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Micro-managing myocyte mitosis

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In the adult mammalian heart, cardiomyocyte renewal is rare[1, 2] and insufficient to restore normal pump function after significant myocardial damage. Recent studies, however, suggest that zebrafish hearts[3–5] and neonatal mouse hearts[6] can regenerate after injury via enhanced cardiomyocyte proliferation. In mice, this restorative potential is lost shortly after birth[6]. In this issue of *Circulation Research*, Porrello et al. identify the miR-15 family of microRNAs as potential mediators of the post-natal loss of proliferative potential[7].

Most (if not all) adult mammalian cardiomyocytes permanently exit the cell cycle and do not proliferate even when challenged by injury or stress. Scientists have long sought to understand the mechanisms that prevent cardiomyocyte cell cycle re-entry and to overcome these blocks in order to generate new functional myocytes (reviewed in[8]). Experimental approaches in the past have included manipulation of direct cell cycle regulators such as cyclins and cyclin dependent kinase inhibitors, and regulation of myofibril disassembly that is thought to be necessary for the mature cardiac myocyte to undergo cell division (cytokinesis)[9].

Excitement and enthusiasm for seeking a deeper understanding of this phenomenon have been enhanced by the discovery that adult zebrafish hearts can regenerate even after significant injury[4], and the more recent observation by Porrello et al. that newborn mouse hearts can also regenerate[6]. These findings indicate that functional cardiac myoctyes, with contractile sarcomeres and myofibrils, are able to re-enter the cell cycle and undergo cytokinesis to generate new myocytes. Importantly, this capacity is largely lost during the first week of post-natal life, suggesting that epigenetic changes during that time period alter the myocyte response to injury.

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Disclosures

None.

Indeed, dramatic changes in gene expression occur shortly after birth as the fetal gene program is silenced and adult isoforms of contractile proteins, calcium transporters and metabolic regulators are expressed (reviewed in[10]). In rodents, most cardiac myocytes undergo one post-natal round of DNA synthesis without cytokinesis, resulting in binucleation and subsequent cell cycle arrest, usually at G0/G1 (see[11]). Altered response to injury, large-scale changes in gene expression and uncoupling of cytokinesis from karyokinesis (nuclear division) that occur in the first week of life could be coordinately or separately regulated by one or more epigenetic modulators, and direct comparison of the presence or activity of candidate effectors in newborn and early post-natal hearts is an attractive approach for identifying relevant reglators.

Porrello et al. adopted this strategy to identify potential microRNAs (miRs) that regulate cardiomyocyte proliferation[7]. MicroRNAs are short, non-coding RNAs that modulate stability or translation of mRNAs and may function to simultaneously target multiple members of a biological pathway. Indeed, several miRs have previously been implicated in the regulation of cardiomyocyte proliferation[12, 13]. By comparing miR expression in P10 and P1 murine hearts, Porrello et al. found that miR-195 (a member of the miR-15 family) is significantly up-regulated in P10 hearts, and remains elevated throughout adulthood. Other members of the miR15 family are similarly regulated. Although detailed analysis by Porrello et al. focused on the miR-15 family, it is worth noting that a total of 71 miRs were found to be altered (up or down regulated) between P1 and P10 in this screen.

In order to determine if members of the miR-15 family can function to regulate cardiomyocyte proliferation, gain- and loss-of-function studies were performed. Transgenic mice over-expressing miR-195 using the ß-myosin heavy chain promoter exhibit cardiomyocyte hypoplasia and ventricular septal defects. At P1, the number of myocytes undergoing mitosis is decreased ~3 fold (as assessed by phospho-histone H3 staining) and the number of multinucleated cells is increased. Micro-array analysis suggests that mitosis and cell cycle genes are repressed both in vivo (in Tg hearts) and in cultured myocytes infected with an adenovirus expressing miR-195. The pattern of gene expression is consistent with the induction of a G2/M arrest.

Although cardiac-specific genetic inactivation (knockout) of the miR-15 family is not reported in this study, the function of these microRNAs was disrupted by the use of locked-nucleic acid (LNA)-modified oligonucelotides directed against miR-15b and miR-16 delivered by subcutaneous injection. Administration of these long-lived inhibitory oligonucleotides once daily from P2–P4 appears to be sufficient to repress all miR-15 family members until at least P12. A significant ~3 fold increase in phospho-H3-stained myocytes is detected after miR-15 inhibition, although evidence for cardiomyocyte cell division is not reported and cardiomyocytes expressing Aurora B kinase, which is necessary for cytokinesis, were not detected. These results suggest that the miR-15 family is likely to regulate important aspects of postnatal withdrawal from cell cycle progression in the heart, but that inhibition of the miR-15 family is not sufficient to induce cytokinesis in adult myocytes. It will be interesting to determine if the response to injury in the adult heart is altered by inhibition or loss of the miR-15 family (i.e. can the proliferative response seen at P1 be restored).

In order to identify mRNA transcripts regulated by miR-195, Porrello and colleagues utilized a modification of the chromatin immunoprecipitation (ChIP) procedure to precipitate the RNA-induced silencing complex (RISC) and associated mRNA transcripts from miR-195 Tg and wild type hearts at P1[14]. This approach, coupled with subsequent validation, identified *Chek1* as a miR-195 target, among other candidates. The finding is of interest because *Chek1* encodes checkpoint kinase 1, a multifunctional kinase implicated in the DNA damage response and in multiple mitotic checkpoints including those regulating spindle assembly and cytokinesis[15, 16]. Indeed, Aurora B is a substrate of Chek1, and *Chek1* haploinsufficiency leads to mitotic defects, binucleation and mis-localization of Aurora B in non-cardiac epithelium[17]. In the post-natal heart, the effects of Chek1 over-expression have not been tested. Hence, it is not clear which aspects of the miR-15 family knockdown phenotype (which is associated with Chek1 over-expression) are attributable to Chek1, nor whether miR-15-mediated inhibition of *Chek1* that occurs during the post-natal period accounts, in whole or in part, for the altered regenerative response to injury that occurs in the first week of life. Indeed, physiological down-regulation of *Chek1* after birth may reflect the lack of an active requirement for its function in a post-mitotic cell, and this change alone is unlikely to account for the loss of proliferative potential.

Nevertheless, the work of Porrello et al. represents an exciting first example of the potential insights that are likely to emerge from careful comparisons of regeneration-competent P1 murine hearts with regeneration-incompetent hearts from slightly older animals. The emerging understanding of cellular reprogramming, and the power of miRs and small molecules to alter epigenetic landscape and cellular characteristics provides strong encouragement for continued investigation of strategies for enhancing cardiac regeneration[18–21]. Indeed, emerging studies suggest that adult cardiac myocytes may be able to divide under some circumstances, and this process may be enhanced by growth factors such as neuregulin, periostin, fibroblast growth factor or by unknown factors associated with exercise[22–25]. It has long been known that cardiac stress (such as pressure overload or beta-adrenergic stimulation) can lead to cardiac myocyte DNA synthesis, polyploidy or multinucleation – as if the heart is trying to mount a proliferative response, but is blocked from undergoing cytokinesis[26, 27]. For unknown reasons, pathological hypertrophic stimuli also induce cardiac expression of several regulators of cytokinesis, including RhoA, Rac and Cdc42[28], and the precise reasons why cell division remains blocked is unknown. Further detailed understanding of the epigenetic regulation of cardiomyocyte cytokinesis and how it is coupled to cell cycle progression and myofibrillar disassembly will be an important and exciting area of future investigation.

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