Revealing Pathways of Cardiac Regeneration

See Article by Adamowicz, Morgan, and Haubner et al

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Major cardiac injuries like a large myocardial infarction lead to systolic heart failure because of loss of cardiomyocytes. Unlike during embryonic development,[1] adult mammalian cardiomyocytes have limited ability to proliferate and replenish the adult mammalian heart. Although there was once excitement over possible adult cardiac stem cells residing in the mammalian myocardium, it now seems that there are insufficient cardiac progenitor cells in the heart[2] to generate any meaningful regeneration. More recently, it was discovered that the heart's regenerative ability is preserved in the first hours after birth in mice[3] and rats.[4] Indeed, cardiac regeneration may be recapitulated in vitro using immature human heart organoids,[5] indicating an innate capacity of some cardiomyocytes for regeneration. These and other recent discoveries have advanced our understanding of how the mammalian heart can regenerate under certain circumstances, potentially setting the stage for us to appreciate why adult human hearts fail to regenerate.

Modern genomic technology can provide a broad molecular characterization of specific cell and tissue states. In this issue of *Circulation: Genomic and Precision Medicine*, Adamowicz et al[6] present a wealth of data regarding the total transcriptomic profile of newborn mouse hearts under normal (physiological) development and during the regenerative response after myocardial infarction. Here, using RNASeq technology, they reveal not only the relative amount of messenger RNAs but also lncRNAs and microRNAs in a time-course study. Left ventricular samples were collected within at least 4 time intervals and pairwise compared between the conditions (Figure).

The transition from the fetal–perinatal to the adult period is highlighted by several microenvironmental changes that require adaptation by cardiomyocytes. There is an abrupt increase in the supply of oxygen to cardiomyocytes after birth, which progressively leads to cell-cycle arrest and a metabolic shift.[7] In addition, the heart has an important progressive increment in the strength of mechanical stimuli because of increasing cardiac work required to pump blood to the growing body. Changes in the transcriptional profile reflect these major postnatal stimuli. A large number of genes have their expression levels modulated, and there are also changes in gene isoforms,[8] all part of the adaptation and maturation of the cardiac tissue.

Adamowicz et al confirmed that thousands of mRNAS and hundreds of lncRNAs and miRNAs undergo variation during the 10 days of the developmental period assessed. Surprisingly, most differently expressed molecules in post-myocardial infarction samples overlap with those related to development. This likely indicates that there are common developmental pathways being activated when needed to regenerate the tissue. In particular, the authors reveal a relative enrichment of oxi-

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Key Words: Editorials ■ heart ■ regeneration ■ RNA ■ transcriptome

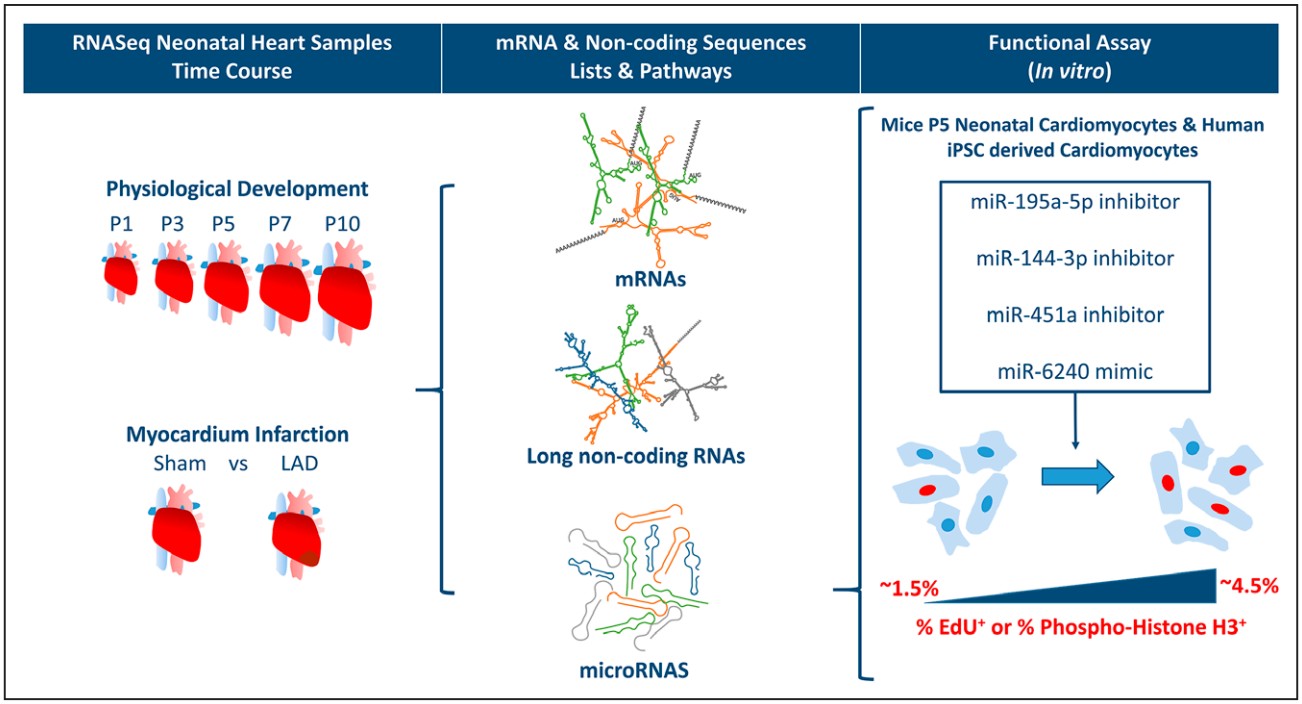

Figure. Deep molecular characterization of heart regeneration.
Left ventricular samples from 2 conditions (physiological development or myocardial infarction) were collected and pairwise compared within at least 4 time intervals. Lists of most differentially expressed mRNAS, long noncoding RNAs, and miRNAs were generated and enriched pathways described. Ten miRNA candidates were functionally tested for interference on proliferation of mouse neonatal cardiomyocyte or human-induced pluripotent stem cell (iPSC)-derived cardiomyocytes. Finally, 4 miRNAs (mir-195a-5p, mir-144-3p, mir-451a, and mir-6240) changed the percentage of EdU (5-ethynyl-2′-deoxyuridine) and PH3 (phospho-histone H3 proteins)-positive cardiomyocytes, indicating their potential roles in heart regeneration. LAD indicates ligation of the left anterior descending coronary artery.

dative phosphorylation, phagosome, and focal adhesion pathways in these samples.

FOCAL ADHESION AS PROXY FOR NEONATAL MOUSE CARDIAC REGENERATION?

Focal adhesion complexes are specialized structures on the plasma membrane that connects the extracellular matrix to the cellular cytoskeleton and it contributes to both anchoring, transmission of mechanical tension and signaling.[9] One of the central molecules of the complex, FAK (focal adhesion kinase), is ubiquitously expressed and responsible for regulating the response to hypertrophic agonists and biomechanical stress. In cardiac fibroblasts, when FAK is deleted, there is abnormally low proliferation and higher migration.[10] In both neonatal and adult cardiomyocytes, FAK is highly expressed and, within this context, plays a role as a biomechanical sensor. When FAK is overactivated by mechanical stimuli[11] or conditionally deleted,[12] cardiac hypertrophy results, showing FAK's central role in cardiomyocyte size. The activation of FAK promotes the maturation of human-induced pluripotent stem cell-derived cardiomyocytes in vitro.[13] When FAK is activated by specific cell substrates, polyploidy, reduced proliferation, and more terminally differentiated-like cardiomyocytes can result.[13] This activation also participates in intercalated disk assembly, resulting in more electrophysiologically connected cardiomyocytes in engineered heart tissues generated when using rat neonatal cardiomyocytes.[14] Alternatively, when FAK is inhibited, cardiomyocyte size is reduced and less β-MHC (beta myosin heavy chain) expression is observed in human-induced pluripotent stem cell-derived cardiomyocytes in vitro.[13]

Here, Adamowicz et al show upregulation of focal adhesion-related gene expression during early postnatal development (as denoted by higher *Z*-score values of P10 versus P7 and P7 versus P5 comparison), presumably because of activation of the FAK pathway while the heart matures after birth. It is also noteworthy that some differently expressed lncRNAs are associated with gene sets enriched for focal adhesion pathway and miRNA targeting this pathway overlap in infarcted hearts and during development. Based on this, it is possible to conjecture that the activation of the focal adhesion pathway, specifically in this circumstance, might lead to a more mature cardiomyocyte during physiological development. In contrast, inhibition of this pathway, as

detected at P3–P5-infarcted animals, might permit the proliferation of immature cardiomyocytes—a necessary event for heart regeneration.

FUNCTIONAL SCREENING

Genomics yields many insights, but specific gene experiments provide validation and more mechanistic insights. Adamowicz et al tested the functional influence of at least 10 of the detected miRNAs. Mouse P5 neonatal cardiomyocytes and human-induced pluripotent stem cell-derived cardiomyocytes were treated with these candidates to test whether there would be modulation of cyclin gene family mRNA expression, DNA synthesis, measured in vitro by incorporation of EdU (5-ethynyl-2′-deoxyuridine) and also entry into mitosis (G2/M transition), indicated by the overexpression of PH3 (phospho-histone H3 protein). Among candidates, when the microRNAs miR-22-5p, miR-144-3p, miR-195a-5p, and miR-451a were transduced, there was a ≈3-fold increase in both parameters, with proliferative cardiomyocytes increasing from ≈1.5% to 4.5% and a significant alteration of the Ccna2, Ccnd2, and Ccne2 expression levels. The 3 first miRNAS appear to be overexpressed in P5 hearts during development (when comparing with P3), perhaps impeding cell proliferation on this stage. In particular, miR-22-5p targets some genes related to FAK pathway, as indicated by the authors. miR-22-5p has also been shown to be strongly related to differentiation and cardiomyocyte hypertrophy when upregulated.[15] On the other hand, miR-6240 overexpression was mimicked in vitro (as observed in infarcted hearts at P5), increasing the proliferation of cardiomyocytes. Although the FAK pathway has other well-defined roles and signaling regulation in other settings, based on the data presented, it is possible that specifically during neonatal regeneration, it has a mediating role on cardiomyocyte proliferation regulated by miRNAs. Considering the multiple number of targets that miRNAs have and some contradictory[12] and lack[16,17] of evidences to this hypothesis, the exact details and mechanisms deserve to be further explored.

Taken together, the present study brings new and unexpected molecules and pathways to the complex regulation of mammalian heart regeneration, and this leads to new intriguing questions. What would happen to neonatal heart regeneration if the focal adhesion pathway is specifically abrogated during the neonatal period? Would it be possible to extend the regenerative time window, as per example of inhibition of DNA damage response?[7]

We are learning the road map of successful heart regeneration from neonatal animals and adult organisms like zebrafish and axolotls that can regenerate their hearts through adulthood.[18] We know that many features of successful heart regeneration are evolutionarily conserved,[18] including regulation of the hippo pathway,[19] an immune cell response,[20] and a wave of cardiomyocyte proliferation. Completing the road map will likely tell us why adult humans run off the road and fail to regenerate their hearts.

DISCLOSURES

None.

AFFILIATION

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FOOTNOTES

Circ Genom Precis Med is available at http://circgenetics.ahajournals.org.

REFERENCES

1. Drenckhahn JD, Schwarz QP, Gray S, Laskowski A, Kiriazis H, Ming Z, et al. Compensatory growth of healthy cardiac cells in the presence of diseased cells restores tissue homeostasis during heart development. *Dev Cell*. 2008;15:521–533. doi: 10.1016/j.devcel.2008.09.005.
2. Eschenhagen T, Bolli R, Braun T, Field LJ, Fleischmann BK, Frisén J, et al. Cardiomyocyte regeneration: a consensus statement. *Circulation*. 2017;136:680–686. doi: 10.1161/CIRCULATIONAHA.117.029343.
3. Porrello ER, Mahmoud AI, Simpson E, Hill JA, Richardson JA, Olson EN, et al. Transient regenerative potential of the neonatal mouse heart. *Science*. 2011;331:1078–1080. doi: 10.1126/science.1200708.
4. Zogbi C, Saturi de Carvalho AE, Nakamuta JS, Caceres Vde M, Prando S, Giorgi MC, et al. Early postnatal rat ventricle resection leads to long-term preserved cardiac function despite tissue hypoperfusion. *Physiol Rep*. 2014;2:pii: e12115. doi: 10.14814/phy2.12115.
5. Voges HK, Mills RJ, Elliott DA, Parton RG, Porrello ER, Hudson JE. Development of a human cardiac organoid injury model reveals innate regenerative potential. *Development*. 2017;144:1118–1127. doi: 10.1242/dev.143966.
6. Adamowicz M, Morgan CC, Haubner BJ, Noseda M, Collins MJ, Abreu Paiva M, et al. Functionally conserved noncoding regulators of cardiomyocyte proliferation and regeneration in mouse and human. *Circ Genom Precis Med*. 2018;11:e001805. doi: 10.1161/CIRCGENETICS.117.001805.
7. Puente BN, Kimura W, Muralidhar SA, Moon J, Amatruda JF, Phelps KL, et al. The oxygen-rich postnatal environment induces cardiomyocyte cell-cycle arrest through DNA damage response. *Cell*. 2014;157:565–579. doi: 10.1016/j.cell.2014.03.032.
8. Gilsbach R, Preissl S, Grüning BA, Schnick T, Burger L, Benes V, et al. Dynamic DNA methylation orchestrates cardiomyocyte development, maturation and disease. *Nat Commun*. 2014;5:5288. doi: 10.1038/ncomms6288.
9. Burridge K. Focal adhesions: a personal perspective on a half century of progress. *FEBS J*. 2017;284:3355–3361. doi: 10.1111/febs.14195.
10. Manso AM, Kang SM, Plotnikov SV, Thievessen I, Oh J, Beggs HE, et al. Cardiac fibroblasts require focal adhesion kinase for normal proliferation and migration. *Am J Physiol Heart Circ Physiol*. 2009;296:H627–H638. doi: 10.1152/ajpheart.00444.2008.

11. Torsoni AS, Constancio SS, Nadruz W Jr, Hanks SK, Franchini KG. Focal adhesion kinase is activated and mediates the early hypertrophic response to stretch in cardiac myocytes. *Circ Res*. 2003;93:140–147. doi: 10.1161/01.RES.0000081595.25297.1B.
12. Peng X, Wu X, Druso JE, Wei H, Park AY, Kraus MS, et al. Cardiac developmental defects and eccentric right ventricular hypertrophy in cardiomyocyte focal adhesion kinase (FAK) conditional knockout mice. *Proc Natl Acad Sci USA*. 2008;105:6638–6643. doi: 10.1073/pnas.0802319105.
13. Herron TJ, Rocha AM, Campbell KF, Ponce-Balbuena D, Willis BC, Guerrero-Serna G, et al. Extracellular matrix-mediated maturation of human pluripotent stem cell-derived cardiac monolayer structure and electrophysiological function. *Circ Arrhythm Electrophysiol*. 2016;9:e003638. doi: 10.1161/CIRCEP.113.003638.
14. Sun H, Tang J, Mou Y, Zhou J, Qu L, Duval K, et al. Carbon nanotube-composite hydrogels promote intercalated disc assembly in engineered cardiac tissues through β1-integrin mediated FAK and RhoA pathway. *Acta Biomater*. 2017;48:88–99. doi: 10.1016/j.actbio.2016.10.025.
15. Huang ZP, Chen J, Seok HY, Zhang Z, Kataoka M, Hu X, et al. MicroRNA-22 regulates cardiac hypertrophy and remodeling in response to stress. *Circ Res*. 2013;112:1234–1243. doi: 10.1161/CIRCRESAHA.112.300682.
16. DeLaughter DM, Bick AG, Wakimoto H, McKean D, Gorham JM, Kathiriya IS, et al. Single-cell resolution of temporal gene expression during heart development. *Dev Cell*. 2016;39:480–490. doi: 10.1016/j.devcel.2016.10.001.
17. Quaife-Ryan GA, Sim CB, Ziemann M, Kaspi A, Rafehi H, Ramialison M, et al. Multicellular transcriptional analysis of mammalian heart regeneration. *Circulation*. 2017;136:1123–1139. doi: 10.1161/CIRCULATIONAHA.117.028252.
18. Uygur A, Lee RT. Mechanisms of cardiac regeneration. *Dev Cell*. 2016;36:362–374. doi: 10.1016/j.devcel.2016.01.018.
19. Heallen T, Zhang M, Wang J, Bonilla-Claudio M, Klysik E, Johnson RL, et al. Hippo pathway inhibits Wnt signaling to restrain cardiomyocyte proliferation and heart size. *Science*. 2011;332:458–461. doi: 10.1126/science.1199010.
20. Aurora AB, Porrello ER, Tan W, Mahmoud AI, Hill JA, Bassel-Duby R, et al. Macrophages are required for neonatal heart regeneration. *J Clin Invest*. 2014;124:1382–1392. doi: 10.1172/JCI72181.

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Circ Genom Precis Med. 2018;11:
doi: 10.1161/CIRCGEN.117.002053
Circulation: Cardiovascular Genetics is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

Print ISSN: 1942-325X. Online ISSN: 1942-3268