cardiomyocytes proliferation control and CPCs biology.

miRNA		Organism	Molecular Activity	Cardiac Function	Reference (Main Evidences)
miR-1		Mouse	Binds Hand2 and Irx5 transcription factor	Promotes mesoderm differentiation from ESCs and then promotes the differentiation of mesoderm into CMs	Liu N, *et al.* Genes Dev 2008
miR-133		Mouse	Represses SRF and Cyclin D2 expression	Promotes mesoderm differentiation from ESCs, promotes CM proliferation and protects CPCs against apoptosis	Liu N, *et al.* Genes Dev 2008 Izarra A, *et al.*, Stem Cell Rep 2014
miR-17~92 - miR-17 - miR-20	Cluster	Mouse	Target Bim, BMP 2/4, Isl1 and tbx1	Control the differentiation of SHF progenitors into right ventricular myocytes, limit CPCs expansion potential	Fuller N, *et al.* Cells 2014 Sirish P, et al J Mol Cell Cardiol, 2012 Hosoda T, *et al.* Circulation 2011 Wang J, *et al.* Dev Cell 2010
miR-208 miR-209b miR-499	Family	Human Mouse	Strongly regulate myosin heavy chain genes: Myh6 Myh7, Myh7b	Regulate muscle growth and enhance conversion of fibroblasts into CM-like-cells	England J, *et al.* Mol Life Sci 2010
miR-15a/b miR-16 1/2 miR-195 miR-497	Family	Mouse	Regulate the expression of Chek1, Cdk9, CyclinD1, Cyclin D2 and others	Play a central role in CM cell cycle progression	Porrello ER, *et al.* Circulation 2011
miR-590 miR-199	Family	Mouse Rat	Multiple targets; of note YAP pathway components TAOK1 and β-TrCP	Induce cell cycle re-entry of fully differentiated CM	Eulalio A, *et al.* Nature 2012 Tao Y, *et al.* BBRC 2019 Torrini C, *et al.* Cell Rep 2019
miR-302-367	Cluster	Mouse	Targets Mst1, Lats2, and Mob1b	Regulates CM proliferation through the inhibition of Hippo signaling	Tian J, *et al.* Sci Transl Med 2015
miR-1825		Rat	Targets p16, Rb1 and Meis2	Induces proliferation of adult CM and promotes cardiac regeneration post ischemic injury	Pandey R, *et al.* Am J Transl Res 2017
miR-29a		Rat	Targets Cyclin D2	Involves in cell cycle control in CM aged up to 4 weeks	Cao X, *et al.* FEBS Lett 2013
miR-128		Mouse	Targets SUZ12, which suppress p27 and activates Cyclin E and CDK2 expression	Impairs cardiac regeneration	Huang W, *et al.* Nat Commun 2018
miR-34a		Mouse	Targets Bcl2, CyclinD1 and Sirt1	Impairs cardiac regeneration	Yang Y, *et al.* Circ Res 2015
miR-31a5p		Rat	Targets RhoBTB1	Promotes CM proliferation	Xiao J, *et al.* Exp Mol Med 2017
miR-499		Human Mouse	Targets SOX6 and ROD1	Enhances cardiac stem cells differentiation into CM	Hosoda T, *et al.* Circulation 2011
miR-193a		Mouse	Mediates c-Kit downregulation with unknown mechanism	Decreases c-Kit+ progenitors proliferation activity	Sun Y, *et al.* Stem Cell Res Ther 2018
miR-21 miR-221 miR-24	Cocktail	Mouse	Multiple targets, including Bim in CPCs	Increase Sca1+ progenitor cells survival in cell therapy applications	Hu S, *et al.* Circulation 2011
miR-210	CDCs Exosomes	Mouse	Targets multiple genes involved in CM apoptosis pathways	Cardioprotective, angiogenetic and anti-apoptotic	Barile L, *et al.* Cardiovasc Res 2014 Wang L, et al. J Cel Mol Med 2019
miR-146a			Targets p38 mediated MAPK phosphorilation	Anti-apoptotic, recapitulates some of the beneficial effects provided by CDCs in cell therapy	Ibrahim AG, e*t al. S*tem Cell Rep 2014

repressing the SRF (serum response factor) and Cyclin D2 expression, both required for cell cycle progression [34]. The miR-17~92 cluster controls the differentiation of the second heart field (SHF) progenitors into right ventricle myocytes and the formation of the cardiac outflow tract [35]. Studies on the inactivation of the bone morphogenic protein (BMP) signalling, a well-known cardiac development regulator, showed that Bmp2 and Bmp4 were responsible for the transcriptional activation through Smad1/5 binding to the conserved region upstream of miR-17~92 cluster. In particular, two members of the cluster, miR-17 and miR-20a, directly repress Isl1 and Tbx1 [36]. These two transcription factors are expressed in cardiac progenitor cells and their downregulation limits cardiac progenitor cell's capacity to expand [37]. Furthermore, during heart development, contractile protein such as Myh6 (alpha-myosin heavy chain), Myh7 (beta-myosin heavy chain) and Myh7b are strongly regulated by the group of myomiRs, which includes miR-208a, miR-209b and miR-499, respectively. Deregulated expression of these miRNAs leads to an abnormal expression of myosin proteins which causes pathological cardiac remodeling [38]. In the last phase of heart development, the miR-15 family which includes miR-15a/b, miR-16-1/2, miR-195 and miR-497 plays a pivotal role in cardiomyocytes cell cycle progression regulating the expression of many genes such as Chek1, Cdk9, Cyclin D1, Cyclin D2 and others [39]. Thus, miRNA expression during organogenesis allows a timely control of transcriptional programs guiding differentiation by modulating the expression of key transcription factors (e.g. Hand2, Irx5, Smad1/5, Tbx1) and of genes necessary for the muscular function (e.g. Myosin genes).

4. FUNCTIONAL miRNAs IN POST-NATAL PROLIFERATION CONTROL

4.1. Adult Cardiomyocytes

The heart has been classically described as a post-mitotic organ and adult mammalian cardiomyocytes have limited regeneration capacity [40]. Probably, there is a strong intrinsic mechanism which keeps the heart homeostasis but also precludes cardiomyocytes cell cycle re-entry. Nowadays, it is clear that chronic, as well as acute cardiac injury, can stimulate endogenous repair mechanisms, and cardiomyocyte cell cycle re-entry is the main mechanism allowing the production of new cardiomyocytes in the adult heart [41]. Some of the miRNAs described as regulators of developmental programs (i.e. miR-1, miR-133, miR-17~92 cluster and others) were shown to be involved also in the activation and control of mature cardiomyocyte proliferation [42]. Fate mate mapping studies indicated that this mechanism can provide a viable pool of cardiomyocytes to the adult cardiac muscle after injury [43], but the specific localization of potentially cycling cardiomyocytes and their biology is still under investigation. In many instances, miRNAs implicated in cell cycle regulation during development, trigger the proliferative capacity of adult cardiomyocytes when induced or repressed.

A comprehensive screening of miRNAs function in neonatal cardiomyocytes revealed that miR-590 and miR-199a are key proliferation regulators in these cells by inducing cell cycle re-entry leading to a substantial increase in cardiomyocytes number. In addition, these same genes promoted also adult cardiomyocyte proliferation and regeneration [44]. Another example is represented by the miR-302-367 cluster, whose expression decreases during postnatal development and its re-activation was shown to be sufficient to induce cardiomyocyte proliferation. This was also achieved through ectopic expression of miR-302-367 mimics in therapeutic settings, which provided benefit after cardiac injury through targeting Hippo pathway components Lats2, Mst1, and Mob1 [45].

Also, components of the miR-199 family showed the ability to induce cell cycle re-entry of fully differentiated cardiomyocytes, upon the overexpression *in vitro*. Specifically, overexpression of miR-199 and miR-590, showed an increase up to 20% in the number of mature cardiomyocytes and demonstrated that these miRNAs can, on their own, induce proliferation of cardiomyocytes postnatally [44]. A recent study on the functional effects of pro-proliferative miRNA revealed that all of these miRNAs activate nuclear localization of the master transcriptional cofactor Yes-associated protein (YAP) and ultimately induce expression of YAP-responsive genes. miR-199a targets the upstream YAP inhibitory kinase TAOK1, and the E3 ubiquitin ligase β-TrCP, which leads to YAP degradation, therefore, the YAP pathway seems to play a central role in cardiac regeneration [46]. A recent report showed the effect of genetic overexpression of miR-199a in a gene therapy preclinical study in pigs, where the miRNA overexpression resulted in increased heart regeneration after MI, giving proof of concept evidence of the potential therapeutic use of miRNAs in heart failure [47]. The evidence presented and other evidence collected so far [48] revealed how the Hippo-YAP pathway, which has a pivotal role in heart regeneration, is regulated through miRNAs action at many levels, making miRNAs additional important players in both adult and neonatal cardiomyocytes proliferation control through the Hippo pathway.

A recent paper strengthened the notion that miR-199a is a powerful activator of proliferation in rodent cardiomyocytes, by affecting multiple genes acting on proliferation control. In detail, miR-199a targets negatively CD151, which induces p38 expression, a known negative regulator of cardiomyocyte proliferation [49]. In addition, recent evidences show an interesting regulatory loop where miR-199a is under control of miR-1825. Upon miR-1825 overexpression, the direct targets of miR-199a, namely p16, Rb1, and Meis2 are downregulated, making miR-1825 a master regulator of cardiomyocytes proliferation [50].

Many miR and miR-families act instead as negative regulators of cardiomyocytes proliferation, and this makes them good candidates for pharmacological intervention using antisense molecules. For instance, the miR-15 family has been implicated in postnatal cardiomyocyte proliferation block. One of its members, miR-195, is physiologically upregulated after birth and was shown to block cardiomyocyte proliferation; conversely, its overexpression during murine heart development, leads to congenital heart abnormalities [51]. The inhibition of miR-15 family members (including miR-195) after MI in neonatal mouse heart can increase the organ regenerative capacity through the proliferation of preexisting cardiomyocytes [39, 51]. The effects of its depletion in neo-

natal mice are associated with depression of Chek1 and an increased number of mitotic cardiomyocytes [39]. The evidences collected so far indicate that the miR-15 family contributes to the postnatal loss of regenerative capacity of the heart, and specific inhibitors can indeed provide therapeutic benefit against cardiac ischemic injury [52]. A recent study investigated the function of p53 and Mdm2, classically involved in cancer cell cycle derangement, in the maintenance of cardiac function and cell cycle arrest. Genetic ablation of both p53 and Mdm2 in a single mouse model showed that a set of miRNAs (miR-30 a, miR-30b, miR-30c, miR-30e, let-7a, let-7f, miR-181, miR-26b, miR-204, miR-149, miR-194) acts on cell cycle activation and is indeed responsible for cardiomyocyte cell cycle arrest. Specific inhibition of these miRNAs besides inducing cardiomyocyte cell cycle re-entry, can support the completion of this pathway, providing a way of generating new cardiomyocytes [53]. Similarly, miR-29a, highly expressed in cardiomyocytes aged up to 4 weeks, is involved in these cells' cycle control. Specific miR-29a inhibition induced proliferation through accelerated G1/S and G2/M transition, and up-regulated the cell cycle gene expression such as Cyclin D2 (CCND2) which is a direct target of miR-29a [54]. As already mentioned, members of the miR-1 and miR-133 family are expressed during cardiogenesis and act as crucial regulators of proliferation and differentiation in cardiac muscle [42]. Genetic ablation studies revealed how miR-133 also acts as a brake for the cardiomyocyte cell cycle and blocks their growth and proliferation. This is due to the inhibition of specific target genes such as SRF2 and Cyclin D2 [55]. Cardiomyocytes from miR-133a-1/miR-133a-2 double knockout mice show proliferation increase and the opposite results were obtained with transgenic overexpression of both genes, indicating that these miRNAs function as inhibitors of these cells proliferation *in vivo* [34]. Recent studies found that miR-128 is responsible for the proliferation block in cardiomyocytes. miR-128 is overexpressed in cardiomyocytes after terminal differentiation and blocks expression of SUZ12, a chromatin modifier which suppresses p27 (cyclin-dependent kinase inhibitor) and activates Cyclin E and CDK2 expression, both positive cell cycle regulators. In mice, deletion of miR-128 promotes cell cycle re-entry of adult cardiomyocytes and attenuates cardiac dysfunction in response to MI [56]. Another miRNA that impairs cardiac regeneration is miR-34a;, in the heart of neonatal mice, a miR-34a overexpression inhibits cardiac regeneration after MI, through targeting of Bcl2, Cyclin D1 and Sirt1, three main pro-survival and pro-regeneration genes in cardiomyocytes. This suggests that suppression of miR-34a in adult heart could increase post-MI cardiac regeneration and function. The evidence discussed above shows that miRNA can inhibit cardiomyocyte proliferation of post-natal cardiomyocytes through targeting cell cycle regulators (e.g. Cyclin genes, CDK, p27) as well as chromatin modifiers (e.g. SUZ12, Sirt1), making miRNAs a linking hub among these different mediators of cardiac cells proliferative capacity [57].

Besides canonical cell cycle regulation, other pathways affected by miRNA expression levels can induce CMs growth. For example, a recent study reported that miR-31a, normally lost during the early postnatal period, when re-expressed, can promote CMs proliferation acting through downregulation of RhoBTB1, a tumor-suppressor gene whose function is still unknown in the cardiac system. The same phenotype could also be induced by specific knockdown of RhoBTB1 gene which was shown to be the main mediator of the pro-proliferative effect of miR-31a on CMs [58].

4.2. Adult Cardiac Progenitor Cells (CPCs)

In addition to the mature cardiomyocytes cell cycle re-entry, the heart can achieve regeneration through specific proliferation and differentiation of resident progenitor cells, which are a very small and diffuse population responsible for some regenerative capacity of the heart. Resident progenitors can be activated by cardiac injury and mediate cardiac repair also through miRNAs [59]. Data on human cardiac-derived cardiomyocytes' progenitor cells (CMPCs) showed that miRNAs regulate the proliferation of these cells and their differentiation into cardiomyocytes. In particular cardiac derived cardiomyocytes express miR-1 and miR-499 at a high level, and their overexpression reduced proliferation and induced differentiation of CMPCs and embryonic stem cells [60]. miR-499 was shown to enhance cardiac stem cell differentiation into cardiomyocytes by targeting SOX6 and ROD1 [61]. In addition the expression level of miR-17~92 cluster is higher in neonatal CPCs as compared to the adult counterpart, and the cluster re-expression in adult cells might increase their proliferative potential [62]. Modulating progenitor cells expansion capacity is one of the main goals of regenerative medicine, and miR-17 and miR-20a inhibition might be a useful tool in this sense.

Molecular profiling of miRNAs expressed in resident CPCs responsible for keeping heart homeostasis [63], showed that they express many of the miRNAs acting on heart development. However, these cells don't go down the differentiation path, but stay in a paused state until triggered by specific stimuli. The pathways responsible for keeping them in this intermediate state are not fully known, but some studies revealed that manipulation of miRNAs involved in early development (miR-1, miR-17~92 cluster, miR-499) can modulate their fate choice [60-62]. Both miR-1 and miR-133 act on adult progenitor cells proliferative potential, are progressively upregulated during *in vitro* differentiation of adult cardiac progenitor cells (CPCs) and miR-1 family overexpression enhanced the cardiac differentiation of CPCs, whereas overexpression of the miR-133 family protected CPCs against apoptosis [64]. A recent paper described a loop mechanism providing proliferation potential to c-Kit$^+$ CPCs, involving miR-193a. In particular c-Kit$^+$ progenitor cells isolated from adult mouse heart showed an increased proliferation ability when treated with Insulin-like growth factor-1 (IGF-1), which resulted in increased c-Kit expression decreased miR-193a expression. The loop mechanism induced by IGF-1 stimulus drives an increased proliferation and migration of c-Kit$^+$ CPCs by epigenetic silencing the expression of miR-193a, which was shown to negatively modify the c-Kit expression level [65]. A set of miRNAs has been included in CPCs survival and apoptosis control. For example, a functional screening revealed that adult Sca$^+$ CPCs have an increased survival to stress after overexpression of miR-21, miR-221 and miR-24, achieved through targeting Bim protein. An increased Sca$^+$ CPCs survival was also reported after *in vivo* transplantation of cells overexpressing the three miRNAs

in ischemic heart, revealing a potential role for these miRNAs in ameliorating cell therapy approaches [66].

One of the cellular populations used in current clinical protocols for heart regenerative medicine approaches are Cardiosphere Derived Cells (CDCs). These are generated using a specific culture method allowing 3D culture of spheroids from stromal cardiac cells, which provides a niche-like structure that allows the expansion of cells with regenerative potential currently used in clinical trials (recently reviewed in [19]) for cardiac regenerative therapy of heart failure. It has been shown how the paracrine action of CDCs is crucial for their biological activity, and also that their phenotype, as well as expression of some miRNAs, is influenced, for example, by the pharmacological intervention on patients that undergo surgery [67]. In addition it has been shown that specific miRNAs are found in extracellular vesicles and exosomes secreted by CDCs [68, 69], among which are miR-210, and miR-146a. A cardioprotective role has been described for miR-210, which acts by improving angiogenesis and inhibiting cardiomyocytes apoptosis through targeting apoptosis pathway genes, cell cycle regulators as well as chromatin modifiers [70]. An anti-apoptotic role in cardiac cells after an injury has been also attributed to miR-146a [71, 72] which blocks, in combination with miR-21, p38-MAPK activity by inhibiting its phosphorylation. Importantly it has been shown that the administration of the sole miR-146a to the damaged heart of mice after MI can recapitulate, at least in part, the beneficial action provided using CDCs [69], which makes miR-146a one of the miRNAs to be studied in more detail as a possible surrogate for therapeutic cells usage in regenerative medicine protocols.

4.3. Functional Long Noncoding RNAs in Cardiomyogenesis

Differently from mRNAs, which mainly localize to cytoplasm, lncRNAs can have a mixed distribution within the cell or can preferentially occupy either the nuclear (chromatin, nucleoplasm, subnuclear foci) or the cytoplasmic compartment (Table **2**). Despite the differences in localization and mechanism of action, both nuclear and cytoplasmic lncRNAs are involved in all cellular processes that concur to the correct exertion of cardiovascular functions.

Table 2. Functional lncRNAs and circRNAs in cardiomyogenesis.

ncRNA	Organism	Subcellular Compartment	Molecular Activity	Loss of Function Phenotype	References
bvht	Mouse	Nuclear	Binds SUZ12 subunit of PRC2 complex preventing its repressive activity on MesP1 promoter	Decrease in beating cardiomyocytes in ESC derived cultures	Klattenhoff CA, *et al* Cell 2013
Fendrr	Mouse, Human	Nuclear	Binds PRC2 and TrxG/MLL to influence histone marks on lateral mesoderm specific gene promoters	Embryonic lethality	Grote P, *et al* Dev Cell 2013
carmen	Mouse, Human	Nuclear	Interacts with SUZ12 an EZH2 subunits of PRC2 and influences the expression of cardiomyogenic genes	Impairment of cardiac differentiation in ESCs	Ounzain S, *et al* J Mol Cell Cardiol 2015
Charme	Mouse, Human	Nuclear	Acts as a chromatin architect to promote cell differentiation	Cardiac hyperplasia	Ballarino M, *et al* EMBO J. 2018
cpr	Mouse, Human	Nuclear	Recruits DNMT3A and promotes CpG methylation of MCM3 promoter	Increased proliferation in adult heart	Ponnusamy, M *et al* Circulation. 2019
uph	Mouse	Nuclear	Its transcription maintains a permissive chromatin environment for the expression of nearby Hand2 gene	Embryonic lethality	Anderson KM, *et al* Nature 2016 Han X, *et al* Development 2019
Tbx5ua	Mouse, Chicken, Human	Not clearly localized	Upstream and divergent to Tbx5 gene	Embryonic lethality, hypoplastic left ventricle	Hori Y, *et al* BMC genomics 2018
YylncT	Mouse, Human	Nuclear	Binds and prevents DNMT3B activity on BRACHYURY (T) locus	hESC inability to differentiate into mesoderm cells	Frank S, *et al* Cell Stem Cell 2019
HBL1	Human	Cytoplasmic	Binds miR-1 to repress its functions in cell differentiation	Increase in differentiation of PSC in cardiomyocytes	Juli L, *et al* Dev Cell 2017
CAREL	Mouse, Human	Cytoplasmic	Competes for the binding with Trp53inp1 and Itm2a mRNAs	Promotes cardiac regeneration in the neonatal heart after injury, induces cardiomyocytes proliferation	Cai B, JACC, 2018
Crrl	Rat, Human	Cytoplasmic	Binds miR-199a-3p, increasing the activity of the HopX in mediating the deacetylation of Gata4	Alleviation of remodeling after myocardial infarction and increased cardiomyocytes proliferation	Chen G, *et al* J Mol Cell Cardiol. 2018
NR_045363	Mouse, Human	Cytoplasmic	Binds miR-216a and activates the miR-216a/JAK2-STAT3 pathway and cell proliferation	Decrease in primary fetal cardiomyocytes proliferation	Wang J, *et al* J Mol Cell Cardiol. 2019

Table (2) contd….

ncRNA	Organism	Subcellular Compartment	Molecular Activity	Loss of Function Phenotype	References
ECRAR	Rat, Human	Cytoplasmic	Binds ERK1/2 inducing its phosphorylation, activation and translocation in the nucleus.	Decreased proliferation	Chen Y, *et al* Mol Therapy 2019
Galont	Mouse	Cytoplasmic	Competes for the binding of miR-338 with ATG5	Decrease of autophagic vesicles and cell death after anoxia/reoxygenation treatment	Yin G, *et al*. J of Cell Biochem. 2018
SNHG1	Human	Cytoplasmic	Binds miR-195, regulating BCL2L2 translation and enhancing cell viability	Reduced viability in apoptotic cardiomyocytes	Zhang N, *et al* Cell Physiol Biochem 2018
CTBP1-AS2	Rat, Mouse	Cytoplasmic	Binds FUS in the cytoplasm to stabilize TLR4 mRNA and induce cardiomyocyte hypertrophy	Attenuation of AngII-induced hypertrophy	Luo X, *et al* Cardiov Path. 2019
Linc1405/Meteor	Mouse	Nuclear	Interacts with Eomes, WDR5, and GCN5 to regulate histone modification of MESP1 enhancer region	Impairs cardiac differentiation in ESC	Guo X, *et al*.Cell Stem Cell 2018 Engreitz JM *et al*, Nature 2016 Alexanian M, *et al* Nat Commun 2017
chfr	Mouse, Human	Cytoplasmic	Sponges miR-489 regulating Myd88 translation	Reduction of cardiomyocyte hypertrophy	Wang K, *et al* Circ Res. 2014
alien	Zebrafish, Mouse, Human	Not clearly localized	Specifically expressed in vascular progenitors, long interspersed noncoding RNA located next to FOXA2	Impairment in mesodermal specification, in particular, defective vascular patterning and heart chamber formation (Zebrafish),	Kurian L, *et al* Circulation 2015
punisher	Zebrafish, Mouse, Human	Not clearly localized	Specifically expressed in mature endothelial cells, antisense lncRNA of the AGAP2 gene	Severe defects in vasculature and heart development (Zebrafish), impaired human vessel maturation (Human)	Kurian L, *et al* Circulation 2015
terminator	Zebrafish, Mouse, Human	Nuclear	Specifically expressed in pluripotent stem cells, intergenic lncRNA located next to ZNF281	Embryonic lethality, severe cardiovascular defects (zebrafish), high cell mortality (hES)	Kurian L, *et al* Circulation 2015
SENCR	Human	Cytoplasmic		Attenuation of the SMC contractile phenotype and enhanced cell motility	Bell RD *et al* Arter Thromb Vasc Biol. 2014
circ-Slc8a1, circ-NCX1	Mouse, Rat, Human	Cytoplasmic	Sponges miR-133a and miR-133a-3p, inhibiting the regulation of the latter on CDIP1	Reduction of cardiac hypertrophy, reduction of apoptosis in oxidative-stress induced cardiomyocytes and *in vivo* amelioration of myocardial ischemia-reperfusion damage	Lim TB, *et al* Cardiov Res. 2019 Li M, *et al* Theranostics 2019
circ-Nfix	Mouse, Rat, Human	Cytoplasmic	Interacts with Ybx1 causing its cytoplasmic retention, ubiquitination by Nedd4l and degradation. Sponges miR-214 regulating Gsk3β expression	Increased cardiomyocytes proliferation, reduction of apoptosis in oxidative-stress induced cardiomyocytes, *in vivo* cardiomyocytes de-differentiation and proliferation followed by redifferentiation, increased regeneration and surviving after cardiac injury	Huang S, *et al* Circulation 2019
circ-Ttc3	Mouse, Rat	Cytoplasmic	circ-Ttc3 sponges miR-15b upregulating Arl2	Promotes cardiomyocyte apoptosis under cardiac ischemia and therefore cardiac dysfunction and remodeling after myocardial infarction	Cai L, *et al* J of Mol and Cell Cardiol 2019
circ-Amotl1	Human	Cytoplasmic, Nuclear translocation	Binds AKT and PDK, induces AKT phosphorylation and pAKT nuclear translocation	Decreased proliferation and survival and increased apoptosis	Zeng Y, *et al* Theranostics 2017
circ-HIPK3	Mouse, Rat, Human	Cytoplasmic	Sponges miR-29b-3p upregulating α-SMA, COL1A1 and COL3A1 expression	Inhibition of cardiac fibroblasts proliferation and migration. Reduction of cardiac fibrosis and improvement in diastolic function, together with a decrease in cardiomyocyte size	Ni H, *et al* Int J of Cardiology 2019

4.4. *Nuclear*-Enriched lncRNAs

Nuclear lncRNAs (Fig. **1A**) have demonstrated to exert significant biological functions during cardiovascular development. Even though only a few transcripts have been mechanistically characterized, many of them were shown to regulate cardiac-specific gene expression programs by influencing epigenetic marks, active or repressive transcription factor activities or the tri-dimensional architecture of specific chromatin domains [73]. These activities are temporally and spatially regulated by the formation of secondary and tertiary folds also assisted by the dynamic interaction with several partners. In this regard, biochemical high-throughput approaches revealed that lncRNAs may serve as structural scaffolds, to bring proteins and nucleic acids in proximity by the formation of ribonucleoprotein aggregates [74]. This versatility is exceptional and further increased by splicing regulation, which expands the variety of RNA structures by joining alternative combinations of exonic and intronic sequences. In the nucleus, some lncRNAs act *in cis*, thus influencing the expression of neighboring genes; other species diffuse from their transcription site to act *in trans* on chromatin loci which are located on different chromosomes.

Several lncRNAs influence pluripotency and cardiac lineage specification by regulating the expression of important master regulators. Earlier examples include Braveheart [75] and Fendrr [76], two *trans*-acting lncRNAs which activity in mouse is required for the commitment to the cardiac lineage and, ultimately, to heart development. Mechanistically, Braveheart regulates the progression of nascent mesoderm towards a cardiac fate by displacing the repressive Polycomb Repressive Complex 2 (PRC2) from the promoter of the master transcription factor (TF) mesoderm posterior 1 (MESP1). Concomitantly, Fendrr binds PRC2 as well as the activating Trithorax group (TrxG/MLL) complex, to turn on the expression of lateral plate mesoderm specific genes. MESP1 expression is further regulated by Linc1405, a lncRNA which transcription site is located nearby EOMES (Eomesodermin), a gene required for early embryonic mesoderm differentiation [77]. Mechanistically, the exon 2 of Linc1405 binds EOMES and promotes the assembly of the activating EOMES/WDR5/GCN5 complex at the MESP1 enhancer. Thus, as shown for Braveheart, Linc1405 also has a permissive function in cardiogenesis and knockdown results in decreased MESP1 expression [78]. For complete information, similar results were described shortly before by the Pedrazzini's lab, which reported in ESCs the modulation of EOMES-dependent mesendodermal specification and cardiac differentiation by the enhancer-associated lncRNA Meteor (MesEndoderm Transcriptional Enhancer Organizing Region) [79].

As Meteor, a number of cardiac lineage-determining lncRNAs is produced by cardiac-specific enhancer and super-enhancers (SE) [80]. An example is CARMEN (CARdiac Mesoderm Enhancer-associated Noncoding RNA), an evolutionary conserved SE-associated lncRNA whose expression is significantly induced during fetal CPCs differentiation. The enhancing activity of CARMEN, which is upstream of both Braveheart (in mouse) and EOMES/MESP1 regulatory pathway, places CARMEN at the top of a molecular hierarchy which controls the ability of precursor cells to differentiate into cardiomyocytes[81]. In addition to the above studies, other examples that support the important role of lncRNAs in cell specification from ESCs to CPCs and differentiation of cardiac cell subtypes (i.e. endothelial [82], cardiomyocytes [83] and smooth muscle cells [84]) were also identified. The balance between cardiac cell proliferation and differentiation, when dysregulated, may cause pathological remodeling, in which morphological and functional alterations of the cardiac muscle lead to chronic heart failure. In literature, several lncRNAs were described to be implicated in this process when deregulated [83, 85-87]. A recent example is represented by *Charme* (Chromatin architect of muscle expression), a muscle-restricted and chromatin-associated lncRNA which ablation in mouse causes a pronounced phenotype of cardiac remodeling [88]. It remains to be addressed whether the cardiac hyperplasia seen in *Charme* knockout mice simply arises from developmental dysfunctions or rather additional changes in the cardiac cell subtypes contribute post-natally to the observed phenotype. However, since *Charme* was shown to influence the three-dimensional organization of sub-nuclear domains by acting as an architect RNA, overall, these studies contributed to interpret the pathogenesis of cardiac remodeling from an epigenetic perspective.

LncRNAs which regulate cardiomyocyte proliferation may represent promising targets for effective cardiac repair and regeneration. In this direction, Wang's lab recently identified and functionally characterized the cardiomyocyte proliferation suppressor (CPR) lncRNA, which silencing restores heart performances upon myocardial injury by increased cardiomyocyte proliferation [89]. Although these results demonstrate that lncRNAs take part in cardiac repair, the incorporation into clinical trials still remains a challenge as a deeper understanding of their mechanism of actions is yet needed.

In humans, genomic loci encoding for developmental cell fate regulators are often hosting sites for divergently transcribed noncoding RNAs [90, 91]. Although the function of these transcripts is poorly known, their non-random distribution across the genome as well as the correlation of their expression with the accompanying genes, imply *cis*-acting strategies underlying their regulation [92]. Accumulating evidence from independent studies indicate that divergent lncRNA-mediated transcription regulation of neighboring genes represents a general phenomenon during cardiac commitment. A typical example is Upperhand (Uph), a long noncoding RNA co-expressed and associated with HAND2, a transcription factor that controls the reprogramming of fibroblasts into cardiomyocytes. In 2016, Anderson and colleagues found that Upperhand transcription, but not the mature transcript, governs the expression of HAND2 by maintaining a permissive epigenetic signature on HAND2 upstream enhancers [93]. In line with the importance of this regulation in the coordination of heart development, Uph knockout leads to ventricular hypoplasia by E10.5 and embryonic lethality. Of note, the ablation of Uph expression by the use of different knockout mouse models was recently found to produce distinct phenotypes [94]. These findings emphasize the complexity of Uph/HAND2 regulation and point out the need for complementary genetic approaches to understand the physiological relevance of lncRNA *in vivo*. In

analogy to HAND2, Takeuchi's lab found many divergent lncRNAs associated with genes with crucial functions for heart development (i.e. NKX-2.5, GATA6 and TBX5). Among them, the Tbx5 upstream anti-sense product (Tbx5ua) is a divergent lncRNA transcribed from one of the promoters of Tbx5, which genomic position is conserved in human. Although they are close, Tbx5ua and Tbx5 exhibit a different expression pattern being Tbx5ua, unlike Tbx5, equally expressed in both ventricles. Nevertheless, Tbx5ua knockdown by premature transcription leads to embryonic lethality probably caused by severe ventricular hypoplasia [95]. Differently to Tbx5ua, yin-yang lncRNAs (yylncRNAs) represent a subclass of divergent lncRNAs having an almost identical pattern of expression of their neighboring genes [96]. The fact that yylncRNAs are preferentially produced from the genomic loci of key developmental cell fate regulators, implies the potential significance of these transcripts in embryonic lineage decisions. A good example is represented by yylncT, a yylncRNA which was found to control mesoderm differentiation of ESCs by controlling the activation DNMT3B-dependent of the adjacent BRACHYURY (T) locus.

4.5. *Cytoplasmic* Enriched lncRNAs

Recent studies characterizing cytoplasmic lncRNAs involved in cardiomyocyte proliferation are various (Fig. **1B**). Heart Brake LncRNA 1 (HBL1) is a cytoplasmic lncRNA which expression in human is controlled by the transcription factor Sox2. In human pluripotent stem cells, HBL1 acts as a competing endogenous RNA (ceRNA) by physically interacting with miR-1, a key activator of human-induced pluripotent stem cells (hiPSCs) differentiation in cardiomyocytes. Mechanistically, HBL1 repress cardiomyocyte differentiation by counteracting miR-1 [97]. The cardiac regeneration-related lncRNA CAREL is upregulated in murine cardiomyocytes after a few days post-birth and this increase in expression is concomitant with a decrease in cardiomyocyte proliferation and heart regeneration ability. CAREL mediates these processes competing for the binding of miR-296 with Trp53inp1 and Itm2a mRNAs, whose proteins have been shown to regulate cell proliferation, differentiation and apoptosis in different cell types. These data suggest CAREL as a suitable therapeutic target to enhance cardiac regeneration after injury [98]. Similar function and activity are possessed by the Cardiomyocyte Regeneration-Related lncRNA (CRRL). CRRL is upregulated during adult life in cardiomyocytes and its silencing causes the overexpression of many cell cycle-related genes and the attenuation *in vivo* of cardiac remodeling after MI. CRRL functions as a sponge for miR-199a, preventing its activity on HopX mRNA. HopX acts together with the chromatin modifying enzyme histone deacetylase 2 (HDAC2) mediating the deacetylation of GATA4 in order to regulate the proliferation of cardiomyocytes. Like CAREL, CRRL may be a suitable target to activate adult cardiomyocyte proliferation in adult injured hearts [99]. An opposite effect on cardiomyocyte proliferation is exerted by lncRNA NR_045363. This transcript is abundant in developing cardiomyocytes and it is upregulated after cardiac injury in neonatal hearts. In addition, NR_045363 overexpression is able to ameliorate heart functionality after MI enhancing cardiomyocyte proliferation, whereas its silencing reduces DNA synthesis, mitotic activity and cytokinesis in cardiomyocytes. The molecular mechanism of NR_045363 consists of competing for the binding of miR-216a with JAK2, a kinase that phosphorylates STAT3, which is a transcription activator involved in cell proliferation [100]. Another lncRNA that positively influences cardiomyocyte proliferation is ECRAR. This transcript is transcriptionally activated by E2F1 and binds ERK1/2, which performs an essential role in cell proliferation and G1/S transition, inducing its phosphorylation, activation and translocation in the nucleus. ERK1/2 induces the expression of different cell cycle-related genes such as cyclins and E2F1 itself generating a feedback loop together with ECRAR [101]. Cytoplasmic lncRNAs have also been discovered to be involved in pathological conditions. For instance, lncRNA Galont is induced upon anoxia/reoxygenation (A/R) stimulus. This transcript enhances autophagy and cell death in cardiomyocytes by sponging miR-338, that has an opposite effect on these processes, thus preventing its activity on ATG5. This protein is an E3 ubiquitin ligase fundamental in autophagy and is the downstream effector of Galont [102]. lncRNA SHNG1 has an opposite effect on cell viability. This transcript is downregulated by H_2O_2 treatment in cardiomyocytes and its overexpression ameliorated the outcomes of the treatment on cell functionality. Mechanistically, SHNG1 acts as a ceRNA binding miR-195, which in turn negatively regulates the translation of BCL2L2, a mediator of apoptotic cell death. The alleviation of the miRNA effects by SHNG1 increases cell viability and reduces apoptosis [103]. Another example of lncRNA acting in pathological conditions is CTBP1-AS2 that has been found to be upregulated in hypertrophic cardiomyocytes. Loss-of-function studies demonstrated that this lncRNA is able to promote cardiomyocyte hypertrophy induced by Angiotensin II (AngII) treatment. Additionally, researchers discovered that Sp1, a transcription factor involved in a plethora of human diseases including cardiac hypertrophy whose expression is induced by AngII treatment, is able to enhance the expression of CTBP1-AS2. In turn, the transcript activity lies in its ability to bind FUS to stabilize the toll-like receptor 4 (TLR4) mRNA, that is critical in sterile inflammation, which is a common process during cardiac hypertrophy and contributes to the progression of the disease, and to hypertrophy itself [104].

4.6. Functional Circular RNAs in Cardiomyogenesis

Although there is not a general consensus on their function, many cardiac-specific circRNAs have been characterized in the latest years. Two different research groups worked on the characterization of a circRNA that arises from the second exon of the sodium-calcium exchanger gene Slc8a1, named as circSlc8a1/circNCX1 [105, 106]. Both studies demonstrated that this molecule acts as a miR-133a sponge. circSlc8a1 is constitutively expressed in cardiomyocytes, but its activity is of particular relevance in stress response after pathological conditions. Indeed, the studies show that inhibition of circSlc8a1 ameliorates pathological hypertrophy and decreases apoptosis induction in cardiomyocytes. Furthermore, Li and colleagues observed that circSlc8a1 endogenous upregulation by apoptotic stress causes an enhancement in pro-apoptotic factor cell death-inducing factor (CIDP1) expression, which is due to the re-

duced activity of miR-133 caused by the circular RNA, and that this process enhanced apoptotic death of cardiomyocytes [106]. An opposite role in cardiomyocytes survival is exerted by circ-Ttc3. It has been demonstrated that circ-Ttc3 is overexpressed after MI in cardiomyocytes under hypoxia and that its knockdown in cells undergoing this condition causes increased apoptosis. The same result is obtained through its induced downregulation *in vivo*, a process that causes cardiac dysfunction and remodeling. Circ-Ttc3 acts as a miRNA sponge too, competing for the binding of miR-15b with ADP Ribosylation Factor Like GTPase 2 (Arl2) mRNA, which is involved in mitochondrial functionality and cell division [107], therefore exerting a cardioprotective role [108]. Specific silencing of circHIPK3 in cardiac fibroblasts after AngII treatment counteracts these processes and additionally reduces cardiac hypertrophy. circHIPK3 mechanism of action consists of sponging miR-29b and upregulating the expression of its target genes α-SMA, COL1A1 and COL3A1 [109]. Circular RNA activity is not restricted to the class of competing endogenous RNAs, for instance, the recently characterized circNfix interacts both with proteins and miRNAs in the cytoplasm. This transcript is associated with a super-enhancer and is mainly expressed in adult hearts. circNfix expression is controlled by Meis1, which is a transcription factor that controls cell cycle arrest in adult cardiomyocytes, that is able to bind to the super-enhancer activating the RNA expression. circNfix is involved in cell cycle arrest, first interacting with Ybx1, a transcription factor associated with cell proliferation, and causing its retention in the cytoplasm. Here, Ybx1 is degraded after ubiquitination by the ubiquitin ligase Nedd4l. Furthermore, circNfix influences cell proliferation and angiogenesis sponging miR-214, which regulates glycogen synthase kinase 3 β (Gsk3β) expression. Gsk3β is involved in β-catenin degradation and VEGF release from cardiomyocytes, thus representing the main downstream effector of circNfix activity [110]. Another possible circRNA-mediated mechanism of action is the one performed by circAmotl1. This molecule is mainly expressed in neonatal cardiomyocytes and acts binding both AKT and PDK1 in the cytoplasm, thus facilitating AKT phosphorylation by pPDK1 and the subsequent pAKT activation and translocation in the nucleus. The AKT pathway is essential in the regulation of cell proliferation and survival and this makes circAmotl1 a potential powerful target to increase cardiomyocyte proliferation after injury [111].

4.7. ncRNAs and Future Challenges in Regenerating the Heart

Since the early 2000s, the discovery of many functional miRNAs acting as development regulators, oncogenes or tumor suppressors stimulated the interest of the scientific community on the involvement of ncRNAs in biomedical research. Over the years, this has led to a deeper understanding of the mechanisms behind the establishment and progression of many pathologies with the ambition to develop novel biotechnological tools for counteracting clinically relevant diseases. Above all, the emphasis was given to the cardiomyopathies as they are projected to be the leading cause of death worldwide. The main outcome of cardiac diseases is represented by the loss of millions of cardiomyocytes. Although at least in mammals, the cardiac muscle has a reduced regenerative potential, the presented literature highlights how often in pathological conditions regeneration can be sustained by miRNAs, which are essentially the same acting during ontogenesis [34, 42, 55, 60-62]. This might be applicable to lncRNAs as well, although their impact on heart remodeling is less known and a thorough description of their biology is then necessary.

Pharmacological treatments are frequently used for restoring cardiac functionality, but they usually only relieve symptoms without repairing the damaged tissue [112]. A more effective way is the one envisaged by regenerative medicine, whose ambition is to develop feasible strategies to replenish the pool of functional cardiomyocytes, thus repairing the damaged tissue. In this regard, an emerging issue is to obtain regeneration without the transplant of exogenous cells but by enhancing endogenous cardiomyocyte proliferation, possibly through expression or inhibition of specific genes. This kind of approach would be as challenging as the stem cell transplantation, especially given the low number and proliferation rate of the resident adult cardiac progenitor cells. Heart failure gene therapy approaches, currently at different stages of the clinical testing, have been developed mainly to target cardiac excitation-contraction coupling in cardiomyocytes [113]. Some recent evidence also showed promising results with gene therapy approaches designed on specific miRNAs, which can be efficiently delivered to post-ischemic hearts and induce regeneration (reviewed in [114]). Apart from miRNAs, several lncRNAs and circRNAs have been suggested to regulate cardiomyocytes proliferation proliferation [86, 89, 98, 99, 101, 110, 111]. Even though these studies hold the promise for the future, still some issues remain before translation into routine clinical practice. Firstly, there is a need for tailoring these strategies for feasibility in human hearts, evaluating the real efficacy in regenerating the damaged tissue *in vivo*. In addition, possible unwanted long-term drawbacks on the health of patients possibly due to the permanent reactivation of proliferation in cardiomyocytes have to be taken into account. However, the high tissue specificity of these molecules may favor their use in gene therapy, because it could prevent side effects on cell types other than those under treatment.

Besides boosting proliferation and endogenous repair mechanisms, it is well documented how the heart regenerative capacity can be influenced epigenetically by environmental factors, specifically oxidative stress, through ncRNAs [115-117]. Given the multiple functions of ncRNAs, and the documented interaction between different classes of ncRNAs (e.g. miRNAs and lncRNA or circRNA as summarized in Fig. **1**) it is of pivotal importance to gather as much information as possible on their involvement in all the variables which lead to heart failure progression.

Further studies on the involvement of noncoding RNAs in cardiomyocytes and progenitor cells proliferation, differentiation and secretion pathways, as well as in oxidative stress response, may lead to a better understanding of the mechanisms by which stem/progenitor cells treatments affect cardiac regeneration, thus helping in enhancing their efficacy. On the other hand, these studies can unravel the functionality of many other unknown noncoding RNAs that may be used in future gene therapy approaches either alone or in synergic combination with other molecules.

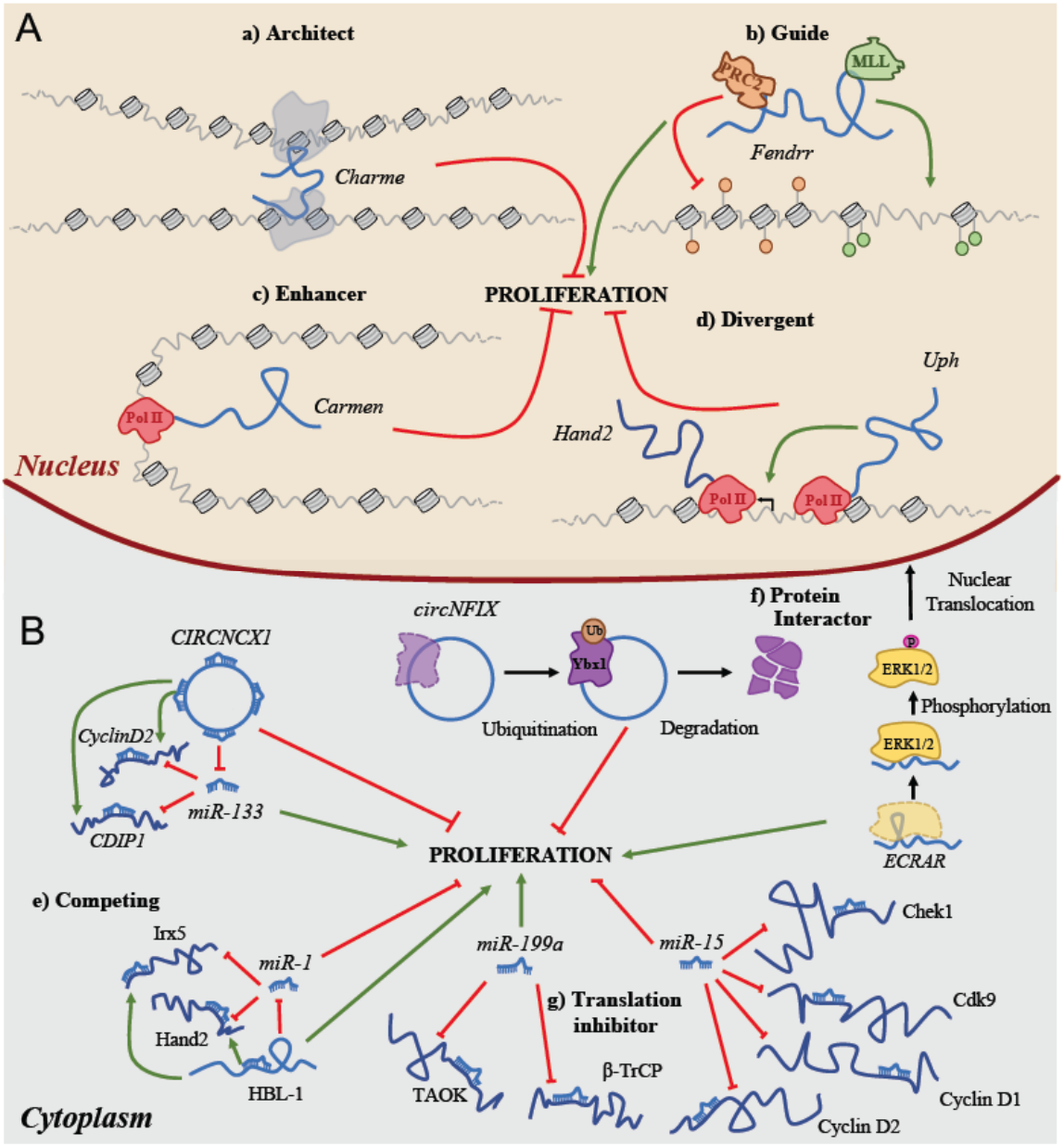

Fig. (1). Mechanisms of noncoding RNA activity in the nucleus (A) and in the cytoplasm (B) of cardiac cells. In the nucleus, cardiac lncRNAs can act as **a)** architects of chromatin 3D structures (*Charme* [88]), **b)** guides for chromatin modifying complexes (*Fendrr* [76]), **c)** enhancer RNAs (eRNAs) that are transcribed from enhancer regions (*Carmen* [80, 81]), **d)** divergent RNAs that are involved in the regulation of their neighbouring genes (*Uph* [93, 94]). In the cytoplasm, together with lncRNAs, also miRNAs and circRNAs exert their activity. LncRNAs can act as **e)** competing endogenous (ce)RNAs that bind miRNAs preventing their activity on mRNAs (*HBL1* [97], *CIRCNCX1* [105, 106]), **f)** protein interactors modulating the functionality of proteins (*ECRAR* [101], *circNFIX* [110]), while miRNAs are **g)** translation inhibitors for mRNAs (miR-1 [31], miR-15 [37], *miR-133* [32], *miR199a* [44]). The images show how the different noncoding RNAs influence cell proliferation. Green arrow represents a positive activity towards proliferation, while the red points out a negative effect on the process. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

The possibility of using ncRNAs as new therapeutic targets for cardiac diseases has produced a huge interest in the scientific community. With this in mind, in the last years, several clinical studies have been privately and publicly funded with the primary objective to identify distinct miRNA and lncRNA signatures in cardiac myectomies from cardiomyopathy patients (Table **3**). These studies will help to understand how tissue dysregulated RNAs influence both the clinical outcomes of the pathology and the response to pharmaceutical treatments. In addition, they will contribute to identifying new potential biomarkers which measurement in plasma samples will surely improve the diagnosis and prognosis of cardiomyopathies. Although very few data from preclinical studies are available so far, we expect to analyze much more data in the years to come given the interest in both cardiac and noncoding RNA research fields.

Table 3. Clinical Trials conducted for Cardiomyopathies (Disease) and ncRNAs (miRNAs and lncRNAs). Source: clinicaltrials.gov/Clinical Studies

Identifier (NCT number)	Status	Study Title	Locations
NCT03268135	*Recruiting*	Heart Failure and Aortic Stenosis Transcriptome	IRCCS Policlinico San Donato San Donato Milanese, Ospedale San Raffaele Milan, Italy
NCT02611336	*Recruiting*	Endocrine Cardiomyopathy: Response to Cyclic GMP PDE5 Inhibitors in Acromegaly Cardiomyopathy cardiomyogenic genes	Sapienza University of Rome, Policlinico Umberto I, Department of Experimental Medicine Rome, Italy
NCT02611258	*Recruiting*	Endocrine Cardiomyopathy in Cushing Syndrome: Response to Cyclic GMP PDE5 inhibitors	Sapienza University of Rome, Policlinico Umberto I, Department of Experimental Medicine Rome, Italy
NCT03356756	*Recruiting*	PET MRI Study in Patients With Cardiac Sarcoidosis	University Health Network Toronto, Ontario, Canada
NCT02149316	*Completed*	Remote Ischemic Preconditioning With Postconditioning in Heart Transplantation Surgery	State Key Laboratory of Cardiovascular Disease, Fuwai Hospital, National Center for Cardiovascular Diseases, CAMS and PUMC Beijing, China
NCT01798992	*Completed*	Effect of Beta-blockers on Structural Remodeling and Gene Expression in the Failing Human Heart	University of Colorado Hospital Denver, Colorado, United States University of Utah Medical Center Salt Lake City, Utah, United States
NCT02973594	*Recruiting*	Pulse Reduction On Beta-blocker and Ivabradine Therapy	University of Colorado Anschutz Medical Campus Aurora, Colorado, United States The Ohio State University Wexner Medical Center Columbus, Ohio, United States
NCT03076580	*Recruiting*	An Integrative-"Omics" Study of Cardiomyopathy Patients for Diagnosis and Prognosis in China	Beijing Institute of heart, lung and blood vessel diseases Beijing, Beijing, China Shijie You Beijing, China
NCT03838237	*Active, not recruiting*	Effect of Migalastat on Cardiac Involvement in Fabry Disease	IRCCS Policlinico San Donato San Donato Milanese, Milan, Italy
NCT03049254	*Recruiting*	Mayo AVC Registry and Biobank	Mayo Clinic Rochester, Minnesota, United States Royal Papworth Hospital NHS Foundation Trust Papworth Everard, Cambridge, United Kingdom

CONSENT FOR PUBLICATION

Not applicable.

FUNDING

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

This work was partially supported by grants from Sapienza University (prot. RM11715C7C8176C1) and FFABR Anvur (2017) to M.B.

REFERENCES

[1] Abugessaisa I, Noguchi S, Hasegawa A, *et al.* FANTOM5 CAGE profiles of human and mouse reprocessed for GRCh38 and GRCm38 genome assemblies. Sci Data 2017; 4170107 http://dx.doi.org/10.1038/sdata.2017.107 PMID: 28850105

[2] Carninci P, Kasukawa T, Katayama S, Gough J, Frith MC, Maeda N, *et al.* Molecular biology: The transcriptional landscape of the mammalian genome. Science 2005; (80): http://dx.doi.org/10.1126/science.1112014

[3] Beermann J, Piccoli M-T, Viereck J, Thum T. Non-coding RNAs in Development and Disease: Background, Mechanisms, and Therapeutic Approaches. Physiol Rev 2016; 96(4): 1297-325. http://dx.doi.org/10.1152/physrev.00041.2015 PMID: 27535639

[4] Ha M, Kim VN. Regulation of microRNA biogenesis. Nat Rev Mol Cell Biol 2014; 15(8): 509-24. http://dx.doi.org/10.1038/nrm3838 PMID: 25027649

[5] Jonas S, Izaurralde E. Towards a molecular understanding of microRNA-mediated gene silencing. Nat Rev Genet 2015; 16(7): 421-33. http://dx.doi.org/10.1038/nrg3965 PMID: 26077373

[6] Lujambio A, Lowe SW. The microcosmos of cancer. Nature 2012; 482(7385): 347-55. http://dx.doi.org/10.1038/nature10888 PMID: 22337054

[7] Pagano F, De Marinis E, Grignani F, Nervi C. Epigenetic role of miRNAs in normal and leukemic hematopoiesis. Epigenomics 2013; 5(5): 539-52. http://dx.doi.org/10.2217/epi.13.55 PMID: 24059800

[8] Small EM, Olson EN. Pervasive roles of microRNAs in cardiovascular biology. Nature 2011; 469(7330): 336-42. http://dx.doi.org/10.1038/nature09783 PMID: 21248840

[9] Rinn JL, Chang HY. Genome regulation by long noncoding RNAs. Annu Rev Biochem 2012; 81: 145-66. http://dx.doi.org/10.1146/annurev-biochem-051410-092902 PMID: 22663078

[10] Ulitsky I, Bartel DP. lincRNAs: Genomics, Evolution, and Mechanisms. Cell 2013; 154(1): 26-46.

[11] Fatica A, Bozzoni I. Long non-coding RNAs: new players in cell differentiation and development. Nat Rev Genet 2014; 15(1): 7-21. http://dx.doi.org/10.1038/nrg3606 PMID: 24296535

[12] Thum T, Condorelli G. Long noncoding RNAs and microRNAs in cardiovascular pathophysiology. Circ Res 2015; 116(4): 751-62. http://dx.doi.org/10.1161/CIRCRESAHA.116.303549 PMID: 25677521

[13] Kristensen LS, Andersen MS, Stagsted LVW, Ebbesen KK, Hansen TB, Kjems J. The biogenesis, biology and characterization of circular RNAs. Nat Rev Genet 2019; 20(11): 675-91. http://dx.doi.org/10.1038/s41576-019-0158-7 PMID: 31395983

[14] Patop IL, Wüst S, Kadener S. Past, present, and future of circRNAs. EMBO J 2019; 38(16)e100836 http://dx.doi.org/10.15252/embj.2018100836 PMID: 31343080

[15] Li M, Ding W, Sun T, *et al.* Biogenesis of circular RNAs and their roles in cardiovascular development and pathology. FEBS J 2018; 285(2): 220-32. http://dx.doi.org/10.1111/febs.14191 PMID: 28783257

[16] Zhou Q, Zhang Z, Bei Y, Li G, Wang T. Circular RNAs as Novel Biomarkers for Cardiovascular Diseases. Adv Exp Med Biol 2018; 1087: 159-70. http://dx.doi.org/10.1007/978-981-13-1426-1_13 PMID: 30259365

[17] Huang Y. The novel regulatory role of lncRNA-miRNA-mRNA axis in cardiovascular diseases. J Cell Mol Med 2018; 22(12): 5768-75. http://dx.doi.org/10.1111/jcmm.13866 PMID: 30188595

[18] Li M, Duan L, Li Y, Liu B. Long noncoding RNA/circular noncoding RNA-miRNA-mRNA axes in cardiovascular diseases. Life Sci 2019; 233116440 http://dx.doi.org/10.1016/j.lfs.2019.04.066 PMID: 31047893

[19] Pagano F, Picchio V, Chimenti I, *et al.* On the Road to Regeneration: "Tools" and "Routes" Towards Efficient Cardiac Cell Therapy for Ischemic Cardiomyopathy. Curr Cardiol Rep 2019; 21(11): 133. http://dx.doi.org/10.1007/s11886-019-1226-5 PMID: 31673821

[20] Pagano F, Picchio V, Angelini F, *et al.* The Biological Mechanisms of Action of Cardiac Progenitor Cell Therapy. Curr Cardiol Rep 2018; 20(10): 84. http://dx.doi.org/10.1007/s11886-018-1031-6 PMID: 30105430

[21] Wernly B, Mirna M, Rezar R, *et al.* Regenerative cardiovascular therapies: Stem cells and beyond. Int J Mol Sci 2019; 20(6): 1-13. http://dx.doi.org/10.3390/ijms20061420 PMID: 30901815

[22] Menasché P. Cell therapy trials for heart regeneration - lessons learned and future directions. Nat Rev Cardiol 2018; 15(11): 659-71. http://dx.doi.org/10.1038/s41569-018-0013-0 PMID: 29743563

[23] Fernández-Avilés F, Sanz-Ruiz R, Climent AM, *et al.* TACTICS (Transnational Alliance for Regenerative Therapies in Cardiovascular Syndromes) Writing Group; Authors/Task Force Members. Chairpersons; Basic Research Subcommittee; Translational Research Subcommittee; Challenges of Cardiovascular Regenerative Medicine Subcommittee; Tissue Engineering Subcommittee; Delivery, Navigation, Tracking and Assessment Subcommittee; Clinical Trials Subcommittee; Regulatory and funding strategies subcommittee; Delivery, Navigation, Tracking and Assessment Subcommittee. Global position paper on cardiovascular regenerative medicine. Eur Heart J 2017; 38(33): 2532-46. http://dx.doi.org/10.1093/eurheartj/ehx248 PMID: 28575280

[24] Marbán E. A mechanistic roadmap for the clinical application of cardiac cell therapies. Nat Biomed Eng 2018; 2(6): 353-61. http://dx.doi.org/10.1038/s41551-018-0216-z PMID: 30740264

[25] Chen C, Termglinchan V, Karakikes I. Concise Review: Mending a Broken Heart: The Evolution of Biological Therapeutics. Stem Cells 2017; 35(5): 1131-40. http://dx.doi.org/10.1002/stem.2602 PMID: 28233392

[26] Micheu MM, Scarlatescu AI, Scafa-Udriste A, Dorobantu M. The Winding Road of Cardiac Regeneration-Stem Cell Omics in the Spotlight. Cells 2018; 7(12): 255. http://dx.doi.org/10.3390/cells7120255 PMID: 30544622

[27] Arnone B, Chen JY, Qin G. Characterization and analysis of long non-coding rna (lncRNA) in *In vitro*- and Ex Vivo-derived cardiac progenitor cells. PLoS One 2017; 12(6)e0180096 http://dx.doi.org/10.1371/journal.pone.0180096 PMID: 28640894

[28] Thum T. Translational Opportunities and Challenges of Long Noncoding RNAs in Cardiac Regeneration. J Am Coll Cardiol 2018; 72(5): 551-2. http://dx.doi.org/10.1016/j.jacc.2018.05.039 PMID: 29945752

[29] Saxena A, Tabin CJ. miRNA-processing enzyme Dicer is necessary for cardiac outflow tract alignment and chamber septation. Proc Natl Acad Sci USA 2010; 107(1): 87-91. http://dx.doi.org/10.1073/pnas.0912870107 PMID: 20018673

[30] Chen J-F, Murchison EP, Tang R, *et al.* Targeted deletion of Dicer in the heart leads to dilated cardiomyopathy and heart failure. Proc Natl Acad Sci USA 2008; 105(6): 2111-6. http://dx.doi.org/10.1073/pnas.0710228105 PMID: 18256189

[31] da Costa Martins PA, Bourajjaj M, Gladka M, *et al.* Conditional *dicer* gene deletion in the postnatal myocardium provokes spontaneous cardiac remodeling. Circulation 2008; 118(15): 1567-76. http://dx.doi.org/10.1161/CIRCULATIONAHA.108.769984 PMID: 18809798

[32] Kalsotra A, Wang K, Li PF, Cooper TA. MicroRNAs coordinate an alternative splicing network during mouse postnatal heart development. Genes Dev 2010; 24(7): 653-8. http://dx.doi.org/10.1101/gad.1894310 PMID: 20299448

[33] Ivey KN, Muth A, Arnold J, *et al.* MicroRNA regulation of cell lineages in mouse and human embryonic stem cells. Cell Stem Cell 2008; 2(3): 219-29. http://dx.doi.org/10.1016/j.stem.2008.01.016 PMID: 18371447

[34] Liu N, Bezprozvannaya S, Williams AH, *et al.* microRNA-133a regulates cardiomyocyte proliferation and suppresses smooth muscle gene expression in the heart. Genes Dev 2008; 22(23): 3242-54. http://dx.doi.org/10.1101/gad.1738708 PMID: 19015276

[35] Fuller AM, Qian L. MiRiad Roles for MicroRNAs in Cardiac Development and Regeneration. Cells 2014; 3(3): 724-50. http://dx.doi.org/10.3390/cells3030724 PMID: 25055156

[36] Wang J, Greene SB, Bonilla-Claudio M, *et al.* Bmp signaling regulates myocardial differentiation from cardiac progenitors through a MicroRNA-mediated mechanism. Dev Cell 2010; 19(6): 903-12. http://dx.doi.org/10.1016/j.devcel.2010.10.022 PMID: 21145505

[37] Dornian IJ, Chiravuri M, Meer VDP, Feinberg AW, Shi X, Shao Y, *et al.* Generation of functional ventricular heart muscle from mouse ventricular progenitor cells. Science 2009; (80):

[38] England J, Loughna S. Heavy and light roles: myosin in the morphogenesis of the heart. Cell Mol Life Sci 2013; 70(7): 1221-39. http://dx.doi.org/10.1007/s00018-012-1131-1 PMID: 22955375

[39] Porrello ER, Johnson BA, Aurora AB, *et al.* MiR-15 family regulates postnatal mitotic arrest of cardiomyocytes. Circ Res 2011; 109(6): 670-9. http://dx.doi.org/10.1161/CIRCRESAHA.111.248880 PMID: 21778430

[40] Porrello ER, Mahmoud AI, Simpson E, Hill JA, Richardson JA, Olson EN, *et al.* Transient regenerative potential of the neonatal mouse heart. Science 2011; (80): http://dx.doi.org/10.1126/science.1200708

[41] Heallen TR, Kadow ZA, Kim JH, Wang J, Martin JF. Stimulating Cardiogenesis as a Treatment for Heart Failure. Circ Res 2019; 124(11): 1647-57. http://dx.doi.org/10.1161/CIRCRESAHA.118.313573 PMID: 31120819

[42] Katz MG, Fargnoli AS, Kendle AP, Hajjar RJ, Bridges CR. The role of microRNAs in cardiac development and regenerative capacity. Am J Physiol - Hear Circ Physiol 2016; 310: H528-41. http://dx.doi.org/10.1152/ajpheart.00181.2015

[43] Leach JP, Martin JF. Cardiomyocyte Proliferation for Therapeutic Regeneration. Curr Cardiol Rep 2018; 20(8): 63. http://dx.doi.org/10.1007/s11886-018-1011-x PMID: 29904823

[44] Eulalio A, Mano M, Dal Ferro M, *et al.* Functional screening identifies miRNAs inducing cardiac regeneration. Nature 2012; 492(7429): 376-81. http://dx.doi.org/10.1038/nature11739 PMID: 23222520

[45] Tian Y, Liu Y, Wang T, *et al.* A microRNA-Hippo pathway that promotes cardiomyocyte proliferation and cardiac regeneration in mice. Sci Transl Med 2015; 7(279)279ra38 http://dx.doi.org/10.1126/scitranslmed.3010841 PMID: 25787764

[46] Torrini C, Cubero RJ, Dirkx E, *et al.* Common Regulatory Pathways Mediate Activity of MicroRNAs Inducing Cardiomyocyte Proliferation. Cell Rep 2019; 27(9): 2759-2771.e5. http://dx.doi.org/10.1016/j.celrep.2019.05.005 PMID: 31141697

[47] Gabisonia K, Prosdocimo G, Aquaro GD, *et al.* MicroRNA therapy stimulates uncontrolled cardiac repair after myocardial infarction in pigs. Nature 2019; 569(7756): 418-22. http://dx.doi.org/10.1038/s41586-019-1191-6 PMID: 31068698

[48] Wang J, Liu S, Heallen T, Martin JF. The Hippo pathway in the heart: pivotal roles in development, disease, and regeneration. Nat Rev Cardiol 2018; 15(11): 672-84. http://dx.doi.org/10.1038/s41569-018-0063-3 PMID: 30111784

[49] Tao Y, Zhang H, Huang S, *et al.* miR-199a-3p promotes cardiomyocyte proliferation by inhibiting Cd151 expression. Biochem Biophys Res Commun 2019; 516(1): 28-36. http://dx.doi.org/10.1016/j.bbrc.2019.05.174 PMID: 31186138

[50] Pandey R, Velasquez S, Durrani S, *et al.* MicroRNA-1825 induces proliferation of adult cardiomyocytes and promotes cardiac regeneration post ischemic injury. Am J Transl Res 2017; 9(6): 3120-37. PMID: 28670398

[51] Porrello ER, Mahmoud AI, Simpson E, *et al.* Regulation of neonatal and adult mammalian heart regeneration by the miR-15 family. Proc Natl Acad Sci USA 2013; 110(1): 187-92. http://dx.doi.org/10.1073/pnas.1208863110 PMID: 23248315

[52] Hullinger TG, Montgomery RL, Seto AG, *et al.* Inhibition of miR-15 protects against cardiac ischemic injury. Circ Res 2012; 110(1): 71-81. http://dx.doi.org/10.1161/CIRCRESAHA.111.244442 PMID: 22052914

[53] Stanley-Hasnain S, Hauck L, Grothe D, *et al.* p53 and Mdm2 act synergistically to maintain cardiac homeostasis and mediate cardiomyocyte cell cycle arrest through a network of microRNAs. Cell Cycle 2017; 16(17): 1585-600. http://dx.doi.org/10.1080/15384101.2017.1346758 PMID: 28745540

[54] Cao X, Wang J, Wang Z, *et al.* MicroRNA profiling during rat ventricular maturation: A role for miR-29a in regulating cardiomyocyte cell cycle re-entry. FEBS Lett 2013; 587(10): 1548-55. http://dx.doi.org/10.1016/j.febslet.2013.01.075 PMID: 23587482

[55] Pipes GCT, Creemers EE, Olson EN. The myocardin family of transcriptional coactivators: versatile regulators of cell growth, migration, and myogenesis. Genes Dev 2006; 20(12): 1545-56. http://dx.doi.org/10.1101/gad.1428006 PMID: 16778073

[56] Huang W, Feng Y, Liang J, *et al.* Loss of microRNA-128 promotes cardiomyocyte proliferation and heart regeneration. Nat Commun 2018; 9(1): 700. http://dx.doi.org/10.1038/s41467-018-03019-z PMID: 29453456

[57] Yang Y, Cheng HW, Qiu Y, *et al.* MicroRNA-34a Plays a Key Role in Cardiac Repair and Regeneration Following Myocardial Infarction. Circ Res 2015; 117(5): 450-9. http://dx.doi.org/10.1161/CIRCRESAHA.117.305962 PMID: 26082557

[58] Xiao J, Liu H, Cretoiu D, *et al.* miR-31a-5p promotes postnatal cardiomyocyte proliferation by targeting RhoBTB1. Exp Mol Med 2017; 49(10)e386 http://dx.doi.org/10.1038/emm.2017.150 PMID: 29053138

[59] Barile L, Milano G, Vassalli G. Beneficial effects of exosomes secreted by cardiac-derived progenitor cells and other cell types in myocardial ischemia. Stem Cell Investig 2017; 4: 93. http://dx.doi.org/10.21037/sci.2017.11.06 PMID: 29270419

[60] Sluijter JPGG, van Mil A, van Vliet P, *et al.* MicroRNA-1 and -499 regulate differentiation and proliferation in human-derived cardiomyocyte progenitor cells. Arterioscler Thromb Vasc Biol 2010; 30(4): 859-68. http://dx.doi.org/10.1161/ATVBAHA.109.197434 PMID: 20081117

[61] Hosoda T, Zheng H, Cabral-da-Silva M, *et al.* Human cardiac stem cell differentiation is regulated by a mircrine mechanism. Circulation 2011; 123(12): 1287-96. http://dx.doi.org/10.1161/CIRCULATIONAHA.110.982918 PMID: 21403094

[62] Sirish P, López JE, Li N, *et al.* MicroRNA profiling predicts a variance in the proliferative potential of cardiac progenitor cells derived from neonatal and adult murine hearts. J Mol Cell Cardiol 2012; 52(1): 264-72. http://dx.doi.org/10.1016/j.yjmcc.2011.10.012 PMID: 22062954

[63] Brás-Rosário L, Matsuda A, Pinheiro AI, *et al.* Expression profile of microRNAs regulating proliferation and differentiation in mouse adult cardiac stem cells. PLoS One 2013; 8(5)e63041 http://dx.doi.org/10.1371/journal.pone.0063041 PMID: 23690977

[64] Izarra A, Moscoso I, Levent E, *et al.* miR-133a enhances the protective capacity of cardiac progenitors cells after myocardial infarction. Stem Cell Reports 2014; 3(6): 1029-42. http://dx.doi.org/10.1016/j.stemcr.2014.10.010 PMID: 25465869

[65] Sun Y, Xu R, Huang J, *et al.* Insulin-like growth factor-1-mediated regulation of miR-193a expression promotes the migration and proliferation of c-kit-positive mouse cardiac stem cells. Stem Cell Res Ther 2018; 9(1): 41. http://dx.doi.org/10.1186/s13287-017-0762-4 PMID: 29467020

[66] Hu S, Huang M, Nguyen PK, *et al.* Novel microRNA prosurvival cocktail for improving engraftment and function of cardiac progenitor cell transplantation. Circulation 2011; 124(11)(Suppl.): S27-34. http://dx.doi.org/10.1161/CIRCULATIONAHA.111.017954 PMID: 21911815

[67] Pagano F, Angelini F, Siciliano C, *et al.* Beta2-adrenergic signaling affects the phenotype of human cardiac progenitor cells through EMT modulation. Pharmacol Res 2018; 127: 41-8. http://dx.doi.org/10.1016/j.phrs.2017.01.016 PMID: 28099883

[68] Barile L, Lionetti V, Cervio E, *et al.* Extracellular vesicles from human cardiac progenitor cells inhibit cardiomyocyte apoptosis and improve cardiac function after myocardial infarction. Cardiovasc Res 2014; 103(4): 530-41. http://dx.doi.org/10.1093/cvr/cvu167 PMID: 25016614

[69] Ibrahim AG-E, Cheng K, Marbán E. Exosomes as critical agents of cardiac regeneration triggered by cell therapy. Stem Cell Reports 2014; 2(5): 606-19. http://dx.doi.org/10.1016/j.stemcr.2014.04.006 PMID: 24936449

[70] Wang L, Jia Q, Xinnong C, *et al.* Role of cardiac progenitor cell-derived exosome-mediated microRNA-210 in cardiovascular disease. J Cell Mol Med 2019; 23(11): 7124-31. http://dx.doi.org/10.1111/jcmm.14562 PMID: 31557390

[71] Pan J-A, Tang Y, Yu J-Y, *et al.* miR-146a attenuates apoptosis and modulates autophagy by targeting TAF9b/P53 pathway in doxorubicin-induced cardiotoxicity. Cell Death Dis 2019; 10(9): 668. http://dx.doi.org/10.1038/s41419-019-1901-x PMID: 31511497

[72] Huang W, Tian S-S, Hang P-Z, Sun C, Guo J, Du Z-M. Combination of microRNA-21 and microRNA-146a Attenuates Cardiac

Dysfunction and Apoptosis During Acute Myocardial Infarction in Mice. Mol Ther Nucleic Acids 2016; 5e296
http://dx.doi.org/10.1038/mtna.2016.12 PMID: 26978580

[73] Engreitz JM, Ollikainen N, Guttman M. Long non-coding RNAs: spatial amplifiers that control nuclear structure and gene expression. Nat Rev Mol Cell Biol 2016; 17(12): 756-70.
http://dx.doi.org/10.1038/nrm.2016.126 PMID: 27780979

[74] Ribeiro DM, Zanzoni A, Cipriano A, *et al.* Protein complex scaffolding predicted as a prevalent function of long non-coding RNAs. Nucleic Acids Res 2018; 46(2): 917-28.
http://dx.doi.org/10.1093/nar/gkx1169 PMID: 29165713

[75] Klattenhoff CA, Scheuermann JC, Surface LE, *et al.* Braveheart, a long noncoding RNA required for cardiovascular lineage commitment. Cell 2013; 152(3): 570-83.
http://dx.doi.org/10.1016/j.cell.2013.01.003 PMID: 23352431

[76] Grote P, Wittler L, Hendrix D, *et al.* The tissue-specific lncRNA Fendrr is an essential regulator of heart and body wall development in the mouse. Dev Cell 2013; 24(2): 206-14.
http://dx.doi.org/10.1016/j.devcel.2012.12.012 PMID: 23369715

[77] Engreitz JM, Haines JE, Perez EM, *et al.* Local regulation of gene expression by lncRNA promoters, transcription and splicing. Nature 2016; 539(7629): 452-5.
http://dx.doi.org/10.1038/nature20149 PMID: 27783602

[78] Guo X, Xu Y, Wang Z, *et al.* A Linc1405/Eomes Complex Promotes Cardiac Mesoderm Specification and Cardiogenesis. Cell Stem Cell 2018; 22(6): 893-908.e6.
http://dx.doi.org/10.1016/j.stem.2018.04.013 PMID: 29754779

[79] Alexanian M, Maric D, Jenkinson SP, *et al.* A transcribed enhancer dictates mesendoderm specification in pluripotency. Nat Commun 2017; 8(1): 1806.
http://dx.doi.org/10.1038/s41467-017-01804-w PMID: 29180618

[80] Ounzain S, Pezzuto I, Micheletti R, *et al.* Functional importance of cardiac enhancer-associated noncoding RNAs in heart development and disease. J Mol Cell Cardiol 2014; 76: 55-70.
http://dx.doi.org/10.1016/j.yjmcc.2014.08.009 PMID: 25149110

[81] Ounzain S, Micheletti R, Arnan C, *et al.* CARMEN, a human super enhancer-associated long noncoding RNA controlling cardiac specification, differentiation and homeostasis. J Mol Cell Cardiol 2015; 89(Pt A): 98-112.
http://dx.doi.org/10.1016/j.yjmcc.2015.09.016 PMID: 26423156

[82] Kurian L, Aguirre A, Sancho-Martinez I, *et al.* Identification of novel long noncoding RNAs underlying vertebrate cardiovascular development. Circulation 2015; 131(14): 1278-90.
http://dx.doi.org/10.1161/CIRCULATIONAHA.114.013303 PMID: 25739401

[83] Wang K, Liu F, Zhou LY, *et al.* The long noncoding RNA CHRF regulates cardiac hypertrophy by targeting miR-489. Circ Res 2014; 114(9): 1377-88.
http://dx.doi.org/10.1161/CIRCRESAHA.114.302476 PMID: 24557880

[84] Bell RD, Long X, Lin M, *et al.* Identification and initial functional characterization of a human vascular cell-enriched long noncoding RNA. Arterioscler Thromb Vasc Biol 2014; 34(6): 1249-59.
http://dx.doi.org/10.1161/ATVBAHA.114.303240 PMID: 24578380

[85] Han P, Li W, Lin C-HC-JCHC-YCJCY, *et al.* A long noncoding RNA protects the heart from pathological hypertrophy. Nature 2014; 514(7520): 102-6.
http://dx.doi.org/10.1038/nature13596 PMID: 25119045

[86] Wang Z, Zhang XJ, Ji YX, *et al.* The long noncoding RNA Chaer defines an epigenetic checkpoint in cardiac hypertrophy. Nat Med 2016; 22(10): 1131-9.
http://dx.doi.org/10.1038/nm.4179 PMID: 27618650

[87] Viereck J, Kumarswamy R, Foinquinos A, *et al.* Long noncoding RNA Chast promotes cardiac remodeling. Sci Transl Med 2016; 8(326)326ra22
http://dx.doi.org/10.1126/scitranslmed.aaf1475 PMID: 26888430

[88] Ballarino M, Cipriano A, Tita R, *et al.* Deficiency in the nuclear long noncoding RNA *Charme* causes myogenic defects and heart remodeling in mice. EMBO J 2018; 37(18)e99697
http://dx.doi.org/10.15252/embj.201899697 PMID: 30177572

[89] Ponnusamy M, Liu F, Zhang YH, *et al.* Long Noncoding RNA CPR (Cardiomyocyte Proliferation Regulator) Regulates Cardiomyocyte Proliferation and Cardiac Repair. Circulation 2019; 139(23): 2668-84.
http://dx.doi.org/10.1161/CIRCULATIONAHA.118.035832 PMID: 30832495

[90] Hung T, Wang Y, Lin MF, *et al.* Extensive and coordinated transcription of noncoding RNAs within cell-cycle promoters. Nat Genet 2011; 43(7): 621-9.
http://dx.doi.org/10.1038/ng.848 PMID: 21642992

[91] Ponjavic J, Oliver PL, Lunter G, Ponting CP. Genomic and transcriptional co-localization of protein-coding and long non-coding RNA pairs in the developing brain. PLoS Genet 2009; 5(8)e1000617
http://dx.doi.org/10.1371/journal.pgen.1000617 PMID: 19696892

[92] Luo S, Lu JY, Liu L, *et al.* Divergent lncRNAs regulate gene expression and lineage differentiation in pluripotent cells. Cell Stem Cell 2016; 18(5): 637-52.
http://dx.doi.org/10.1016/j.stem.2016.01.024 PMID: 26996597

[93] Anderson KM, Anderson DM, McAnally JR, Shelton JM, Bassel-Duby R, Olson EN. Transcription of the non-coding RNA upperhand controls Hand2 expression and heart development. Nature 2016; 539(7629): 433-6.
http://dx.doi.org/10.1038/nature20128 PMID: 27783597

[94] Han X, Zhang J, Liu Y, *et al.* The lncRNA *Hand2os1*/*Uph* locus orchestrates heart development through regulation of precise expression of *Hand2*. Development 2019; 146(13)dev176198
http://dx.doi.org/10.1242/dev.176198 PMID: 31273086

[95] Hori Y, Tanimoto Y, Takahashi S, Furukawa T, Koshiba-Takeuchi K, Takeuchi JK. Important cardiac transcription factor genes are accompanied by bidirectional long non-coding RNAs. BMC Genomics 2018; 19(1): 967.
http://dx.doi.org/10.1186/s12864-018-5233-5 PMID: 30587117

[96] Frank S, Ahuja G, Bartsch D, Russ N, Yao W, Kuo JCC, *et al.* yylncT Defines a Class of Divergently Transcribed lncRNAs and Safeguards the T-mediated Mesodermal Commitment of Human PSCs. Cell Stem Cell 2019; 24: 318-327.e.8.

[97] Liu J, Li Y, Lin B, Sheng Y, Yang L. HBL1 Is a Human Long Noncoding RNA that Modulates Cardiomyocyte Development from Pluripotent Stem Cells by Counteracting MIR1. Dev Cell 2017; 42(4): 333-348.e5.
http://dx.doi.org/10.1016/j.devcel.2017.07.023 PMID: 28829943

[98] Cai B, Ma W, Ding F, *et al.* The Long Noncoding RNA CAREL Controls Cardiac Regeneration. J Am Coll Cardiol 2018; 72(5): 534-50.
http://dx.doi.org/10.1016/j.jacc.2018.04.085 PMID: 30056829

[99] Chen G, Li H, Li X, *et al.* Loss of long non-coding RNA CRRL promotes cardiomyocyte regeneration and improves cardiac repair by functioning as a competing endogenous RNA. J Mol Cell Cardiol 2018; 122: 152-64.
http://dx.doi.org/10.1016/j.yjmcc.2018.08.013 PMID: 30125571

[100] Wang J, Chen X, Shen D, *et al.* A long noncoding RNA NR_045363 controls cardiomyocyte proliferation and cardiac repair. J Mol Cell Cardiol 2019; 127: 105-14.
http://dx.doi.org/10.1016/j.yjmcc.2018.12.005 PMID: 30553885

[101] Chen Y, Li X, Li B, *et al.* Long Non-coding RNA ECRAR Triggers Post-natal Myocardial Regeneration by Activating ERK1/2 Signaling. Mol Ther 2019; 27(1): 29-45.
http://dx.doi.org/10.1016/j.ymthe.2018.10.021 PMID: 30528086

[102] Yin G, Yang X, Li Q, Guo Z. GATA1 activated lncRNA (Galont) promotes anoxia/reoxygenation-induced autophagy and cell death in cardiomyocytes by sponging miR-338. J Cell Biochem 2018; 119(5): 4161-9.
http://dx.doi.org/10.1002/jcb.26623 PMID: 29247537

[103] Zhang N, Meng X, Mei L, Hu J, Zhao C, Chen W. The Long Non-Coding RNA SNHG1 Attenuates Cell Apoptosis by Regulating miR-195 and BCL2-Like Protein 2 in Human Cardiomyocytes. Cell Physiol Biochem 2018; 50(3): 1029-40.
http://dx.doi.org/10.1159/000494514 PMID: 30355909

[104] Luo X, He S, Hu Y, Liu J, Chen X. Sp1-induced LncRNA CTBP1-AS2 is a novel regulator in cardiomyocyte hypertrophy by interacting with FUS to stabilize TLR4. Cardiovasc Pathol 2019; 42: 21-9.
http://dx.doi.org/10.1016/j.carpath.2019.04.005 PMID: 31220774

[105] Lim TB, Aliwarga E, Luu TDA, *et al.* Targeting the highly abundant circular RNA circSlc8a1 in cardiomyocytes attenuates pressure

overload induced hypertrophy. Cardiovasc Res 2019; 115(14): 1998-2007.
http://dx.doi.org/10.1093/cvr/cvz130 PMID: 31114845

[106] Li M, Ding W, Tariq MA, *et al.* A circular transcript of *ncx1* gene mediates ischemic myocardial injury by targeting miR-133a-3p. Theranostics 2018; 8(21): 5855-69.
http://dx.doi.org/10.7150/thno.27285 PMID: 30613267

[107] Newman LE, Schiavon CR, Zhou C, Kahn RA. The abundance of the ARL2 GTPase and its GAP, ELMOD2, at mitochondria are modulated by the fusogenic activity of mitofusins and stressors. PLoS One 2017; 12(4)e0175164
http://dx.doi.org/10.1371/journal.pone.0175164 PMID: 28380071

[108] Cai L, Qi B, Wu X, *et al.* Circular RNA Ttc3 regulates cardiac function after myocardial infarction by sponging miR-15b. J Mol Cell Cardiol 2019; 130: 10-22.
http://dx.doi.org/10.1016/j.yjmcc.2019.03.007 PMID: 30876857

[109] Ni H, Li W, Zhuge Y, *et al.* Inhibition of circHIPK3 prevents angiotensin II-induced cardiac fibrosis by sponging miR-29b-3p. Int J Cardiol 2019; 292: 188-96.
http://dx.doi.org/10.1016/j.ijcard.2019.04.006 PMID: 30967276

[110] Huang S, Li X, Zheng H, *et al.* Loss of Super-Enhancer-Regulated circRNA Nfix Induces Cardiac Regeneration After Myocardial Infarction in Adult Mice. Circulation 2019; 139(25): 2857-76.
http://dx.doi.org/10.1161/CIRCULATIONAHA.118.038361 PMID: 30947518

[111] Zeng Y, Du WW, Wu Y, *et al.* A circular RNA binds to and activates AKT phosphorylation and nuclear localization reducing apoptosis and enhancing cardiac repair. Theranostics 2017; 7(16): 3842-55.
http://dx.doi.org/10.7150/thno.19764 PMID: 29109781

[112] Machaj F, Dembowska E, Rosik J, Szostak B, Mazurek-Mochol M, Pawlik A. New therapies for the treatment of heart failure: a summary of recent accomplishments. Ther Clin Risk Manag 2019; 15: 147-55.
http://dx.doi.org/10.2147/TCRM.S179302 PMID: 30774351

[113] Kieserman JM, Myers VD, Dubey P, Cheung JY, Feldman AM. Current Landscape of Heart Failure Gene Therapy. J Am Heart Assoc 2019; 8(10)e012239
http://dx.doi.org/10.1161/JAHA.119.012239 PMID: 31070089

[114] Gabisonia K, Recchia FA. Gene Therapy for Heart Failure: New Perspectives. Curr Heart Fail Rep 2018; 15(6): 340-9.
http://dx.doi.org/10.1007/s11897-018-0410-z PMID: 30238397

[115] Leisegang MS, Schröder K, Brandes RP. Redox Regulation and Noncoding RNAs. Antioxid Redox Signal 2018; 29(9): 793-812.
http://dx.doi.org/10.1089/ars.2017.7276 PMID: 28816061

[116] Wang X, Shen C, Zhu J, Shen G, Li Z, Dong J. Long Noncoding RNAs in the Regulation of Oxidative Stress. Oxid Med Cell Longev 2019; 20191318795
http://dx.doi.org/10.1155/2019/1318795 PMID: 30911342

[117] Angelini F, Pagano F, Bordin A, *et al.* The Impact of Environmental Factors in Influencing Epigenetics Related to Oxidative States in the Cardiovascular System. Oxid Med Cell Longev 2017; 20172712751
http://dx.doi.org/10.1155/2017/2712751 PMID: 28607629