MicroRNAs as therapeutic targets in heart diseases

CHAPTER 5

SUMMARY
and
GENERAL DISCUSSION

The events leading the cardiac muscle towards sustained dysfunction, which could precipitate in the HF syndrome, have a wide range of origins. We described the variety of heart diseases causing pathological remodeling through recognized molecular pathways **(chapter 1)**. However, we are still far from having a complete picture of the effectors responsible of the orchestration and coordination of these processes. An attractive research field is the one that deals with non-coding RNA. In particular miRNAs represent versatile tools able to efficiently regulate the gene expression underlining specific cellular responses. Once we are able to recognize the role of these molecular players, this would give us the knowledge to produce gene modulatory therapies aimed to target defined pathological conditions.

Acting with this purpose, we started reviewing the recent and most relevant findings on miRNAs-based treatments inducing heart tissue regeneration and cardiac function improvement specifically after myocardial injury **(chapter 2)**. We addressed in detail the three possible strategies for myocardial regeneration: cardiac stem/progenitor cell differentiation, fibroblast reprogramming and cardiomyocyte proliferation. Moreover, we reported the interesting achievements obtained by consolidated or emerging therapeutic heart delivery strategies such as rAAV-vectors and nanoparticles. Furthermore, we indicated possible future research directions in order to improve their specificity.

From hyperplasia to hypertrophy and back

The heart is endowed with a remarkable capacity of increasing its size during its entire life span. This process is governed by the increase of cardiomyocyte number (hyperplasia) during the embryonic/fetal phase, while it moves to an increase of cardiomyocyte size (hypertrophy) after birth and for the rest of life. Precisely, even in humans, cardiomyocytes keep a limited, but still present, proliferative/regenerative ability for a short time span after birth [1, 2]. After this short period they enter in cell cycle arrest and acquire a hypertrophic and polyploid phenotype in response to overload or myocardial insults [3]. This reduced cellular plasticity is at the basis of the higher vulnerability of the heart when faced with pathological conditions with the effects of extended cell death and pathological hypertrophy [4]. The resulting clinical consequence is HF. In this work we described the potential of an evolutionary conserved miRNA cluster miRNA-106b~25 that is operative in this pathological condition **(chapter 3)**. We reported that miRNA-106b~25 is highly expressed in the heart during the early phases of post-natal life, then the expression levels of all three miRNAs decrease in the following days and remain low during adulthood. This dynamic is in accordance with the mentioned reported trend of cardiomyocyte proliferation. Remarkably, in murine models, the complete knockout of miRNA-106b~25 results in cardiomyocyte hypertrophy and

eccentric remodelling. This phenotype matches with the even further reduction of expression in human cardiac biopsies of end-stage heart failure patients. The molecular result of this pathological condition is a derepression of the pro-hypertrophic target bHLH transcription factor Hand2[5]. Moreover, we detected myocyte enhancer factor-2 d (MEF2d) as an additional downstream target of miRNA-106b~25. In accordance, MEF2d overexpression was reported to be responsible of reprogramming gene expression towards pathological cardiac remodeling [6]. Conversely, miRNA-106b~25 overexpression, using adeno-associated virus vector serotype 9 (AAV9), enhanced cardiomyocyte proliferation targeting a network of genes involved in cell cycle progression such as the E2F5, Cdkn1c, Ccne1 and Wee1. These findings reflect the reported observations of expression, both during fetal and neonatal life, of proto-oncogenes, specific cyclins and cyclin-dependent kinases, while, in adulthood, cell cycle inhibitors are more predominant [7, 8]. Taken together, our results suggest a molecular explanation for the underlining mechanism driving cardiomyocytes towards cell cycle arrest and terminal differentiation. In the early phase of life, miRNA-106b~25 is higher expressed and consequently down-regulates several cell cycle inhibitors and pro-hypertrophic effectors promoting a phenotype of cardiomyocyte hyperplasia. On the contrary, moving towards adulthood and in conditions of cardiac damage, the lower expression of miRNA-106b~25 no longer represses the mentioned targets resulting in a phenotype of cardiomyocyte hypertrophy. Guided by this knowledge, we treated a mouse model of chronic ischemic injury with the administration of AAV9-miRNA-106b~25, resulting in the regeneration of the injured adult myocardium and the conservation of heart function. In conclusion, all these findings demonstrate the possibility of using an endogenous epigenetic mechanism as a therapy able to induce cardiac regeneration.

Between concentric and eccentric hypertrophy

We already discussed how, on the cellular and morphological level, a certain kind of pathological hypertrophy can arise from specific heart insults (chapter 1). Pressure overload is the main origin of concentric hypertrophy, while volume overload conditions tend to display eccentric hypertrophy [9]. Here, using samples from HF patients and HF mouse models, we reported a differential expression of the evolutionary conserved miRNA-148a showing higher level in concentric remodelling and lower expression in conditions with eccentric remodelling **(chapter 4)**. Our data also shows that miRNA-148a is able to regulate the cardiac IL-6 superfamily signalling cascades by targeting the co-receptor gp130. In line, miRNA-148a down-regulation, using a specific antagomir approach, resulted in increased expression of gp130 in the heart and the coupled intracellular signalling combined with a maladaptive cardiac phenotype characterized by worsened function, wall thinning and chamber

dilatation following stress conditions. Conversely, cardiac-restricted miRNA-148a overexpression, by AAV9-148a delivery, resulted in reduced gp130 expression with a protective effect promoting wall thickness towards concentric remodelling. These data give indications of molecular events underling distinct forms of cardiac hypertrophy and able to balance the morphological remodelling of the heart.

The activation of these cardiac pathological mechanisms should be connected with agonists enhanced in the stress response. Cardiotropin-1 (CT-1), a member of the IL-6 cytokine superfamily, acts via gp130 homodimer or gp130/leukemia inhibitory factor receptor-β (LIFRβ) heterodimer interaction [10]. In cultured cardiomyocytes, it is described to promote the assembly of sarcomeres in series. The consequence is the hypertrophic increase in cell length but not a significant modification of cell width, and this longitudinal elongation involves the intercellular signal transducers STAT3 and ERK5 [11]. In accordance, we performed a study with continuous CT-1 infusion at a dose of 20 μg/kg/day resulting in an eccentric hypertrophic phenotype with reduced cardiac function and wall thinning. The transfection of miRNA-148a precursor on CT-1 treated cardiomyocytes induced a reduction of the hypertrophic effect indicating the interaction between CT-1 signalling and miRNA-148a. Regarding the involvement of the subunit gp130, it is described to be pivotal in a variety of signalling events activated by the IL-6 cytokine superfamily [12, 13]. In particular, on one side gp130-signaling is necessary for cardiomyocyte survival pathways but on the other side it allows for the transmission of pro-hypertrophic signals. In fact, mice with ventricular-restricted deficiency of gp130 demonstrate a rapidly progressing heart failure phenotype driven by a massive cardiomyocyte apoptosis [14]. On the contrary, over-activation of the transducer MEK5 in the murine heart results in severe eccentric cardiac hypertrophy progressing towards dilated cardiomyopathy and sudden death [15]. Taken together these observations suggest that the IL-6 cytokine/gp130 signalling plays an important and modulatory role in the regulation of distinct subtypes of cardiac hypertrophic. In this scenario, our work provides a framework to understand the action of miRNA-148a and reveals a biphasic mechanistic regulation that can shed new light on the clinical observation of the transition from a compensatory, early stage hypertrophic response to later stage dysfunctional heart failure.

Concluding remarks and future perspectives

Despite a variety of different heart diseases driving to HF syndrome, there is still a lack of specific treatments able to efficiently cure the injured heart. The precise action of these future therapies will become reality through a better understanding of the molecular mechanisms regulating the different morphological modifications of the heart. In this thesis we presented examples indicating the involvement of

specific miRNAs, a defined class of the non-coding RNAs, to regulate processes in these mechanisms. Specifically, our data show how the miRNA106b~25 cluster enhances cardiomyocyte hyperplasia instead of cardiomyocyte hypertrophy by targeting a network of cell cycle inhibitors and pro-hypertrophic factors. Moreover, we reported how the overexpression of miRNA106b~25 is able to promote cardiac regeneration and heart function in a model of permanent myocardial infarction. In the second part, we focused on the dynamic of expression and regulation of miRNA-148a in the concentric and eccentric hypertrophy response. We showed how miRNA-148a exerts a protective effect against eccentric remodelling progression. Our findings support the idea that miRNA-148a can target the co-receptor gp130 and participates at the modulation of the biphasic-regulating mechanism represented by the IL-6 cytokine/gp130 signalling cascades. In conclusion, and recognizing that further tests and trials are needed, we consider our work a valid contribution aimed to expand the concept of tailored, more personalized medicine by using microRNAs as therapeutic targets in the treatment of heart diseases.

References

1. Porrello, E.R., et al., *Transient regenerative potential of the neonatal mouse heart.* Science, 2011. **331**(6020): p. 1078-80.
2. Haubner, B.J., et al., *Functional Recovery of a Human Neonatal Heart After Severe Myocardial Infarction.* Circ Res, 2016. **118**(2): p. 216-21.
3. Brodsky, V., et al., *Polyploidy in cardiac myocytes of normal and hypertrophic human hearts; range of values.* Virchows Arch, 1994. **424**(4): p. 429-35.
4. Olson, E.N. and M.D. Schneider, *Sizing up the heart: development redux in disease.* Genes Dev, 2003. **17**(16): p. 1937-56.
5. Dirkx, E., et al., *Nfat and miR-25 cooperate to reactivate the transcription factor Hand2 in heart failure.* Nat Cell Biol, 2013. **15**(11): p. 1282-93.
6. Kim, Y., et al., *The MEF2D transcription factor mediates stress-dependent cardiac remodeling in mice.* J Clin Invest, 2008. **118**(1): p. 124-32.
7. Busk, P.K., et al., *Involvement of cyclin D activity in left ventricle hypertrophy in vivo and in vitro.* Cardiovasc Res, 2002. **56**(1): p. 64-75.
8. Pasumarthi, K.B., et al., *Targeted expression of cyclin D2 results in cardiomyocyte DNA synthesis and infarct regression in transgenic mice.* Circ Res, 2005. **96**(1): p. 110-8.
9. Gaasch, W.H. and M.R. Zile, *Left ventricular structural remodeling in health and disease: with special emphasis on volume, mass, and geometry.* J Am Coll Cardiol, 2011. **58**(17): p. 1733-40.
10. Wollert, K.C., et al., *Cardiotrophin-1 activates a distinct form of cardiac muscle cell hypertrophy. Assembly of sarcomeric units in series VIA gp130/leukemia inhibitory factor receptor-dependent pathways.* J Biol Chem, 1996. **271**(16): p. 9535-45.
11. Lopez, N., J. Diez, and M.A. Fortuno, *Differential hypertrophic effects of cardiotrophin-1 on adult cardiomyocytes from normotensive and spontaneously hypertensive rats.* J Mol Cell Cardiol, 2006. **41**(5): p. 902-13.
12. Heineke, J. and J.D. Molkentin, *Regulation of cardiac hypertrophy by intracellular signalling pathways.* Nat Rev Mol Cell Biol, 2006. **7**(8): p. 589-600.
13. Sugden, P.H. and A. Clerk, *"Stress-responsive" mitogen-activated protein kinases (c-Jun N-terminal kinases and p38 mitogen-activated protein kinases) in the myocardium.* Circ Res, 1998. **83**(4): p. 345-52.
14. Hirota, H., et al., *Loss of a gp130 cardiac muscle cell survival pathway is a critical event in the onset of heart failure during biomechanical stress.* Cell, 1999. **97**(2): p. 189-98.
15. Nicol, R.L., et al., *Activated MEK5 induces serial assembly of sarcomeres and eccentric cardiac hypertrophy.* EMBO J, 2001. **20**(11): p. 2757-67.