The heart of adult humans and other mammals possesses a limited regenerative capacity, which prevents reconstitution of cardiac contractility after loss of functional myocardium thereby facilitating heart failure. In contrast, the hearts of zebrafish and newts exhibit a remarkable ability to regenerate after injury. Fetal and neonatal mouse hearts can also repair and regenerate myocardial damage after various types of injuries, for example, apical resection, cryoinjury, and myocardial infarction, raising hopes that a better understanding of repair processes in neonates might help to stimulate regeneration in adult mammalian hearts. The response to cardiac injury in neonatal mouse hearts was found to be associated with cardiomyocyte dedifferentiation as defined by the disassembly of sarcomeric structures, increased propensity for cell-cycle entry, and expression of markers characteristic for immature cardiomyocytes. However, a comprehensive characterization of transcriptional changes in damaged neonatal mouse hearts was missing to date.

In the current issue of Circulation Research, O’Meara et al analyzed the transcriptional signature of mouse hearts at different stages of postnatal development (p0, p4, p7, and 8- to 10-week-old mice) and of embryonic stem cells undergoing cardiac differentiation. RNA-sequencing analysis revealed increased expression of multiple genes, which function in mitochondrial, sarcoplasm, and sarcomere-specific processes whereas genes primarily involved in RNA processing (RNA transport, RNA splicing, and RNA capping) and cell cycle were downregulated. Acquired data sets were compared with transcriptional profiles of explanted adult mouse cardiomyocytes, which dedifferentiate for several days of ex vivo culture. The authors found that genes activated during differentiation (eg, genes involved in metabolism and cardiac contraction) were decreased during explant culture and genes downregulated during differentiation were activated (eg, genes involved in the cell cycle and RNA processing). Using a model of cardiac repair/regeneration based on apical resection of neonatal mouse hearts (p1), O’Meara et al compared transcriptional profiles 1 and 7 days after resection (1 and 7 dpr) in relation to sham-operated hearts. The analysis revealed striking differences with only a small overlap of differentially expressed genes: 1 dpr hearts were characterized by inflammatory and wound healing responses, whereas at 7 dpr activation of cell-cycle genes and contractility-related genes dominated. Interestingly, the authors found a significant overlap of genes that were downregulated during differentiation and upregulated during the course of ex vivo culture and those that were upregulated at 7 dpr indicating reversion of differentiation and acquisition of a more immature state of newborn hearts at 7 dpr. Finally, systematic integration of differentially expressed genes and pathway analysis using all available data sets identified 150 potential upstream regulators of cardiomyocyte differentiation/dedifferentiation and cell-cycle activity. This set of genes comprised molecules, which have been implicated previously in the regulation of cardiomyocyte cell-cycle activity, dedifferentiation, and regeneration such as neuregulin 1, oncostatin M, Let-7, and others. Oncostatin M plays a protective role after myocardial infarction by promoting cardiomyocyte dedifferentiation although it does not stimulate cardiomyocyte proliferation on its own. Neuregulin 1 has been claimed to induce cell-cycle re-entry of cardiomyocytes, thereby improving cardiac function after ischemic damage of the heart although this function was disputed by other studies. Further potential regulators included Hoxa9, which forms a complex with Meis1 that regulates postnatal cardiomyocyte cell-cycle arrest by activation of the cyclin-dependent kinase inhibitors p15, p16, and p21. Another interesting candidate was the mRNA Let-7a/c, which together with miR-99/100 was demonstrated to serve a critical function in cardiomyocyte dedifferentiation during zebrafish heart regeneration. In vivo silencing of both Let-7a/c and miR-99/100 in adult mice after myocardial infarction promoted cardiomyocyte dedifferentiation and improved heart function. Altogether, the systematic integrative transcriptional profiling approach by O’Meara et al identified a core set of factors with proven and potential functions in the regulation of cell-cycle re-entry and cardiomyocyte dedifferentiation in damaged hearts (Figure 1).

Next, O’Meara et al asked whether interleukin (IL)-13, one of the predicted upstream regulators whose function in the context of cardiac regeneration has not been analyzed to date, induces cell-cycle re-entry of neonatal cardiomyocytes. Interestingly, IL-13 treatment increased the number of BrdU (bromodeoxyuridine)-incorporating and Ki67-positive neonatal cardiomyocytes via the IL-13Ra1/IL-4Ra, STAT3/STAT6 pathway and stimulated expression of peristin, a potential STAT3 target. Peristin has been claimed to induce cell-cycle re-entry of differentiated cardiomyocytes and promote repair after myocardial infarction, although several other groups
have questioned these functions. Increased periostin expression downstream of STAT3 might therefore reflect activation of the IL-13Ra1/IL-4Ra, STAT3/STAT6 pathway rather than serving a dedicated function in stimulation of cardiomyocyte cell-cycle activity in vivo. STAT3 plays also a major role in the regeneration of adult zebrafish hearts, which together with the identification of Let-7 as a crucial regulator of heart regeneration clearly indicates that key regeneration pathways are conserved in different animal model systems.

The study by O’Meara et al provides detailed insights into transcriptional changes during neonatal heart repair and confirms previous studies postulating that reversal of the differentiated state of cardiomyocytes is closely associated with cell-cycle re-entry and myocardial regeneration. It also provides a core set of potential regulators that might control repair/regeneration of the neonatal mouse heart. Functional analysis of the IL-13Ra1/IL-4Ra, STAT3/STAT6 pathway demonstrated the relevance of such an approach to influence cardiomyocyte proliferation although further in vivo studies in cardiac regeneration models are still pending. In addition, it remains to be demonstrated whether identified regulators of neonatal heart repair/regeneration are also efficient in the adult heart. In fact, there is currently no evidence that IL-13 is able to elicit cell-cycle entry of adult cardiomyocytes. Adult cardiomyocytes are much more refractory to cell-cycle entry than neonatal cardiomyocytes probably because of major changes in chromatin organization, loss of cellular organelles such as centrosomes, lasting metabolic changes, and other reasons. However, transgenic overexpression of cyclin D2 is able to increase the proliferative activity of adult cardiomyocytes resulting in infarct regression, although it has been difficult to exclude that constitutive expression of cyclin D2 before terminal differentiation sustains the proliferative capacity of developing cardiomyocytes rather than inducing de novo cell-cycle entry of postmitotic cells. Combinatorial approaches stimulating cardiomyocyte dedifferentiation and inducing cell-cycle entry seem promising in this respect taking into account the reversal of the differentiated state in a physiological model of mammalian heart regeneration as disclosed by the work of O’Meara et al.

A better understanding of the mechanisms that integrate and coordinate the large-scale shifts in gene expression between proliferation-competent neonatal and primarily postmitotic adult cardiomyocytes will be instrumental to decipher and control the processes of dedifferentiation and regeneration. Most likely, epigenetic regulatory events will play a key role as also suggested by the current study, which identified a significant number of genes involved in chromatin modification that were downregulated during cardiomyocyte differentiation and reactivated during dedifferentiation in explant culture. Epigenetic regulatory mechanisms contribute to the maintenance of distinct chromatin states and cell-type–specific gene expression patterns, a phenomenon referred to as epigenetic memory. For example, it has been shown that enrichment of the repressive mark H3K9me3 at promoters of cell-cycle genes in cardiomyocytes leads to irreversible silencing. Removal of such repressive marks and other epigenetic modifications must be achieved to rewire the highly dynamic changes in DNA methylation and chromatin modifications characterizing the transition between embryonic, neonatal, and adult stages.

Cardiomyocytes do not exist in splendid isolation in the myocardium but require constant interactions with the local environment and neighboring cells, which might be particularly important for understanding regenerative processes. Transcriptional profiling of different homogeneous cell populations or single cells at different time points of heart regeneration will further improve our understanding of the molecular and cellular basis of heart regeneration and extend the current study, which primarily focuses on cardiomyocytes. In addition, it is important to increase the number of biological replicates to define the physiological range of variation between animals. Comparison and integration of the growing number of transcriptome datasets from hearts of different regeneration-competent model organisms will also help to obtain a more complete picture of the key regulators of cardiac regeneration.
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