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REVIEW

Mechanisms of Myocardial Regeneration

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The plasticity of bone marrow-derived progenitor cells (BMPCs) and their ability to acquire the myocyte lineage and regenerate dead myocardium after infarction has been challenged. Similarly, although several laboratories have identified cardiac progenitor cells (CPCs), the controversy concerning myocyte regeneration in the adult heart has not been resolved. The therapeutic efficacy of these 2 classes of progenitor cells depends on their ability to (1) survive in the hostile milieu of the damaged heart, (2) engraft within the myocardium and (3) grow and differentiate. BMPCs may have a growth potential that is superior to that of CPCs, but transdifferentiation could affect this characteristic and CPCs may constitute a more powerful form of therapy for cardiac repair. The process of transdifferentiation may alter the growth behavior of BMPCs, which may result in losing part of their capability of dividing through alterations of the telomere–telomerase system, premature cellular senescence and apoptosis. Moreover, myocytes derived from BMPCs may possess inherent limitations in the acquisition of the adult phenotype. The opposite may also be true and BMPCs may retain a stronger regenerative capacity than CPCs, representing the most appropriate cells for the damaged heart even after transdifferentiation. Ultimately, the question to be addressed is whether BMPCs are superior, equal or inferior to CPCs for the regeneration of cardiomyocytes and coronary vessels in acute and chronic ischemic heart failure. (*Circ J* 2010; **74:** 13–17)

Key Words: Myocardial regeneration; Plasticity; Stem cells

Endothelial progenitor cells, mononuclear bone marrow cells and CD34-positive cells have been administered to patients affected by acute myocardial infarction or chronic ischemic heart failure. These interventions have had positive outcomes and document not only the feasibility and safety of this therapeutic approach, but also beneficial effects on cardiac function.[1–3] While patients are currently being enrolled in large clinical trials, the documentation of cardiac progenitor cells (CPCs) has created great expectation concerning the utilization of this new cell for the management of the human disease. Theoretically, the most logic and potentially powerful cell to be employed for cardiac repair is the CPC. Cardiac regeneration would be accomplished by enhancing the normal turnover of myocardial cells.[4,5] However, difficulties exist in the acquisition of myocardial samples in humans, and in the isolation and expansion of CPCs in quantities that can be employed therapeutically. Conversely, bone marrow-derived progenitor cells (BMPCs) constitute an appealing form of cell intervention because they can be easily collected from bone marrow aspirates or the peripheral blood upon their mobilization with cytokines and utilized clinically.

At present, it is unknown whether CPCs and BMPCs are similarly effective in reconstituting damaged myocardium or whether limitations exist in CPC and BMPC growth resulting in inadequate restoration of the injured heart. Although several laboratories have identified CPCs and documented their critical role in myocardial turnover and regeneration,[6,7] controversy persists concerning the plasticity of BMPCs and their ability to acquire the cardiomyocyte lineage after infarction.[8] Therefore, a fundamental question to be addressed is whether adult BMPCs transdifferentiate into cardiomyocytes and coronary vessels forming a functionally competent myocardium that repairs the acutely and chronically diseased heart. Additionally, BMPCs may constitute a necessary initial form of intervention for ischemic myocardial injury, whereas CPCs might be employed later during the chronic evolution of the cardiac myopathy. In all cases, however, the recipient tissue and the delivered progenitor cells have to interact at multiple levels before a substantial therapeutic effect with recovery of function can occur.

BMPC Homing

The therapeutic efficacy of BMPCs is dictated by 3 interrelated phenomena that condition the fate of the implanted cells: engraftment, replication and differentiation. In the absence of cell engraftment, which establishes structural, physical and molecular connections between resident and administered cells, BMPCs as well as any other cell type cannot exert a paracrine effect, a regenerative effect or both.[9] If BMPCs are delivered through the systemic circulation, the journey of these cells involves their migration through the peripheral blood and vessel wall before they can repopulate

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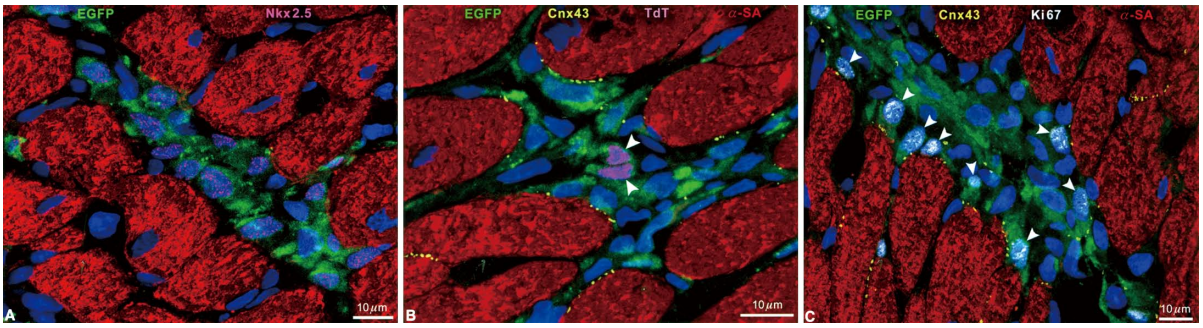

Figure 1. Differentiation of bone marrow-derived progenitor cells (BMPCs). Enhanced green fluorescent protein (EGFP)-positive BMPCs were injected in the border zone of the infarct in wild-type mice shortly after coronary artery occlusion. (**A**) A cluster of EGFP-positive BMPCs (green) express Nkx2.5 (magenta) documenting the acquisition of the myocyte lineage. (**B**) Two EGFP-positive BMPCs are apoptotic (TdT, magenta, arrowheads). Connexin 43 (Cnx43, yellow dots). (**C**) Several EGFP-positive BMPCs are cycling and express Ki67 (white, arrowheads). Confocal microscopy.

the damaged organ. The process by which circulating BMPCs traverse the endothelial barrier and reach the target tissue is termed homing.[10] This may lead to transient retention of cells or to their stable engraftment in niche structures. Homing and engraftment are mediated by similar mechanisms of cell migration and cell adhesion, but engraftment characteristically involves division of the seeded progenitor cells. When BMPCs are delivered via the systemic or coronary circulation, homing precedes cell engraftment, but when BMPCs are injected directly within the myocardium, the 2 events coincide.[9]

So far, documentation showing that BMPCs engraft within the myocardium adjacent to an acute infarct or damaged cardiac tissue is lacking and this information is critical for recognizing whether the delivered cells are taking short- or long-term residence within the heart. This is the premise of successful cell therapy. In the absence of this demonstration, it is impossible to determine whether the contrasting results published in the past few years from different laboratories are the consequence of technical errors in experimental protocols or whether they actually support the controversy in BMPC plasticity and myocardial repair.[11–15] Active engraftment necessitates the formation of gap and adherens junctions between resident and administered cells to promote a number of events leading to BMPC growth and differentiation.

Connexins are gap junction channel proteins that mediate the passage of small molecules and signals involved in cell-to-cell communication.[16] Survival factors and mitogens traverse gap junctions to oppose cell death and favor cell growth. Calcium, ATP, adenosine and cyclic nucleotides can translocate from 1 cell to another via gap junctions. Calcium influx or release from intracellular stores activates progenitor cell proliferation and maturation. Cadherins are calcium-dependent transmembrane adhesion molecules,[17] which have a dual function; they anchor stem cells to the microenvironment and promote interaction between stem cells, and between stem cells and supporting cells. Stem cell anchorage also depends on integrin receptors that bind progenitor cells to the extracellular matrix. Integrins and adherens junctions play a critical role in the maintenance of the quiescent state of primitive cells.[18]

Therefore, the recipient myocardium has a determinant influence on the destiny of the injected BMPCs. The formation of gap junctions between resident cardiomyocytes and implanted BMPCs favors the progressive loss of the hematopoietic phenotype with the acquisition of the cardiogenic fate (**Figure 1A**). However, BMPC differentiation into cardiomyocytes, coronary vascular endothelial cells and smooth muscle cells occurs only in engrafted cells. The lack of engraftment is invariably associated with the activation of apoptosis (**Figure 1B**) and the rapid reduction in the number of BMPCs within the host myocardium. Homed BMPCs commit to the myocyte lineage and divide expanding the pool of newly formed differentiating cells (**Figure 1C**). Additionally, coronary vessels are created. Conversely, N-cadherin is typically located between recipient cardiac cells and BMPCs that are no longer CD45 positive or CD34 positive and may have acquired the properties of resident CPCs. Importantly, these cells are quiescent and do not express the markers of the cell cycle Ki67, MCM5 and phospho-H3.

The pathways that guide BMPC homing within the myocardium are not understood. Cardiac injury and the microenvironment created by ischemic damage appear to enhance the migration and long-term engraftment of stem cells in the myocardium.[11] In the absence of tissue damage, the implanted progenitor cells are at a growth disadvantage with respect to endogenous stem cells. The ischemic myocardium provides a habitat that is particularly rich in cytokines favoring seeding, survival and growth of progenitor cells. The binding of stromal cell-derived factor-1 (SDF-1) to its receptor CXCR4 is critical in promoting homing of BMPCs to the bone marrow and distant organs although insulin-like growth factor-1 (IGF-1) and hepatocyte growth factor (HGF) may be crucial in opposing death signals and facilitating migration, respectively.[19–21] SDF-1, IGF-1 and HGF are acutely upregulated in the border zone after infarction and may enhance BMPC viability, translocation and homing. In this regard, the CD34-positive cell subset coincides with the CXCR4-positive fraction of BMPCs.

Distinct BMPC classes may be differently equipped to home to the injured heart. Unselected bone marrow cells and CD34-enriched BMPCs administered through the coronary circulation have different homing efficiency after infarction.[22] Shortly after delivery, only a small fraction (~2%) of unselected bone marrow cells is present in the myocardium, whereas ~25% of CD34-positive BMPCs are detected within

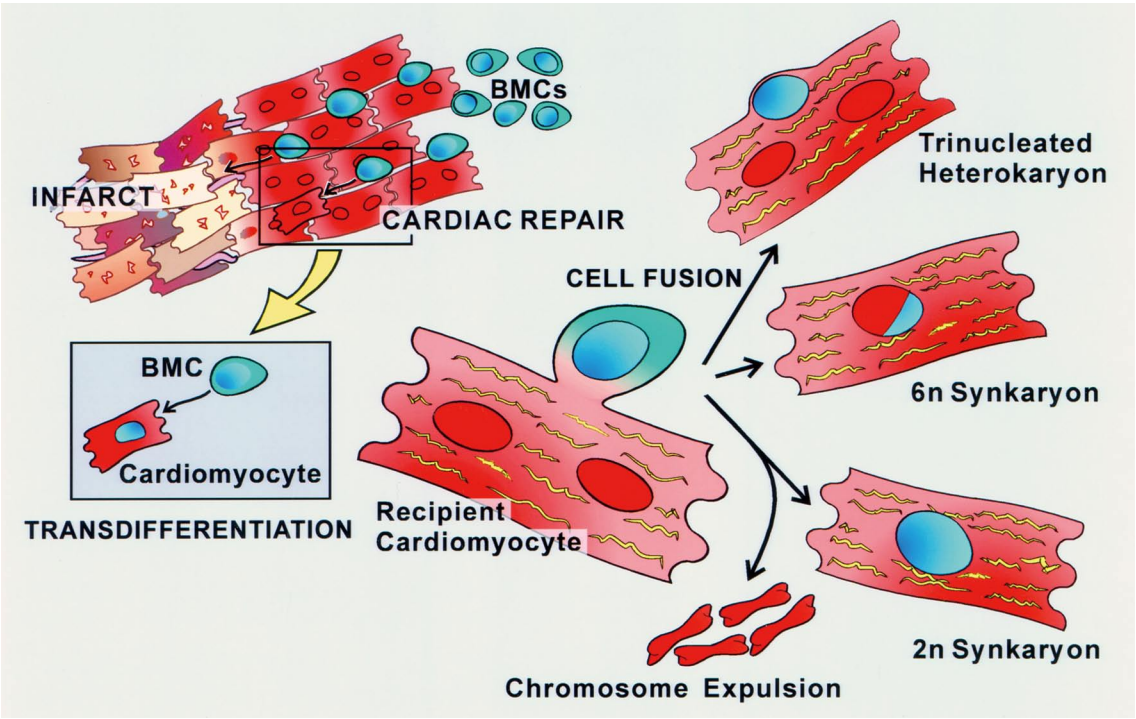

Figure 2. Schematic representation of cell fusion. Cytoplasmic fusion of a terminally differentiated cardiomyocyte with a bone marrow-derived progenitor cell (BMPC) in the absence of nuclear fusion gives rise to a trinucleated heterokaryon. Cytoplasmic fusion followed by nuclear fusion can form a hexaploid synkaryon or a diploid synkaryon if reductive mitosis with chromosomal expulsion occurs.

the infarcted heart. The distribution of the 2 cell classes also differs with a preferential localization of the CD34-positive cells in the border zone while unselected bone marrow cells are located randomly throughout the heart, including both the border zone and infarcted myocardium. These observations strongly indicate that the characteristics of BMPCs dramatically condition their therapeutic impact on the damaged heart and myocardial regeneration.

BMPC Transdifferentiation

The cellular events leading to the contribution of bone marrow cells to specific tissues in vivo is the subject of debate and intense research. Successful engraftment of BMPCs in proximity to or within the infarct is the initial fundamental process of tissue repair. However, this is only the first part of a complex and long journey of progenitor cells. Myocardial reconstitution necessitates the generation of a cardiomyocyte compartment and a well-balanced coronary vasculature. Myocytes alone in the absence of adequate blood supply cannot perform their function and generate force, and coronary vessels alone without muscle mass cannot restore ventricular performance.[6] The question is whether BMPCs commit to cardiac lineages and whether this phenomenon leads to coordinated growth adaptation in which myocytes, resistance arterioles and capillary profiles are developed concurrently to engender functionally competent myocardium.

The contribution of BMPCs to cardiac repair and/or recovery of function can occur through transdifferentiation of progenitor cells into myocytes and coronary vessels, fusion of primitive cells with pre-existing recipient cardiac cells and subsequent myocardial regeneration or through paracrine effects exerted by peptides released by the injected cells on resident CPCs. The most controversial possibility is BMPC transdifferentiation. Stem cell plasticity refers to the ability of adult stem cells to acquire mature phenotypes that are different from their tissue of origin.[23] The concept of transdifferentiation challenges the well-established dogma that adult stem cells undergo a progressive restriction in developmental options and, therefore, their fate is irreversibly specified during prenatal life.[24] Transdifferentiation necessitates nuclear reprogramming, which is mediated by complex extracellular signals together with the exchange of intracellular mediators that are promoted by the interaction of BMPCs with recipient cells.[25] At the molecular level, stem cell plasticity is dictated by the modification in the expression of a master regulatory gene whose normal function is to distinguish different tissues in development.[26] In turn, upregulation or suppression of gene expression is a result of epigenetic mechanisms.[27]

An alternative possibility of cardiac repair involves fusion of BMPCs with resident differentiated cells within the target organ followed by the reprogramming of nuclei in response to intracellular cytoplasmic factors. Ultimately, fusion results in the formation of hybrids (**Figure 2**). The merging of an adult BMPC and a differentiated cardiac cell results in the formation of a binucleated heterokaryon or a mononucleated hyperploid synkarion.[28] The growth of the binucleated heterokaryon seems to depend on the nucleus of the more

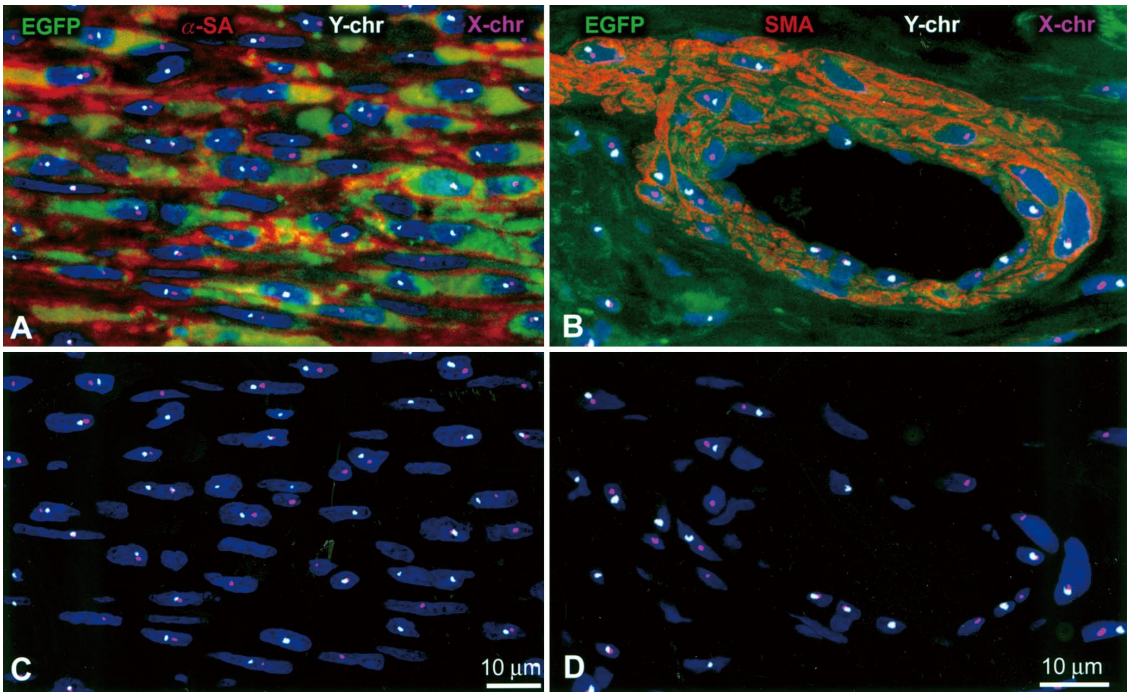

Figure 3. Bone marrow-derived progenitor cells (BMPCs) and cardiac repair. Band of regenerated myocardium following the injection of male enhanced green fluorescent protein (EGFP)-positive BMPCs in an infarcted female mouse heart. (**A**) Newly formed myocytes that are α-sarcomeric actin and EGFP-positive (red-yellow-green). (**B**) A resistance coronary arteriole in which smooth muscle cells are α-smooth muscle actin and EGFP positive (orange-green). (**C**) and (**D**) document the diploid male genotype (Y-chromosome, Y-chr, white dots; X-chromosome, X-chr, magenta dots) of the regenerated structures.

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 differentiated cell. When cellular fusion is accompanied by nuclear fusion, a mononucleated synkarion with a hyperploid DNA content is formed. In this condition, the bulky burden of the high nuclear DNA content leads to genetic instability and reduced or null replicative potential.[28]

Consistently, data in our laboratory and others[11,14,15,29–31] have shown that BMPCs can generate cardiomyocytes and coronary vessels that repair in part the infarcted heart structurally (**Figure 3**) and functionally. Utilizing donor BMPCs from male transgenic mice expressing enhanced green fluorescent protein (EGFP), the newly formed myocardium acquired a male genotype contrasting with the female genotype of the recipient myocardium. Additionally, EGFP expression was apparent and restricted to the regenerated cardiomyocytes and coronary vessels. Importantly, the number of sex chromosomes was consistent with a diploid genotype excluding fusion events between resident female cardiac cells and injected male BMPCs (**Figure 3**).

Conclusions

Currently, a major challenge is to establish whether CPCs and BMPCs have comparable or dissimilar efficacy in cardiac repair after infarction. In particular, the number and phenotypic properties of generated myocytes will have to be characterized and compared. If we assume that the physiological postnatal maturation of the heart represents the gold standard paradigm for effective and successful myocardial regeneration, several criteria will have to be met: (1) shortly after engraftment, progenitor cells would be expected to generate a large number of myocytes resembling neonatal cells, ~1,000 μm^3 in volume; (2) myocyte proliferation should decrease rapidly and cellular enlargement should become the predominant form of expansion of muscle mass, reaching the adult phenotype ~20,000–25,000 μm^3; (3) myocyte apoptosis should be relatively high in the early phases of cardiac repair and minimal when the myocytes have fully matured; (4) because of the small size of cardiomyocytes, there should be approximately 1 capillary every 10–15 myocytes early during regeneration, but a ratio of nearly 1 capillary to 1 myocyte should be reached in a period of 4–6 weeks; and (5) numerous coronary resistance arterioles should develop to decrease coronary resistance, and promote the integration of the new coronary vasculature with the remaining coronary circulation.

The ultimate objective is to define the fundamental criteria that govern successful myocardial regeneration and the replacement of the infarct with functioning tissue. The feasibility to reconstitute an area of infarcted myocardium has been advanced and documented only recently. However, this is a controversial issue and the likelihood of rebuilding the lost tissue in the adult heart has been questioned. Therefore, novel approaches will have to be applied to document whether CPCs and BMPCs lead to myocardial regeneration after infarction and whether they are equally effective in this process, or whether 1 cell type is more powerful than the other. The search for the most appropriate primitive cell for cardiac repair continues.

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