Heart disease often culminates into a decline in function and heart failure as a result of the loss of viable cardiac tissue. Given the global burden of heart disease, a lot of effort is currently dedicated toward the development of strategies that can regenerate the human heart.1 Although the heart is notoriously resistant to regeneration, recent studies have shown that it provides some opportunities to enhance heart repair by the rescue of existing cells or the formation of new cardiomyocytes. These new heart muscle cells can either originate from nonmyocyte cell types or by cardiomyocytes reentering the cell cycle.

Reinitiating Cardiomyocyte Turnover During Ischemic Injury

Although the majority of postnatal cardiomyocytes transition from a proliferative to a differentiated state, a subset of myocytes maintains some regenerative potential albeit at a low capacity. Several years ago, a seminal paper from Bergmann et al showed by carbon-14 birth dating approaches that, of the several billion cardiomyocytes present in the human heart, ≈0.5% turns over every year.3 This is in agreement with the 1% cardiomyocyte turnover that was observed in the adult murine heart.4 The cell cycle withdrawal of mammalian myocytes early in postnatal life has been attributed to the inhibition of cell cycle activators and upregulation of cell cycle inhibitors. It is believed that the local microenvironment induces epigenetic changes that affect the proliferative capacity of cardiac cells under conditions of homeostasis. Stresses, like myocardial injury, cause changes in gene expression that drive reprogramming in a subset of cardiomyocytes, resulting in a significant increase in cardiomyocyte turnover in cells flanking the injured region. The mechanism for this cell cycle reentry may involve cardiomyocyte rejuvenation or the relief of epigenetic gene silencing mechanisms that repress myocyte turnover. Despite the fact that underlying epigenetic changes might be responsible for the inefficient cardiomyocyte turnover, genetic or pharmacological modulation of several individual factors leading to cardiomyocyte proliferation have been successful in enhancing heart regeneration on injury. In vivo overexpression of factors like Cyclin D2 and E2F4, regulation of the Hippo pathway, or administration of factors like neuregulin or oncostatin M have all been shown to enhance cardiomyocyte proliferation and to promote cardiac repair (reviewed in Senyo et al5). More recently, also miRNAs emerged as post-transcriptional regulators of cardiac rejuvenation and regeneration.

MicroRNA Function in Cardiomyocyte Proliferation

Because miRNAs have shown to be process regulators to maintain cell homeostasis, it seems to make sense for them to be involved in the regulation of cardiomyocyte turnover. A few years ago, Porrello et al already showed that the loss in proliferative capacity of cardiomyocytes in the mouse heart during the first few days after birth coincides with an expressional change in several miRNAs, including an increase in the miR-15 family.6 Follow-up studies showed that therapeutic inhibition of miR-15 family members in a model of ischemia–reperfusion in adult mice showed a reduction in infarct size and improved cardiac function 2 weeks after ischemic damage, which was attributed to the derepression of a cohort of prosurvival and pro-proliferative proteins.5,7

More proof for the importance of miRNA function in adult myocyte turnover came from a study by Eulalio et al where they showed that miR-199a-3p and miR-590-3p were able to drive cytokinesis and cell cycle re-entry in postnatal cardiomyocytes.8 Exogenous administration of miR-199a-3p and miR-590-3p exerted beneficial effects on the mouse myocardium after infarction by stimulating cardiomyocyte proliferation as shown by the presence of more EdU-positive nuclei.

Another cluster relevant for myocyte turnover was identified by the Morrissey group, showing that postnatal...
re-expression of the miR-302 cluster is sufficient to induce cardiomyocyte proliferation in the adult heart and promotes cardiac regeneration of the injured heart by the reactivation of the cell cycle in cardiomyocytes. These effects are, at least in part, caused by the repression of the Hippo signal transduction pathway.2

Aguirre et al explained the difference in regenerative potential between zebrafish and mammals by the inability of the mammalian heart to lower miR-99/100 and Let-7a/c after MI as is the case in the regenerating zebrafish heart. Therapeutic inhibition of miR-99/100 and Let-7a/c and a subsequent up-regulation of their protein targets were able to elicit an endogenous regenerative response after MI in the adult heart.10

A common denominator for all these studies is that they all point toward miRNAs as being important regulators of cardiomyocyte proliferation and survival and imply that therapeutic regulation could be useful for enhancing cardiac repair after injury.

MicroRNA-34a Plays a Role in Cardiac Repair

The postnatal loss of cardiac regenerative potential coincides with a change in miRNA expression.6 A recent report by Yang et al showed a postnatal increase in miR-34a levels in the heart, which was induced even further after ischemic damage.2 Based on the loss of cardiomyocyte proliferation, the authors hypothesized that low levels of miR-34a in the neonatal heart contributes to the higher endogenous regenerative capacity. Indeed, an increase in miR-34a blocked regeneration in the neonatal heart, whereas therapeutic inhibition both 6 hours and 2 days after MI in the adult heart improved cardiac function and blunted cardiac remodeling.

Seeking to identify key genes that are responsible for the cardioprotective effect of miR-34a inhibition, Yang et al performed genome-wide expression analysis and focused on genes that were selectively downregulated in adult hearts relative to neonatal counterparts. A set of in vitro and in vivo studies showed that the molecular mechanisms of miR-34a-mediated cardioprotection involved the antiapoptotic and proproliferative actions of Bcl2, Cyclin D1, and Sirt1 as direct targets of miR-34a. All identified target genes individually affect different signaling pathways involved in apoptosis and proliferation. However, because the authors hypothesize that the low level of miR-34a is responsible for the regenerative state, it would have been interesting to know whether there is a link between the developmental increase in miR-34a and the downregulation of these targets, and how this affects the decline in cardiomyocyte proliferation.

Strikingly, Yang et al showed that adenoviral delivery of these individual target genes to cultured cardiomyocytes treated with miR-34a mimic was sufficient to restore the effect on proliferation (as measured by Edu-positive cardiomyocytes) and apoptosis (as measured by TUNEL staining) in vitro. These intriguing effects raise a question regarding the mechanisms by which Bcl2, Cyclin D1, and Sirt1 individually regulate cardiomyocyte proliferation, as well as apoptosis; however, the study did not provide mechanistic explanation for this. This would be interesting to explore further, especially because the cardiomyocyte-specific transgenic models of these factors not all show the benefit observed after miR-34a inhibition.

Previously, the relevance of miR-34 for heart biology was shown in studies from Boon et al and Bernardo et al,11,12 underscoring the potential importance of the miRNA for heart disease. However, the defined mechanism by which this miRNA functions is somewhat divergent for the different studies. Boon et al described a function for miR-34a in cardiomyocyte ageing and cell death.11 They confirmed miR-34a to be the main cardiac isoform of the miR-34 family (consisting of miR-34a, -b, and -c) and also showed it to be mainly expressed in cardiomyocytes, although still detectable in other cell types. AntimiR-mediated inhibition of miR-34a also in their hands induced a reduction in TUNEL-positive cells and infarct size 4 weeks after MI, which they attributed, at least in part, to be because of the regulation of PBNUTS, a factor involved in apoptosis and telomere length.11 It is unclear whether this target was also regulated in the study by Yang et al.

The study by Bernardo et al sketched a somewhat different scenario in that it showed all 3 isoforms to be regulated after MI and that inhibition of only miR-34a had no beneficial effect 8 weeks after MI.12 Only coordinate inhibition of all 3 family members improved heart function after injury. Mechanistically, Bernardo et al validated Vinculin, Pofut1, Sema4b, and Bcl6 as the targets of miR-34 that potentially contribute to the observed cardioprotective phenotype. Because Yang et al studied only the regulation of miR-34a after MI and additionally did not look at these miR-34 target genes, it is difficult to assess whether these genes or miR-34b and-c play a role in their observed effect.

Although the difference in targets might be because of a focused analysis, there could be also some other explanations. Although all studies used LNA-based chemistries to inhibit miR-34, the type of antimiR, route of delivery, dose of administration, timing of analysis, and the cardiac region used for analysis could produce distinct in vivo results in gene regulation and outcome. Nevertheless, although these studies might mechanistically not completely overlap, they provide evidence that inhibition of miR-34a is cardioprotective after ischemic damage in the heart.

Looking to the Future

A large portion of cardiac research is currently dedicated toward finding the solution to repair some of the heart tissue that is lost during disease. It seems likely that miRNAs, as regulators of cell state, are important for the control of cardiomyocyte turnover. Based on the story by Yang et al and additional reports, miR-34a seems to function as a player for cardiomyocyte proliferation and apoptosis, and inhibition after ischemic injury provides therapeutic benefit. As for many drugs, delivery of an antimiR against miR-34 is going to be key in this situation.

As antimiR therapies preferentially end up in the kidney and liver, more localized delivery opportunities could be preferred over systemic delivery. This will not only increase the amount of drug that is being delivered to the heart, but might also circumvent extracardiac side effects because of the regulation of miR-34 in other tissues. This might be especially important because the therapeutic effect of increasing miR-34 with a mimic is now being tested in patients with primary liver cancer or metastatic cancer with liver involvement.13
More locally, also the cellular delivery of miR-34a inhibitors should be taken into account because miR-34a is expressed in multiple cell types. Thus, using an miR-34a inhibitor will result in the reduction of miR-34a levels in all cell types in the heart, and where a pro-proliferative or anti-apoptotic effect is beneficial in cardiomyocytes, it might be detrimental for other cell types. Although no unwanted side effects on noncardiomyocyte cells were observed by Yang et al, this might be a consideration in moving this approach forward into the clinic.

Although we await more mechanistic insight into the proliferative and antiapoptotic function of miR-34a, the data today show strong proof for the beneficial effect of miR-34a inhibition on cardiac repair after ischemic injury. Finding a way to translate these observations into patients could eventually aid to help those suffering from ischemic heart disease.

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**References**

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