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Exploring the role non-coding RNAs during myocardial cell fate

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Abstract

Myocardial cell fate specification takes place during the early stages of heart development as the precardiac mesoderm is configured into two symmetrical sets of bilateral precursor cells. Molecular cues of the surrounding tissues specify and subsequently determine the early cardiomyocytes, that finally matured as the heart is completed at early postnatal stages. Over the last decade, we have greatly enhanced our understanding of the transcriptional regulation of cardiac development and thus of myocardial cell fate. The recent discovery of a novel layer of gene regulation by non-coding RNAs has flourished their implication in epigenetic, transcriptional and post-transcriptional regulation of cardiac development. In this review, we revised the current state-of-the-art knowledge on the functional role of non-coding RNAs during myocardial cell fate.

List of abbreviations

α-SMA	alpha smooth muscle actin
Akt	Protein kinase B
β-catenin	Catenin Beta 1
BMP2	Bone morphogenetic protein 2
Brachyury	T-Box Transcription Factor T
Cacna1c	Calcium Voltage-Gated Channel Subunit Alpha1 C
CARMA	CARdiomyocyte Maturation-Associated lncRNA
Cd31	Platelet And Endothelial Cell Adhesion Molecule 1
CMs	Cardiomyocytes
cTnT	cardiac troponin T
Cx43	Gap Junction Protein Alpha 1
DGCR8	DGCR8 Microprocessor Complex Subunit
Dicer	Dicer 1, Ribonuclease III
Drosha	Drosha Ribonuclease III
EB	embyoid body
Eomes	Eomesodermin
Erk1	Mitogen-Activated Protein Kinase 2
Erk2	Mitogen-Activated Protein Kinase 3
ESCs	embryonic stem cells
Ets2	ETS Proto-Oncogene 2, Transcription Factor
FGF8	Fibroblast growth factor 8
Flk1	Kinase Insert Domain Receptor
Frs2	Fibroblast Growth Factor Receptor Substrate 2
Fzd7	Frizzled Class Receptor 7
Gata4	GATA Binding Protein 4
Gata6	GATA Binding Protein 6
Gcn5	Lysine Acetyltransferase 2
GSK3β	Glycogen Synthase Kinase 3 Beta
Hand2	Heart And Neural Crest Derivatives Expressed 2
HBL1	heart brake lncRNA 1
Hey1	Hairy-Related Transcription Factor 1
Hey2	Hairy-Related Transcription Factor 2
IPSCs	induced pluripotent stem cells
Irx4	Iroquois-class homeodomain protein IRX-4
Isl1	ISL LIM Homeobox 1
Kcnj2	Potassium Inwardly Rectifying Channel Subfamily J Member 2
Lin-28	Lin-28 Homolog A
lncRNA	long non coding RNA
Mef2c	Myocyte enhancer factor 2C
Meis2	Meis Homeobox 2
Mesp1	Mesoderm posterior bHLH transcription factor 1
Mesp2	Mesoderm posterior bHLH transcription factor 2
Myh6	Myosin Heavy Chain 6
Myh7	Myosin Heavy Chain 7
Nkx2.5	NK2 homeobox 5

Notch1	Notch Receptor 1
Oct4	Octamer-binding transcription factor 4
Pik3	Phosphatidylinositol-4,5-bisphosphate 3-kinase
pre-miRNAs	Precursor miRNAs
pri-miRNAs	Primary miRNAs
Ran	RAN, Member RAS Oncogene Family
RISC	RNA-induced silencing complex
Scn5a	Sodium Voltage-Gated Channel Alpha Subunit 5
SHF	Secondary heart field (SHF)
Shox2	SHOX Homeobox 2
Smad3	SMAD Family Member 3
Sox2	SRY-Box Transcription Factor 2
Srf	Serum Response Factor
Suz12	SUZ12 Polycomb Repressive Complex 2 Subunit
Tbx18	T-Box Transcription Factor 18
Tbx2	T-Box Transcription Factor 2
Tbx3	T-Box Transcription Factor 3
Tbx5	T-Box Transcription Factor 5
Tgf-□	transforming growth factor-□
Tgfbr1	Transforming Growth Factor Beta Receptor 1
Tie1	Tyrosine Kinase With Immunoglobulin Like And EGF Like Domains 1
vWF	Von Willebrand Factor
Wnt	wingless related integration site factor
Wrd5	WD Repeat Domain 5
Xpo5	Exportin 5

Background

Myocardial cell fate specification takes place during the early stages of heart development as the precardiac mesoderm is configured into two symmetrical sets of bilateral precursor cells, i.e. the cardiac crescents [1-3]. Soon thereafter, molecular cues of the surrounding embryonic tissue specify and subsequently determine the early cardiomyocytes [1]. Following myocardial cell fate specification, the early cardiomyocytes exhibit a poorly organized cardiac action potential and sarcomere apparatus. As the heart develops, differentiation of the cardiomyocytes takes place, characterized by a complex and dynamic expression of distinct sarcomeric proteins, particularly actin and myosin isoforms [2] as well as distinct ion channels [3] and gap junctional proteins [5-6]. This differentiation leads to the formation of three main different types of cardiomyocytes; atrial, ventricular and nodal. Atrial cardiomyocytes are characterized by fast-contracting properties as compared to ventricular cardiomyocytes both nonetheless with poor electrical automaticity, while nodal cardiomyocytes are characterized by spontaneous beating [7]. Cell-cell coupling is an additional characteristic of fully mature cardiomyocytes, mediated by distinct connexins, that display a highly orchestrated spatial distribution within the different cardiac chambers [8].

Cardiovascular diseases are the main cause of death worldwide, according to the World Health Organization. Notably, heart failure highly contributes to such burden, affecting approximately 1-2% of the adult population [9]. Heart failure is a heterogeneous clinical entity caused by cardiac overload and injury that leads to considerable morbidity and mortality. Myocardial infarction is also a leading cause of cardiovascular disability [10] given the incapacity of the heart to regenerate the damaged myocardium. In both cases, although there are palliative therapeutic approaches, the ultimate solution is heart transplant. Unfortunately, the limited availability of donor´s hearts, coupled with the immune compatibility constrains and the lifelong immunosuppression requirements, restrains heart transplantation as a global therapeutic approach.

Over the last decades, we have witnessed increasing novel approaches to regenerate the damaged heart, particularly those addressing cardiomyocytes renewal during myocardial ischemia. Embryonic stem cells (ESCs), and more recently induced pluripotent stem cells (iPSCs) have been harnessed to undergo into differentiated cardiomyocytes, providing their plausible use in cell therapy, drug discovery and disease modelling as well as novel tools to dissect the regulatory roles that govern cardiomyocyte maturation [11-13]. While it is widely acclaimed that several growth factors such as Bmp, Fgf and Wnt family members play a pivotal role in early phases of cardiomyocyte specification, a core set of transcription factors such as *Mesp1/Mesp2*, *Nkx2.5, Gata4, Srf* and *Mef2c*, are deemed essential for both the early phases of cardiomyocyte specification and determination [14-15], processes that are highly

conserved in evolution [16]. Importantly, the acquisition of distinct atrial, ventricular and nodal characteristics is subsequently orchestrated by Notch regulated *Hey1* and *Hey2* transcription factors [17] as well as by distinct T-box family members, particularly *Tbx2, Tbx3* and *Tbx5*, among others [18-19]. Recently, a novel layer of gene regulation has emerged with the discovery of distinct classes of non-coding RNAs with functional capabilities. Non-coding RNAs are broadly classified according to their transcript length into small non-coding RNAs (<200nt) and long non-coding RNAs (>200nt) [20-21]. Among small non-coding RNAs, and besides tRNAs and rRNAs that have been throughly characterized for many years, microRNAs constitute the most abundant and well-characterized subtype [22-23]. The biogenesis of microRNAs has been studied in detail over the last decades. Most microRNAs are transcribed by polymerase II into primary miRNAs (pri-miRNAs) and then processed to precursor miRNAs (pre-miRNAs) by the microprocessor complex DGCR8/Drosha. Once pre-miRNAs are generated, they are, exported to the cytoplasm by Xpo5/RanGTP complex, matured by Dicer endonuclease and subsequently loaded into the RISC complex, where one of the strands will be degraded [22-23]. The remaining strand will scan for target molecules by base pair complementarity, leading in most cases to RNA instability and/or translational blockage [22-23]. On the other hand, long non-coding RNAs exhibit wide diversity and can be categorized based on their genomic context or their functional capacities [24-25]. LncRNAs can exert distinct and multiple actions, ranging from epigenetic modulation to transcriptional and post-transcriptional regulation [24-25].

It is important to highlight in this context that both microRNAs and lncRNAs are differentially expressed in distinct developmental and adult tissues, thereby contributing therefore to proper cellular and tissue homeostasis [26-27]. The failure or impaired expression of several of these non-coding RNAs is therefore associated with distinct pathological conditions [28-30]. Over the last years the functional contribution of a large number of microRNAs has been reported in distinct biological contexts, including the cardiovascular system [31-34]. Similarly, emerging evidence is also reported for lncRNAs in the cardiovascular system [23,35-36]. In the following paragraphs we will provide a state-of-the-art review of the functional contribution of different non-coding RNAs to cardiomyocyte cell fate acquisition during both *in vivo* and *in vitro* cardiomyogenesis.

The role of microRNAs in embryonic myocardial cell fate

As previously mentioned, myocardial cell fate is a continuous process that initiates soon after gastrulation as precardiac mesodermal cells are specified. Subsequently, as the early cardiac tube is formed, cardiomyocytes become determined and, as the cardiac chambers are formed, they fully differentiate into mature cardiomyocytes. microRNAs have been reported to play key role in different stages of myocardial cell fate, i.e. specification, determination and differentiation. Seminal work by Zhao et al. [37] reported the importance of microRNA biogenesis during heart development. Their

study reported that the deletion of microRNA processing enzyme *Dicer* in *Nkx2.5*-expressing cells was required for normal murine cardiogenesis, highlighting, therefore, the importance of microRNA presence during heart development. Additionally, the authors also demonstrated that deletion of *miR-1-2* impaired cardiac morphogenesis, leading to electrical and cell-cycle control disarrays in mice. Importantly, the functional role of *miR-1* is also conserved during *Drosophila* heart development [38].

Pioneer work by Lopez-Sanchez et al. [39], using the chicken embryo model, demonstrated that *miR-130* plays a pivotal role in regulating an *Fgf8-Bmp2* negative feedback loop during early cardiac specification. Such gene regulatory networks additionally involve *miR-133* since it also targets *Fgf8* and *Bmp2* signaling in the early cardiac crescents [39-40]. Interestingly, Wu et al. [41] demonstrated that *miR-134* can modulate the proliferation of human cardiomyocyte progenitors, without influencing their specification, by targeting the mesodermal-determining transcription factor *Meis2*. Therefore, these studies revealed the essential role of a discrete number of microRNAs in the early phases of cardiomyocyte specification.

The functional role of microRNAs regulating cardiomyogenic determination has been extensively documented in different experimental models. Shen et al. [42] identified enriched microRNAs in *Mesp1* positive early cardiac progenitor cells in mouse embryos, demonstrating that *miR-322-miR-503* cluster was the most enriched microRNA cluster in the *Mesp1* lineage. Ectopic expression of these microRNAs in embryonic stem cells demonstrated an enhancement of cardiomyocyte differentiation as revealed by cardiac troponin immunohistochemistry. Importantly, cardiomyocyte markers such as *Mef2c*, *Nkx2.5* and *Tbx5* were also increased without altering pluripotency (*Oct4, Sox2*) and/or mesendodermal markers (*Eomes, T, Mesp1*). Interestingly, skeletal muscle progenitor markers, but not smooth muscle, were also significantly increased, supporting a role for these microRNAs directing striated muscle determination.

Hoelscher et al. [43] identified *miR-128a* in a microRNA profile of *Nkx2.5* enhancer positive cardiac progenitor cells and functionally demonstrated that *miR-128a* knockdown in zebrafish significantly impaired cardiac development and function. *In vitro* assays in murine pluripotent stem cells demonstrated that *miR-128* knockdown severely diminished the expression of cardiomyogenic markers such as *Isl1*, *Nkx2.5* and *Mef2c* but increased *Irx4*. Conversely, over-expression led to opposite results, including the promotion of nodal-like cardiomyocyte differentiation and increased beating frequencies. Thus *miR-128a* plays a role in different aspects of cardiac development, including differentiation and subtype specialization.

Chen et al. [44] performed a microRNA array screening to identify differentially expressed microRNAs during mouse embryonic stem cell differentiation into cardiomyocytes. Among the microRNAs analyzed, *miR-142-3p* emerged as one of the most markedly downregulated microRNAs. While ectopic expression and inhibition of *miR-142-3p* do not alter the undifferentiated ESCs, the ectopic expression of *miR-142-*

3p impaired cardiomyocyte formation. In addition, the inhibition of *miR-142-3p* results in decreased expression of the mesodermal marker gene *Mesp1*, along with reduce levels of downstream cardiac transcription factors *Nkx2.5, Tbx5*, and *Mef2c*. These authors further demonstrated that *Mef2c* is a direct target of *miR-142-3p*, supporting the notion that *miR-142-3p* is an important regulator of early cardiomyocyte differentiation.

Additionally, evidence supporting of the role of *miR-1* and *miR-133* has also been reported during cardiomyocyte determination. In human induced pluripotent stem cells, the overexpression of *miR-1* has been shown to enhance the expression of key cardiac transcription factors (*Nkx2.5, Isl1* and *Gata4*) as well as sarcomeric genes (*Tnnt2*, *Myh6* and *Myh7*). Importantly, this overexpression also suppressed endothelial cell commitment by modulating Wnt and Fgf signaling pathways, as *Fzd7* and *Frs2* were identified as direct targets of *miR-1* [45]. Crucially, the role of *miR-1* is antagonist to that of *miR-133* during cardiac differentiation, as reported in murine pluripotent stem cells by Izarra et al. [46].

The regulatory role of *miR-335-3p* and *miR-335-5p* have also been reported to influence human embryonic stem cell differentiation into cardiomyocytes, by modulating *Brachyury*, *Gata4* and *Nkx2.5* transcription factors and thus enhancing the expression of cell-cell coupling Cx43 and sarcomeric Tnnt2 proteins [47]. On the other hand, *miR-125b* exhibits abundant expression in undifferentiated mouse embryonic stem cells and is significantly down-regulated during ESC differentiation, displaying a complementary pattern to *Lin-28*. Wang et al. (2012) demonstrated that *miR-125b* overexpression negatively influences endoderm and mesoderm development, while not affecting ectoderm development [48]. In a similar *in vitro* experimental model, Zheng et al. [49] reported that *Srf* directly controls the cardiac expression of *miR-210*. Both *miR-210* and *miR-30c* were found to block the formation of beating cardiomyocytes during murine embryoid body development. While several signaling pathways, such as Pi3k/Akt and Ets2, are proposed to modulate such developmental arrest not additional mechanistic insights have been gained. Negative regulation of human embryonic stem cell-derived cardiomyogenesis has also been reported. In this scenario, *miR-200c* acts as a repressor of cardiomyocyte differentiation and maturation by directly targeting *Gata4*, *Srf* and *Tbx5*. Importantly, the modulation of *miR-200c* significantly alters the expression of genes related to Ca2+, K+ and Na+ ion channel genes (*Cacna1c, Kcnj2* and *Scn5a*) for instance, *Cacna1c* represented a validated *miR-200c* target [50]. Moreover, Kuppusamy et al. [51] evidenced that let-7 family is required, and sufficient, for hESC-CMs maturation and metabolic transition, achieving an equivalent postnatal cardiomyocyte maturation. They indeed reported that overexpression of let-7 family members in hESC-CMs enhances cells size, sarcomere length force of contraction and respiratory capacity.

Additional evidence on the functional role of microRNAs has been gained through studies using cardiosphere-derived cells, in which cardiac stem cell subpopulations are identified. Using this *in vitro* experimental model, Jafarzadeh et al. [52] reported that *miR-497* overexpression impaired human cardiac differentiation. Furthermore, these authors demonstrated that *Tgfbr1* is a direct target of *miR-497*, resulting in negative regulation of *Smad3*, leading to impaired cardiac differentiation. Also, Ekhteraei-Tousi et al. [53] reported that *miR-590* modulates cardiac differentiation in human cardiosphere-derived cells, as evidenced by monitoring cTnt expression through the regulation of Tgf-β signaling pathway.

Finally, Qin et al. [54] reported that *miR-19b* overexpression promoted both proliferation and differentiation into cardiomyocytes, while inhibited apoptosis in P19 pluripotent murine cells. Furthermore, Wnt and β-catenin expression were decreased, while the expression of Gsk3β was increased. These data suggest that *miR-19b* inhibits activation of the Wnt/β-catenin signaling pathway, which may regulate cardiomyocyte differentiation.

In addition to modulating cardiac determining transcription factors and consequently sarcomeric gene expression, microRNAs also play a role in regulating other characteristics of the cardiomyocyte cell fate, such as their electrical and cell-cell coupling characteristics. In this context, Ling et al. [55] demonstrated that *miR-155-3p* expression is down-regulated during cardiogenesis from mouse embryonic stem cells, displaying a complementary pattern to muscle-enriched *Mef2c* transcription factor. Importantly, *miR-155* inhibition increased the percentage of beating embryoid bodies and up-regulated the expression of cardiomyogenic-specific markers, such as *Gata4*, *Nkx2.5* and *Tnnt2*, as well as *Erk1/2* intracellular signaling proteins. While these findings support the notion of a functional role of *miR-155* in cardiogenesis, it also remains undetermined how it modulates the electrical properties of the cardiomyocytes.

In addition, Chen et al. [56] reported the role of *miR-199* during mouse embryonic stem cell-derived cardiogenesis using embryoid bodies formation. The study revealed an inverse correlation between the expression of cardiogenic markers such as *Mef2c, Gata4*, *Nkx2.5, Mhy6* and *cTnt* with *miR-199* expression during embryonic stem cell-derived cardiogenesis. miR-199 inhibition led to increase in beating rates in EB and promoted cardiogenic markers. Moreover, *Mef2c* was verified as a direct *miR-199a* target, supporting the notion that miR-199 influences cardiomyocyte cell fate determination but modulating *Mef2c* expression. It remains nonetheless enigmatic how *miR-199* affects mechanistically the cardiomyocyte beating frequency.

In sum these data illustrate that distinct microRNAs can contribute to modulate, both positively and negatively, distinct characteristics of cardiomyocyte cell fate (**Figure 1**). Such modulatory effects can be exerted at distinct time points including thus processes such as progenitor cell proliferation, specification, determination and

differentiation, involving in the later the acquisition of specific sarcomeric, action potential and cell-cell coupling characteristics.

The role of lncRNAs in myocardial cell fate

In contrast to microRNAs, lncRNAs have a wide variety of functional roles including involvement in epigenetic, transcriptional and post-transcriptional regulatory mechanisms. In most cases, lncRNAs are transcribed by RNA polymerase II as inmature transcripts containing intron and exon sequences, which subsequently are subjected to an splicing process generating thereafter distinct spliced variants. Similar to mRNA transcripts, 5' and 3' ends of lncRNA transcript also display a 5´cap and poly(A) tail respectively. Curiously, some lncRNA have been described to be transcribed by RNA polymerase III, although in those cases, these lncRNAs lack both 5' and 3' post-transcriptional modifications. Despite their functional diversity, lncRNAs are often poorly evolutionary conserved across species, hampering their functional understanding in different species. While the functional role of lncRNAs in cardiomyocyte cell fate is still in its infancy, emerging evidence has been reported in different stages of cardiogenesis. Seminal work by Klattenhoff et al. [35] identified *Braveheart*, a lncRNA that is expressed in the nascent mesoderm and that is essential for cardiac fate acquisition. *Braveheart* is critical for the activation of a core cardiovascular gene network and functions upstream of *Mesp1*, a master regulator of common multipotent cardiovascular progenitor cells in mice, while no human homologue has been found. Mechanistically, *Braveheart* interacts with Suz12 during cardiomyocyte differentiation, supporting an epigenetic role of this lncRNA during cardiovascular lineage determination in mice. Furthermore, expression of *Braveheart* is positively regulated by *Carmn*, a super enhancer associated lncRNA. *Carmn* directly acts during the earliest steps of cardiac lineage commitment regulating cardiac differentiation from nascent mesoderm by modulating the expression downstream of *Mesp1* cardiac gene network [57].

Additional evidence on the role of lncRNAs during early cardiomyocyte specification has been provided by Guo et al. [58] and Kim et al. [59]. Specifically, *linc1405*, a lncRNA that physically interacts with *Eomes* forming a core complex that also includes *Wdr5* and histone acetyltransferase *Gcn5*. This complex plays a regulatory role in the transcription of *Mesp1*. *Linc1405* depletion impairs heart development and function *in vivo* [60], highlighting the critical role of *linc1405*-mediated *Eomes/Wdr5/Gcn5* complexes that contributes to cardiogenesis in mice. These findings highlight the critical roles of lncRNA-based complexes in the epigenetic regulation of cardiogenesis during early cardiomyocyte specification.

Kim et al. [59] identified an antisense lncRNA located upstream of *Gata6*, i.e. *Moshe*. *Moshe* knockdown during mouse heart development caused significant repression of *Nkx2.5* in cardiac progenitor cells. This repression, in turn, resulted in increased expression of secondary heart field (SHF) lineage markers, such as cardiac

transcriptional factors (*Isl1, Hand2, Tbx2*), endothelial-specific genes (*Cd31, Flk1, Tie1, vWF*), α smooth muscle actin (*α-SMA*) and sinoatrial node-specific genes (*Shox2, Tbx18*). Mechanistically, Moshe was found to activate *Nkx2.5* gene expression via directly binding to its promoter region. Interestingly, *Moshe* is conserved across species, including humans, pigs and mice. Thus, this study suggests that *Moshe* is a heart-enriched lncRNA that controls a sophisticated network of cardiogenesis by repressing genes in the SHF via *Nkx2.5* during cardiac development.

In the context of cardiomyocyte determination, Liu et al. [60] identified a novel human-specific lncRNA dubbed, heart brake lncRNA 1 (*HBL1*) that regulates cardiomyocyte development in human induced pluripotent stem cells. *HBL1* overexpression represses cardiomyocyte differentiation, while its inhibition has the opposite effect, promoting cardiomyocyte development. Mechanistically, *HBL1* interacts with *miR-1* and *Sox2* to control cardiomyocyte differentiation.

Similarly, Kay et al. [61] identified *CARMA* (CARdiomyocyte Maturation-Associated lncRNA), as a conserved lncRNA controlling cardiomyocyte differentiation and maturation in human ESCs. *CARMA* is located adjacent to *miR-1* and *miR-133* and transcribed in an antisense orientation. Importantly, the expression of *CARMA* and these miRNAs is negatively correlated. Furthermore, *CARMA* knockdown led to an increase in the expression of *miR1-1* and *miR-133a2*. Cardiomyocyte differentiation by *CARMA* is achieved by regulation of the Notch signaling pathway as well as by the modulation of two additional lncRNAs, *linc1230* and *linc1335* that, if forced expressed, improved ESC-derived cardiomyocyte differentiation.

Therefore, evidence is emerging on the functional role of lncRNAs during early specification and determination phases of cardiomyocyte cell fate (**Figure 1**), while scarce evidence is available about their plausible in action potential configuration and/or cell-cell coupling characteristics, although some evidence is becoming available in other biological contexts [62-63].

Reprogramming of cardiomyocyte cell fate

Cardiovascular diseases are the leading cause of death worldwide. Thus, searching for innovative modes to repair the broken hearts are continuously devised, among which cell therapy has taken advantage during the last decades. In this context, providing cues for myocardial cell fate reprogramming from different cell sources has been investigated. A cocktail of cardiac-enriched transcription factors can induce mouse and human fibroblast to cardiomyocyte reprogramming, respectively [64-65]. Human fibroblast reprogramming has also been gained by a cocktail of non-coding RNAs such as *miR-1, miR-133, miR-208* and *miR-499* [66-67], a process that has recently been demonstrated to be dependent of epigenetic demethylation of H2K27 [68]. Furthermore, the administration of additional microRNAs, such as *miR-2392* together with cardiomyogenic differentiation compounds [69] and *miR-590* together with GMT cocktail (*Gata4, Mef2c* and *Tbx5*) of cardiogenic transcription factors [70], respectively,

can also enhance fibroblast to cardiomyocyte-like cell fate transition. Similarly, Nam et al. [71] demonstrated the conversion of fibroblast to cardiomyocyte cell fate by administration of cardiac transcription factors, including *Gata4, Hand2, Tbx5* and *myocardin*, together with another two microRNAs, *miR-1* and *miR-133*, activating cardiac marker expression in both neonatal and adult human fibroblasts.

Besides reprogramming of fibroblasts into cardiomyocytes, other approaches have also been undertaken. In this context, Nesthti et al. [72] reported that *miR-499a-5p* can promote differentiation of human bone marrow-derived mesenchymal stem cells into cardiomyocytes, as revealed by α-actinin and cardiac troponin I enhanced expression. Huang et al. [73] demonstrated that *miR-1* administration promoted cardiogenic lineage differentiation of mesenchymal stem cells by downregulating *Hes1*, leading thus to upregulation of cardiomyocyte markers such as *Nkx2.5, Gata4, cTnT* and *Cx43*.

In sum these data demonstrate the pivotal role of microRNAs in modulating the reprogramming of distinct cell types, namely, fibroblasts, bone marrow-derived mesenchymal stem cells and mesenchymal stem cells, into cardiomyocytes, opening new avenues for their plausible usage as therapeutic tools to regenerate the damaged heart.

Conclusions

Cardiovascular development is a complex developmental process that requires the contribution of distinct cell subpopulations. Focusing on cardiomyocyte cell fate specification, seminal works at the end of the last century revealed a complex and intricate regulatory networks of growth factors and receptors, particularly those of the Bmp, Fgf and Wnt families. These networks play crucial roles in committing precardiac mesoderm into the cardiomyogenic lineage (reviewed in [74-75]). Concomitantly, a core of cardiac-enriched transcription factors was identified that synergistically direct cardiomyocyte determination, i.e. *Mesp1/Mesp2, Nkx2.5, Gata4, Sfr* and *Mef2c* (reviewed in [76]). However, understanding how chamber-specific cardiomyocyte identity is acquired required additional efforts, revealing that *Isl1* is essential for SHF deployment, and that T-box family members and Notch-related Hey transcription factors are important for chamber regional identity [18-19]. However, while our understanding of the molecular mechanisms driving sarcomeric maturation has greatly increased in recent years, our comprehension of the regulatory mechanisms that provide other key characteristics to the mature cardiomyocytes is still pending, i.e. how the electrical properties that allow the distinct regional cardiac action potential configuration are acquired and how the asymmetric regional cell-cell communication pathways are obtained.

The advent of a novel regulatory layer, such as that achieved by non-coding RNAs, reveals that additional regulatory mechanisms can modulate myocardial cell fate (**Figure 1**). Increasing evidence is emerging that demonstrates the pivotal role of

microRNAs in modulating cardiomyocyte cell fate specification, determination and differentiation. Incipient evidences are also emerging for lncRNAs. Interestingly, scarce evidence is currently reported on the functional impact of microRNAs and lncRNAs modulating regional chamber-specific cardiomyocyte identity. On the other hand, while increasing evidence is available about the functional role of microRNAs, and to a lesser extent, of lncRNAs, in regulating distinct components of the cardiac action potential and of cell-cell interacting proteins in mature cardiomyocytes [77-78], their role in the acquisition of these characteristics during cardiomyocyte cell fate determination remains to be elucidated.

Seminal works have also unraveled that microRNAs can reprogram distinct cell types into the cardiomyogenic lineage [66-67] and can also serve as co-adjuvants to enhance cell reprogramming of fibroblasts into cardiomyocytes [69-71]. However, in most cases, full cardiac differentiation is not achieved, including therein not only the formation of mature and functional sarcomere but also the electrical and cell-cell coupling features of fully differentiated cardiomyocytes.

Thus, in the coming years increasing evidence will be discovered on the functional contribution of these non-coding RNAs to orchestrate cardiomyocyte fate specification, determination and differentiation. The advent of such novel evidence, together with the possibility of using them to reprogram distinct cell types into mature cardiomyocytes will open up new therapeutic avenues to regenerate the damaged heart.

Perspectives

- Dissecting the functional roles of non-coding RNAs during myocardial cell fate might pave the road to design novel therapeutic tools to heal the damaged heart
- Cardiovascular development is complex developmental process that requires the contribution of distinct cell subpopulations, governed by complex and intricate regulatory networks of growth and transcription factors. The advent of a novel regulatory layer, such as that achieved by non-coding RNAs, reveals that additional regulatory mechanisms can modulate myocardial cell fate.
- Increasing evidence of the functional contribution of these non-coding RNAs to orchestrate cardiomyocyte fate specification, determination and differentiation will be achieved in the coming years. Such discoveries, together with the possibility of using them to reprogram distinct cell types into mature cardiomyocytes will open up new therapeutic avenues to heal the damaged heart.

Figure legends

Figure 1. Non-coding RNAs during myocardial cell fate. Inner circle. Schematic representation of cardiac development incluyding the distinct phases of myocardial cell fate, i.e. specification, determination, differentiation and maturation. Outer panels 1-4. Schematic representation of the non-coding RNAs modulation of distinct signaling factors involved in myocardial cell fate progression. Green arrows denote positive regulation while red ones denote inhibitory regulation. Observe that evidence on the role of these non-coding RNAs in the early stages of myocardial cell fate progression, i.e. specification, determination and differentiation is emerging while, no evidence is currently available for cardiomyocyte maturation.

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