Identity Deception: Not a Crime for a Stem Cell

Stem cell transdifferentiation in the adult organism is the most common and questioned mechanism of growth and repair. Recent data suggest that adult stem cells are capable of generating mature cells beyond their own tissue boundaries, a process called developmental plasticity. To date, the most versatile cell discovered is the bone marrow progenitor cell.

Originally, transdifferentiation was defined as an irreversible switch from one differentiated cell to another differentiated cell type (41, 51). It was considered a rare event, mainly observed in cultured cells. A typical example of this paradigm in vivo was found in the ability of the pigmented cells of the iris to transdifferentiate into lens cells following removal of the existing lens in newts (14, 16, 21). From a broader perspective, transdifferentiation corresponds to the conversion of one cell type into another (51). This biological process belongs to the large class of cell transformations defined as metaplasia (41, 51). Metaplasia also includes cases in which stem cells of one tissue acquire the cell phenotype of another tissue (51).

With the recent explosion of the field of regenerative medicine and stem cell therapy, transdifferentiation—that is, stem cell plasticity—is undergoing careful scrutiny. This is because the possibility that adult stem cells can give rise to cells different from the organ of origin has created excitement and skepticism, believers and opponents. Because of controversial findings that are impossible to reconcile (2, 5, 11, 19, 26, 31–33, 46, 48), a consensus about the actual plastic potential of stem cells has not yet been reached. The general view was that once a cell had reached a stable differentiated state it could not change its phenotype. During prenatal development, cells undergo a hierarchical progressive restriction of developmental options (6, 16, 41, 51). This phenomenon was thought to be inviolable in adulthood (41, 51). However, several examples of transition from one cell type to another or from one cell lineage to a different lineage have challenged this accepted dogma in developmental biology (1, 28, 42).

Pancreatic tissue has been found in cirrhotic liver, and this observation has prompted studies about the conversion of liver into pancreatic structures (38). Because liver and pancreas arise from adjacent regions in the embryo, it has been suggested that their specification differs by a single developmental decision, affecting the expression of a relatively small number of master switch genes (17, 39, 49, 50). Surprisingly, the overexpression of a single transcription factor, Pdx1VP16, is sufficient to promote the conversion of the reprogramming of liver into pancreatic tissue that contains both endocrine and exocrine cells (9). This modification in cell fate has been defined as direct transdifferentiation (4, 13, 16), in contrast with transdifferentiation that requires a dedifferentiation step and the transdifferentiation that occurs through the involvement of a stem cell (FIGURE 1).

The form of transdifferentiation that necessitates the dedifferentiation of a mature specialized cell before it assumes a new phenotype has been definitively proven in only a few cases. During transdifferentiation of the iris-pigmented epithelial cells into lentoids (small clusters of lens cells), the starting cells progressively lose the granules of pigment and, after a phase of intense proliferation, dedifferentiate (22). Ascorbic acid favors the formation of mature lens cells and the regeneration of the lost ocular structure (22, 40). Similarly, dedifferentiation of alveolar mammary epithelium may be followed by transdifferentiation into epidermal and pilar structures when the β-catenin signaling pathway is markedly activated (29).

Other cases of phenotype conversion by dedifferentiation belong to the complex context of cancer cell growth. Defects in the control of cell maturation are thought to be of etiological significance in the early stages of carcinogenesis. This possibility is supported by a variety of experimental studies that have established the relationship between metaplasia and preneoplastic lesions (41, 51). In fact, malignancy may involve the uncontrolled growth of undifferentiated, dedifferentiated, or transdifferentiated cells. These abnormalities may be influenced or determined by a variety of factors, including genetic instability, proliferation, apopto-
sis, alterations of extracellular matrix, and growth factor activation. When mesenchymal stem cell-derived preadipocytes are induced to dedifferentiate, they can subsequently become macrophages. A cell derived from a stem cell that is unable to reach full differentiation has an increased proclivity to experience metaplastic changes or neoplastic transformation.

Stem cell transdifferentiation in the adult organism is the most common and questioned mechanism of growth (12, 18, 25, 42, 43, 54, 58). Recent data suggest that adult stem cells are capable of generating mature cells beyond their own tissue boundaries, a process called developmental plasticity. The most versatile cell is the bone marrow progenitor cell (BMPC). In this regard, BMPCs injected in the border zone of a myocardial infarct or mobilized systemically into the circulation with cytokines lead experimentally to the repair of the dead tissue and the formation of functionally competent myocardium (19, 32, 33). The wave of enthusiasm created by this discovery and the lack of effective new drugs for the treatment of heart failure has prompted cardiologists to the rapid implementation of BMPCs in the management of the severely decompensated infarcted human heart (44).

Currently, bone marrow cells or circulating bone marrow cells, including endothelial progenitor cells, are utilized in patients, and several initial clinical trials have consistently shown positive results (27, 34, 37, 60). Because of the compelling need to treat severely ill patients, clinicians are leading the field of cellular therapy and cardiac regeneration. Conversely, statements of caution are advanced by the scientific community concerning the necessity to acquire a better understanding of the mechanisms of recovery of cardiac function and repair before these protocols are introduced in the treatment of the diseased human heart.

However, despite the therapeutic efficacy of BMPCs in heart failure and models mimicking the
human disease, two studies (2, 31) and two commentaries (1a, 11) have presented and discussed negative results, criticizing the early experimental data and clinical trials. They questioned the ability of BMPCs to regenerate dead myocardium and claim that the original findings were a collection of artifacts, that all clinical trials were premature, and that they may have placed a group of very sick patients at high risk (2). Because of the impact that these positive and negative findings can have on the future treatment of the postinfarcted heart in humans, we have implemented a simple protocol that can easily be reproduced in laboratories with experience in models of myocardial infarction in small animals (19). Additionally, we have applied and emphasized the type of analysis that has to be performed to obtain reliable information. By this approach, we have demonstrated once more that BMPCs differentiate into myocytes and coronary vessels, replacing the infarcted myocardium (FIGURE 2) independently of cell fusion (19).

The reintroduction of the notion of cellular fusion, extremely popular in the 1980s (8, 30), has created uncertainty about stem cell plasticity. Cellular and/or nuclear fusion requires the merging of two distinct cells leading to formation of a hybrid cell. Similarly to the evolution of the concept of transdifferentiation, cell fusion originally referred only to the joining of two fully differentiated cells (8). This event was frequently seen following injury of fusogenic organs, such as the skeletal muscle and liver (10, 53, 56, 59). Since the publication of two in vitro reports describing the extremely rare occurrence of fusion in coculture systems of embryonic stem cells with bone marrow cells or neural progenitors (47, 61), the possibility that a phenotype conversion of an adult stem cell could mask cellular fusion in vivo has been promoted. The merging of an adult stem cell and a differentiated cell results in the formation of a binucleated heterokaryon or a mononucleated hyperploid synkarión (35). In this case, the growth of the binucleated heterokaryon seems to depend on the nucleus of the more undifferentiated cell that dominates the nucleus of the somatic cell by transferring its replication properties, whereas the destiny of the heterokaryon is regulated by the differentiat-ed cell (7, 35). When cellular fusion is accompanied by nuclear fusion, a mononucleated synkarión with a hyperploid DNA content is formed. In this condi-tion, the bulky burden of the high nuclear DNA content leads to genetic instability and reduced or null replicative potential (35).

The observations obtained in our laboratory argue strongly in favor of differentiation of BMPCs into the myogenic and vascular lineages as the mechanism of cardiac repair and against cell fusion as the cause of the new cardiac phenotype. The formed cardiac cells in female infarcted hearts have the male phenotype of the injected BMPCs (19, 32). Cell fusion remains essentially an in vitro phenomenon with little implication in vivo. Cell fusion in vivo in different organs, including the skin, the lung, the brain, and the heart, is restricted at most to a few cells that, by inference, have no physiological consequences on baseline function or on tissue repair in pathological states (15). Studies of cell fusion in the liver and skeletal muscle are problematic because cell fusion is an inherent aspect of the growth pathway of hepatocytes and myofibers. Under conditions of normal physiological turnover, fusion of BMPCs with parenchymal cells is an extremely rare event in these tissues (15).

There are several other factors that support BMPC transdifferentiation rather than cell fusion in myocardial regeneration after infarction. Following permanent coronary occlusion, all cells in the supplied myocardium die within 5 h. Essentially, there are no partner cells left for fusion. Adult myocytes have a volume of ~20,000 μm³, and therefore if cell fusion occurred under our condi-

**FIGURE 2.** Bone marrow cells transdifferentiate into myocytes and coronary vessels

This confocal image corresponds to regenerating myocardium in the infarcted area of a female mouse heart 10 days after transplantation of male bone marrow-derived Lin− c-kitPOS cells. Myocytes are depicted by the red fluorescence of α-sarcomeric actin. Smooth muscle cells, single or organized in a developing arteriole, are stained by the yellow fluorescence of α-smooth muscle actin. The presence of erythrocytes (TER-119, green) in the lumen of the newly formed arteriole documents its connection with the coronary circulation. The origin of myocytes and smooth muscle cells from the donor cells is demonstrated by the Y chromosomes in their nuclei (white dots). Nuclei are stained by DAPI.
tions, new myocytes should have a volume of 20,000 μm³ or larger. In contrast, these myocytes have a maximum size of ~2,000 μm³ and a minimum size of ~100 μm³. Donor-derived cells divide rapidly and extensively, whereas tetraploid cells divide slowly and might not divide at all if one of the partners is a terminally differentiated myocyte (35). Fusion of a BMPC to a myocyte that has reached irreversible growth arrest cannot stimulate its reentry into the cell cycle (35). Cell fusion should generate binucleated myocytes, with one tetraploid and one diploid nucleus, or myocytes with three diploid nuclei. This has never been the case (FIGURE 3).

The cre-lox genetic system is frequently used to detect cell fusion. However, this system is not perfect. It is surprising that the possibility of metabolic cooperation (45) was not considered, because this phenomenon may account for some of these observations. By metabolic cooperation, a cell acquires the Cre recombinase from a neighboring cell and undergoes excision of the flox-flanked DNA segment in the absence of cell fusion. The exchange of the enzyme between the donor cell and the recipient cell occurs through intercellular junctions. Metabolic cooperation is important in a tissue that is functionally a syncytium (FIGURE 4).

Theoretically, newly formed cardiomyocytes with a normal 2C DNA content could have resulted from hybrid cells that underwent reductive cell division, converting the hyperploid cell to a diploid karyotype, which concealed their fusion history (55). Reductive cell division of hybrid cells in vivo has only been documented in hepatocytes generated under a stringent selection pressure that conferred them survival and growth advantage (56). These “pseudodiploid” cells accumulate slowly and are found together with a large number of fused cells (15). Conversely, we found several million donor-derived myocytes to be diploid, with an XY chromosome complement as early as 5 and 10 days after cell implantation. The short interval between the injection of BMPCs and the generation of diploid male cells makes reductive mitosis an unlikely possibility (19, 34).

Whether the twist in fate occurs by transdifferentiation or fusion, reprogramming of chromatin configuration is required. The reorganization of chromatin is mediated by an alternate turning on and off of transcription factors to drive the adult stem cell toward the creation of a specific progeny (15, 39). This process could be slow and limited in efficiency. Moreover, if cell fusion is involved, the replicative potential of the fused cell is at best tran-
lineages, i.e., myocytes and vascular smooth muscle and endothelial cells. Additionally, the identification of cardiac stem cells clustered in niches indicates that the heart is a self-renewing organ that possesses an intrinsic growth reserve capable of responding to the physiological and pathological demands of the myocardium. Importantly, resident stem cells should be more efficient and powerful for tissue reconstitution. Because of the ingrained concept of the heart as a postmitotic organ, it has been assumed that attempts to replace lost myocytes require the introduction of exogenous cells into the damaged heart. The demonstration that the heart harbors stem cells capable of creating functional myocardium explains earlier observations of a robust regenerative response in the acute postinfarcted heart in humans (3) but raises the question of why this regenerative response stops before the repair process is completed. However, cardiac stem cells may be coaxed in vivo to home to the damaged region of the heart and then promote the formation of functionally competent myocardium. A rapid and efficient restoration of lost myocardium is often crucial for the survival of the organ and organism. This clinical necessity has its dramatic overtone in patients with large myocardial infarcts in which the immediate

sient. In organs such as the brain and the heart, cell fusion leads only to a temporary rescue of the old, preexisting, terminally differentiated parenchymal cell without additional growth (57). Heterokaryon formation in the Purkinje cells of the brain results in a transient rejuvenation of old or damaged cells that is not associated with the acquisition of replication potential (23, 57). Nuclear reprogramming of transdifferentiated cells involves epigenetic modifications of chromatin. Extrinsic induction signals present in the new environment appear to drive chromatin remodeling. The principal epigenetic mechanisms by which tissue-specific gene-expression patterns and global gene silencing are established or maintained involve chromatin modification. This includes DNA methylation and histone acetylation, phosphorylation, methylation, and ubiquitylation. Dynamic changes in these epigenetic modifications underlie chromatin remodeling that is responsible for cell transdifferentiation (20, 24, 36).

The recent discovery that resident progenitor cells exist in the adult myocardium overcomes the need for the complex and time-consuming process of nuclear reprogramming (35). This is because primitive cells nested in the cardiac microenvironment are predetermined to evolve into cardiac cell lineages, i.e., myocytes and vascular smooth muscle and endothelial cells. Additionally, the identification of cardiac stem cells clustered in niches indicates that the heart is a self-renewing organ that possesses an intrinsic growth reserve capable of responding to the physiological and pathological demands of the myocardium. Importantly, resident stem cells should be more efficient and powerful for tissue reconstitution. Because of the ingrained concept of the heart as a postmitotic organ, it has been assumed that attempts to replace lost myocytes require the introduction of exogenous cells into the damaged heart. The demonstration that the heart harbors stem cells capable of creating functional myocardium explains earlier observations of a robust regenerative response in the acute postinfarcted heart in humans (3) but raises the question of why this regenerative response stops before the repair process is completed. However, cardiac stem cells may be coaxed in vivo to home to the damaged region of the heart and then promote the formation of functionally competent myocardium. A rapid and efficient restoration of lost myocardium is often crucial for the survival of the organ and organism. This clinical necessity has its dramatic overtone in patients with large myocardial infarcts in which the immediate

![Image](https://example.com/image.png)

**FIGURE 4. Cre/lox regulation of the expression of a protein**

The gene lacZ has LoxP sequences containing a stop signal that prevents the expression of the gene. When exposed to the Cre recombinase protein, the LoxP and stop signal are excised and the gene lacZ is expressed, producing β-galactosidase. The formation of gap junctions may lead to the transfer of Cre recombinase to the cell carrying the lacZ gene. The end product is a β-galactosidase-positive cell in the absence of cell fusion.
reduction of infarct size is critical for survival. This possibility offers an alternative or complementary therapeutic approach to exogenous cells. The extraordinary clinical potential of myocardial stem cells makes the dissection of the biology of the cardiac stem cell a challenging and exciting endeavor.

References


