Created Equal?
The Many Facets of Cell Reprogramming

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Reprogramming, transdifferentiation, transdetermination, and dedifferentiation represent multiple aspects of the same phenomenon, ie, the conversion of one cell type into another. Collectively, these cell transformations include (1) the switch from one differentiated cell to another differentiated cell type; (2) the developmental plasticity of stem cells that, by crossing tissue boundaries, commit to lineages different from those of the organ of origin; and (3) the regression of mature cells to a proliferative condition or to a unipotent or multipotent progenitor state. This unexpected biological flexibility has challenged the view that changes in phenotype could not take place once a cell, through a hierarchical progressive restriction of developmental options, had attained its stable mature properties. Similarly, adult stem and progenitor cells may not be discrete “fixed” entities but functional units, and a bidirectional shift between more primitive and more differentiated phenotypes may occur.

In the elegant study by Thal and collaborators in the present issue of Circulation Research, small molecules induced molecular and functional reprogramming of endothelial progenitor cells (EPCs), a class of bone marrow cells currently used in Phase 2 and 3 clinical trials for the treatment of refractory angina and peripheral artery disease. The identification of EPCs in the bone marrow and circulating blood and the encouraging results of the ongoing clinical studies have demanded a reconsideration of traditional vascular biology. Migration and homing of EPCs to regions of damage has been shown to contribute to endothelial cell turnover and vessel growth, providing an alternative mechanism of vessel repair. EPCs generate de novo capillary structures after delivery to infarcted hearts and ischemic hindlimbs and form an endothelial lining in vascular grafts and on the surface of left ventricular assist devices. EPCs, however, are characterized by a limited ability to transdifferentiate into cardiomyocytes and vascular smooth muscle cells. This limitation has been overcome in the present study by the introduction of a simple but extremely effective in vitro treatment of EPCs that prompted the formation of cardiomyocytes and smooth muscle cell–covered vessels in an experimental model of myocardial infarction.

The high vasculogenic potential of EPCs reflects their capacity to create a large number of capillaries in the tissue surrounding the ischemic lesion. In the present study, the spectrum of developmental choices of EPCs has been broadened by modulating the epigenetic control of stemness and lineage-related genes with inhibitors of DNA methyltransferases, histone deacetylases, and histone methyltransferases. The acquisition of a remarkably plastic state did not require the irreversible insertion of proviral integrants in the genome of mouse and human EPCs and was not coupled with unrestrained pluripotency and tumorigenic potential in vivo. The alternative destiny of ex vivo manipulated EPCs remained restricted to cardiovascular lineages; EPCs retained the ability to differentiate into endothelial cells and attained the novel option to give rise to cardiomyocytes and smooth muscle cells. The partial reversion of the endothelial-selective fate of EPCs required the upregulation of transcripts for Oct3/4, Nanog, and Sox2, which converted EPCs in bona fide cardiovascular progenitors.

The preservation of the endothelial identity of EPCs makes the form of reprogramming obtained by Thal and colleagues a true transdifferentiation process. Transdifferentiation is defined as the mechanism by which adult stem/progenitor cells break the law of tissue fidelity and generate mature cells beyond their own organ boundaries. This cellular adaptation may not be a rare event if we consider the nearly infinite number of permutations that occur in each individual during prenatal development and postnatal life. Transdifferentiation may represent a normal aspect of cell turnover, but it remains one of the most questioned modalities of growth and repair in the adult organism.

Recently, reprogramming of somatic differentiated cells into induced pluripotent stem cells capable of committing to most cell types in the organism has received great attention in the scientific community and in the media. This process, however, is not a component of the naturally occurring repair response, calling into question its biological importance. The alluring prospect of using induced pluripotent stem cells therapeutically has been wiped out by the documentation that adoptive transfer of these powerful cells results in the formation of teratomas. Moreover, activation of the T cell–dependent immune reaction against the transplanted graft has been demonstrated, challenging the dogma that syngeneic and autologous induced pluripotent stem cells can bypass immune rejection. Currently, induced pluripotent stem cells are used as a platform for drug screening and human disease modeling; caution, however,
has to be exercised, because the differentiated progeny of the parental induced pluripotent stem cell may fail to faithfully recapitulate in vitro the pathological defects observed in vivo.7

Dedifferentiation of adult postmitotic cardiomyocytes into proliferative immature myocytes or progenitor cells has not been proven to take place physiologically in the mammalian heart.9 In zebrafish and neonatal mice, the poorly organized sarcomeric structure of replicating myocytes has led to the conviction that dedifferentiation constitutes the primary step of cardiac repair after injury.10-11 Unfortunately, the genetic fate mapping strategies used in these studies fail to discriminate the contribution of preexisting parenchymal cells and progenitor cells to organ turnover, leaving unanswered the question of whether newly formed myocytes correspond to cells that underwent dedifferentiation or reflect cells derived from lineage specification of stem cells. Because of the intrinsic limitations of lineage tracing, the evidence of dedifferentiation has been based exclusively on cellular morphology.9 Cells characterized by an intermediate phenotypic fate between a primitive and a fully mature state have arbitrarily been considered the product of dedifferentiation. However, the lack of structural markers typical of a given cell type is not by itself indicative of the developmental stage of the cell; it is impossible to define in vivo whether the cell of interest is undergoing differentiation or whether it is in the process of reverting to an earlier, immature cell function.

The relevance of induced cell pluripotency and myocyte dedifferentiation for the development of novel cell-based therapeutic protocols in patients is, at present, uncertain. This reality is in striking contrast to the biologically and clinically important strategy that has been undertaken in the study by Thal and collaborators,2 in which fundamental issues of progenitor cell biology and myocardial healing have been recognized and addressed. Ideally, cardiac regeneration should coincide with restitutio ad integrum of the damaged organ and formation of a tissue that closely resembles the structural and functional properties of the healthy parenchyma. This objective can be achieved when multipotent functionally competent exogenous or endogenous progenitor cells are involved in the generation of new myocardium composed of cardiomyocytes and coronary vessels. However, chronological aging, cardiac diseases, and comorbidities affect the functional integrity of bone marrow and cardiac progenitors,12 conditioning the functional properties of these primitive cells. The age-dependent changes in the biology of stem cells may include loss of self-renewing capacity, forced entry into an irreversible quiescent state, and biased differentiation.

As documented in the present study,2 the transdifferentiation of EPCs was dictated by epigenetic mechanisms, which are implicated in gene activation and silencing at the level of transcription. Epigenetic processes condition the packaging of DNA and histones into highly condensed heterochromatin or loose unfolded euchromatin.13 Although euchromatin is permissive, heterochromatin is resistant to transcriptional activation. Typically, epigenetics is implicated in the regulation of pluripotency and differentiation of embryonic stem cells by preserving the uncommitted state or promoting the acquisition of specific cell lineages. The work by Thal and colleagues has clarified that the competence of EPCs to form a specific progeny is conditioned by intrinsic repressive and activating epigenetic mechanisms.2 The undifferentiated and differentiated states of EPCs were found to be epigenetically regulated by acetylation and methylation of lysine residues of core histones.14 Histone acetylation is typically associated with increased transcription, whereas histone methylation may condition upregulation or silencing of gene expression.13 Although trimethylation of histone H3 at lysine 27 (H3K27me3) constitutes the major repressive mark in embryonic stem cells, this function may be replaced in adult progenitors by dimethylation of histone H3 at lysine 9 (H3K9me2). This epigenetic modification was significantly reduced in drug-treated mouse and human EPCs and was accompanied by a higher degree of acetylation of histone H3 at lysine 9 (H3K9) and pan-acetylation of histone H4.2 Global lysine acetylation in histone H3 and H4 activates the network of stemness-related genes in the primitive cells of the inner mass.14

Similar epigenetic changes have been documented in a recently identified category of coronary vascular stem cells,15 C-kit–positive cardiac stem cells with significant vasculogenic potential (vCSCs) are characterized by the co-existence of the inactivating marks H3K27me3 and H3K9me2 and the activating mark H3K4me2 (Figure). Moreover, vCSCs exhibit 2 acetylation sites in histone H3 at lysine 9 (H3K9Ac) and lysine 14 (H3K14Ac). The bivalent configuration of EPCs and vCSCs indicates that the chromatin structure of these 2 cell categories has a dynamic configuration and possesses a certain level of plasticity. Whether these epigenetic changes target promoter regions of pluripotency and lineage genes in vCSCs is unknown. In contrast, chromatin immunoprecipitation assays of EPCs in the present study have revealed the specific histone acetylation and methylation pattern in the promoter regions of genes involved in stemness and cardiomyocyte differentiation.2 H3K9Ac was bound to the promoter regions of Oct4, activin 2, ryanodine receptors, and troponin T. DNA methylation of Nkx2.5 did not differ in untreated and treated EPCs, pointing to histone acetylation as the major modulator of EPC plasticity. Thus, both genome-wide and promoter-specific epigenetic modifications provide the molecular bases for the switch in fate of EPCs.2

Reprogrammed mouse EPCs and human CD34-positive cells injected in the border zone of a myocardial infarct led experimentally to the repair of the necrotic tissue and the formation of functionally competent myocardium.2 The regenerated tissue decreased infarct size and cardiac fibrosis, which resulted in enhanced ejection fraction and fractional shortening, as well as attenuation of ventricular dilation. Additionally, the paracrine activity of the delivered cells was enhanced by drug treatment with secretion of a large quantity of proangiogenic factors, which reduced apoptosis and increased cell proliferation. These findings argue in favor of transdifferentiation of EPCs into myogenic and vascular cell lineages, important variables of cardiac repair.

Findings in the study by Thal and collaborators2 contribute to fill a gap in the limited knowledge of the epigenetic
of this state is mediated by the histone demethylase Jmjd3. Silencing of Jmjd3 induces apoptosis and senescence and inhibits hypoxia-mediated upregulation of eNOS in proangiogenic cells. These observations have made apparent that gene silencing and activation in EPCs is more complex than originally thought.

Epigenetic modifications are important determinants of stem cell senescence, organism aging, and cardiac diseases, suggesting that chronological age and heart failure may lead to epigenetic lesions of EPCs. Chromatin remodeling may affect the phenotypic plasticity of EPCs and their ability to respond to alterations in the cardiac microenvironment, which occur in the old heart and with chronic heart failure. Over time, telomeric shortening takes place, and telomere attrition may be coupled with the expression of senescence-associated genes in human EPCs, inhibiting cell replication and triggering cell death. Findings in the present study suggest that epigenetic compounds can be used to modulate the fate of old EPCs, restoring their viability, preventing senescence, and enhancing their fate options. Whether a peculiar histone code characterizes senescent EPCs in old individuals and whether this molecular signature is comparable to that found in EPCs of younger patients with heart failure remain important unanswered questions.

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None.

References


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