Cell Therapy for Cardiac Diseases-Bridging Basic and Clinical Science

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Regenerative medicine is a term given to varied strategies of repairing or replacing damaged or diseased tissues or organs to restore, maintain, or enhance their function. As the average age of the population is rising with the increasing incidence of age-related degenerative conditions, a pressing need for the products of regenerative medicine is imminent. Regenerative medicine promises a more effective solution than current pharmaceutical and interventional therapy. By far the most well-known areas of regenerative medicine have been tissue engineering and cell therapy. Engineering complex organs *ex vivo* by knowledge from tissue engineering is still many years away, but the use of cells to rebuild diseased or damaged tissues by delivering a small number of cells may soon be used to treat some of the world most devastating diseases.

In this review, we first examine the knowledge of regeneration and tissue stem cells. We provide the most updated information about cardiac stem cells, myocyte death and growth and regeneration in diseased and aging heart. Recent research demonstrates replacement and regeneration of functional cardiac muscle can be achieved by either implanting competent cells or stimulating proliferation of endogenous stem cells. We therefore explore the scientific advances, clinical trials and challenges ahead regarding cell therapy for myocardial regeneration. Our goal is not to provide detailed information about regenerative medicine, but rather, using heart repair as an example, to inform the readership about recent advances in this promising field and encourage their active participation in its further development.

Key Words: Cell therapy • Myocardial repair • Regenerative medicine • Stem cells

REGENERATION IN NATURE

Regeneration is a natural power,[1] that is defined as the replacement of lost or diseased tissues, organs or portions of the body. In invertebrates, regeneration can be involved in replacing substantial amounts of the body or constituting asexual reproduction. In vertebrates, regeneration is not a means of asexual reproduction but involves the replacement of tissues or parts of organs.[2] Regeneration also takes place in the mammals, including humans, in some capacity throughout life. For example, blood and skin are continually restored, but liver, bone, muscle, and blood vessels have a limited capacity for self-repair. However when extensive damage is suffered, the regenerative power of most tissues or organs is not sufficient to cope with the damage. The fate is to undergo repair by fibrous scar tissue,[3] which results in a loss of function that can be incapacitating to varying degrees, or even life-threatening, depending on the severity of the injury. Therefore, biomedical research to uncover the magic power of regeneration is eagerly pursued.

Research goals in this field are to understand how regeneration is initiated, what sets of genes are activated to achieve regeneration, which growth factors and hormones

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play roles, and what cell-cell interactions occur in order to ensure replacement of the regenerating components in the correct place and with the appropriate pattern. Application of modern methodologies in molecular and cell biology in experimental and clinical studies of injury and regeneration has resulted in the uncovering of mechanisms underlying these phenomena. Scientists now know that mechanisms of regeneration in many types of tissues closely resemble the pathways of their normal embryonic development.[4] Vertebrates use two different mechanisms to accomplish this recapitulation. The first mechanism is to create progenitor cells by dedifferentiation of differentiated cells to a state where they can proliferate and re-differentiate into new tissue. Liver regeneration is accomplished by the partial dedifferentiation of hepatocytes, and is the only tissue known to regenerate by this mechanism in mammals.[5] By far the most common regenerative mechanism in vertebrates is the second mechanism, that is the activation of undifferentiated tissue stem cells set aside within tissues as they develop from multipotent cells during embryonic and fetal life.[6]

TISSUE STEM CELLS

Tissue stem cells are either multipotent or monopotent stem cells, which have capacities for self-renewal and amplifying committed progenitor cells.[6] Well-known examples are the osteogenic cells in the periostium and endostium of bone and the satellite cells of muscle. Blood vessels contain tissue stem cells called pericytes that differentiate into smooth muscle,[7] and proximal kidney tubules also contain cells that allow them to regenerate.[8] Recent studies demonstrated cells with the capability of differentiating into bone, cartilage, skeletal muscle and adipocytes locate in the bone marrow along with haematopoietic stem cells, but the normal distribution and function of these mesenchymal stem cells (MSCs) are not clear.[9] It has also been proposed that stem cells might reside in the connective tissue compartments of virtually every tissue and organ in the body.[10,11] A subpopulation of connective tissue cells is capable of differentiating into the same cell phenotypes as bone marrow MSCs. Whether or not these cells are derived from bone marrow MSCs is unknown, and the role of these cells in the regeneration of local tissue is still to be determined.

Interestingly, tissue stem cells have now been identified in the mammalian liver, as well as in some mammalian tissues that regenerate poorly or not at all. If most of the liver is destroyed, a population of bipotent stem cells, called oval cells, located in the bile ducts of the liver can be activated for regeneration.[12,13] These cells seem to act as "reserve" when regeneration by dedifferentiation is compromised or not adequate. Multipotent stem cells have also been isolated from different areas of the adult mouse and fetal human brain with proliferative potential and form neurospheres, which can differentiate into both neurons and glial cells.[14] As mentioned above, their roles in poorly regenerated tissue or organ are also unknown.

An even more perplexing feature of tissue stem cells is their plasticity. Data have shown that cells with exceptional and unanticipated capacities to mature into a broad range of phenotypes may be isolated from a variety of organs even in adults, proliferate in culture, and accommodate to new environment after delivered into other tissues.[15,16] For example, some cells within the bone marrow appear to be capable of unexpected differentiation into a variety of tissue types, not only in mice, but also in humans.[17,18] Based on these findings, scientists drew the hypothesis that regeneration of tissues may also derive from other cells which are not tissue-specific stem cells. However, whether these changes in cell function are the result of a random and rare event or result from a biological process remains to be determined, as the frequencies of these events shown in the studies have generally been very low. Nevertheless, the unexpected plasticity and the tremendous therapeutic implications have created a lot of excitement.[19,20]

In order to explore potential clinical applications of these puzzling cells, further characterization of their biological role is necessary. Perhaps the most universally accepted criteria for tissue stem cells are that they are normally slow-cycling (or, perhaps more accurately, rarely cycling) *in vivo*, that they can self-renew and are responsible for the long-term maintenance of the tissue, and that they can be activated by wounding or by *in vitro* culture conditions to regenerate the tissue and to proliferate respectively.[21,22] The slow-cycling attribute is par-

ticularly important in biology because it conserves the cell proliferative potential and minimizes DNA replication-related errors. It has been suggested that the rare divisions of stem cells give rise to one stem cell and one transit amplifying (TA) cell, which has a limited proliferative potential. This is so-called "asymmetric division of adult stem cells." On the exhaustion of their proliferative potential, the rapidly proliferating TA cells undergo terminal differentiation and exit the cell cycle.

Another unique feature of tissue stem cells is that they occupy niches;[23] a specific microenvironment that instructs and supports stem cell self-renewal, proliferation, and differentiation by providing specific cellular neighbors, signaling molecules, and extracellular matrix components.[24,25] Identifying the molecular basis underlying the interaction between stem cells and the niches will contribute to understanding the molecules involved in one of the most important issues in regenerative medicine, that is, regulation of stem cell activity.

Furthermore, identification of stem cell markers, one of the major controversies in this field, is biologically and clinically important. One of the variables in identifying the stem cell markers, which is particularly confounding, relates to the biological state of stem cells in culture. Although *in vitro* clonogenicity and cumulative proliferative potential provide useful assays for stem cells,[26,27] scientists have observed that markers for the *in vivo* tissue stem cells may not be identical to those of *in vitro* colonies. It is logically proposed that cultured cells have been released from the control mechanisms of the *in vivo* stem cell niche designed to keep them in a slow-cycling state. Therefore, tissue stem cells, as we define them *in vivo* in terms of their slow-cycling feature, may completely disappear in most of the mitogenic tissue culture environments, with marked changes in their markers.[28,29]

GROWTH AND DEATH OF CARDIOMYOCYTES

If one wants to know how a machine works, one must disassemble and then reassemble it. The same is applicable and valid for our knowledge of the structure and regeneration of organ and tissue. The identification and characterization of growth and death of cardiomyocytes is important, because they represent the crucial factors of the whole cardiac homeostasis as well as its remodeling response to the disease and injury. A better understanding of myocardial biology is also important because the knowledge evolved will pave the way to the new strategies, including regeneration medicine and cell therapy, for treating cardiac diseases. Based on this sentiment, we reviewed the most updated knowledge about myocyte death, growth and regeneration in diseased and aging hearts. By understanding these advanced views in the biology of myocardium, we hope to figure out how to regenerate functional cardiac tissues for future clinical application.

It was long believed that the heart was a terminally differentiated organ, which excluded any significant turnover of myocytes in the normal and diseased hearts. Even moderate rates of myocyte death would lead to the disappearance of myocardial mass over time. Not surprisingly, the existence of myocyte death has been controversial for years.[30,31] There is still considerable disagreement on the rate and mechanism of myocyte death,[32,33] but the consensus[35,36] that myocyte death is quantifiable in the normal and pathological hearts is slowly evolving by applying the modern techniques of molecular and cellular biology.[32,36]

Cell death can occur by three mechanisms or their combination: apoptosis, necrosis and autophagic cell death.[33] At any given time, 0.002%, 0.06% and 0.08% of the cardiac myocytes in the normal heart are undergoing apoptosis, necrosis and autophagic cell death, respectively.[33,35] Various mechanisms of cell death have different consequences on cardiac remodeling. Myocyte necrosis leads to an inflammatory reaction, vessel proliferation, macrophage infiltration, fibroblast activation, and ultimately, scar formation. Controversially, after apoptosis, the reparative process does not involve collagen accumulation, and apoptotic bodies are removed by neighboring cells with no apparent changes in the morphology of the tissue, though apoptosis can induce acute restructuring of the ventricular wall and depression of tension development of the myocardium.[37] On the other hand, autophagic cell death, in which sequestration of cellular material into double membrane vacuoles was proceeded, dock to and fuse with lysosomes, forming autophagic vacuoles, represents a programmed and dynamic cellular degeneration. Together, these different types of cell death play a significant role in the mechani-

cal behavior and structural composition of the heart, which lead to myocyte disappearance and development of contractile dysfunction in failing heart.

Cellular hypertrophy has been the main, if not the only, acknowledged form of myocyte growth in the adult heart.[38] The nature, extent, and mechanisms of myocyte regeneration, or cellular hyperplasia, have remained obscure.[18,39] One of the main difficulties is to find an actual quantitative estimation of the phenomenon. The strongest evidence in favor of myocyte proliferation in the mature heart is the increase in myocyte number from birth to young adulthood.[40] On the other hand, scientists now can also accurately estimate the magnitude of myocyte regeneration that occurs in the overloaded heart in the absence of coronary artery disease by the absolute increase in cell number in the ventricular myocardium. This also constitutes a demonstration accountable for new myocyte formation in the adult heart.[40] What's more, the underlying hemodynamic conditions may influence the growth response of the myocardium. Ventricular dysfunction and failure prefer to the activation of new myocyte formation, whereas myocyte hypertrophy characteristically is more pronounced in the functionally compensated ventricle.

Remodeling change after myocardial infarction is a good example to examine the cell behavior in response to injury. Occlusion of a major coronary artery leads first to apoptotic myocyte death and, subsequently, to cell necrosis. Diastolic stretch of the surviving myocardium results in the release of angiotensin II and upregulation of the local RAS via activation of p53-regulated genes, which further promote single- and double-strand DNA cleavage, myocyte apoptosis, then cell slippage, wall thinning, and ventricular dilation. In parallel, myocardial infarction also activates myocyte hypertrophy with cell lengthening and more importantly myocyte regeneration that together contribute to the formation of new muscle mass.[40] Though partially restoring the cardiac mass, myocyte hypertrophy and hyperplasia do not significantly invade and substitute the scarred myocardium. Together, the turnover of myocardial mass contributes to the progressive remodeling in anatomy and impairment in the function of the ischemic heart.

The process of aging also offers an extraordinary example of the effects that changing balance between cell death and cell growth on the pathological restructuring of the heart. Evidence shows that myocytes are constantly replaced by newly formed myocytes in the aging heart. Some myocytes undergo proliferation, some hypertrophy, and yet another group experiences apoptosis and necrosis.[40] The rate of cell death increases with age and is not balanced by a concomitant increase in new myocyte formation after middle age. The excess cell death results in a net reduction in myocyte number.[40,41] This smaller number of viable myocytes become hypertrophic to preserve myocardial mass, resulting in an old heart of normal or slightly decreased size but with enlarged parenchymal cells.[42]

These data strongly suggest that myocyte renewal occurs throughout life in the normal or diseased myocardium, exemplified by a subpopulation of proliferating myocytes continuously changing the balance within the cardiac homeostasis, that is, the proportion of young and old cells in the heart.

CARDIAC STEM CELLS

Preview studies didn't provide information about the origin of the increased myocytes. They could originate by replication of preexisting myocytes through dedifferentiation, or they could come from the commitment of precursor cells or stem cells to the myocyte lineage. Most previous controversy about myocyte regeneration has focused on the evidence for or against the replication of existing myocytes through de-differentiation. There is considerable research directed at defining the cellular mechanisms that cause the termination and suppression of cardiac cell division during development.[43] However, no convincing data demonstrates the de-differentiation of mature cardiomyocytes to myocyte precursor cells or stem cells.

On the other hand, recent results in humans and animals have provided evidence of stem cells capable of differentiating into myocytes in the adult heart. It has been shown that primitive cells exist in the diseased or normal heart which co-express stem cell-related surface antigens including c-kit, MDR1, and an epitope related to stem cell antigen-1 (Sca-1)[44] and, amazingly, the transcription factors of the myocyte lineage or receptors typical of endothelial cells and smooth muscle cells.[18] Although the data did not offer information on whether all

cell types originated from one or several different precursors, these findings point to the existence of stem-like cells with the potential of regenerating all components of the myocardium.

An important issue, then, is the origin of these cardiac stem cells. There are three possible origins: (1) the remnants of the multipotentent cells in the developing heart; (2) circulating cells that home to the heart; or (3) both of them. It is an issue of biological and clinical significance to determine whether the cardiac stem cells are resident cells that accumulated in the heart early in development or whether they are the progeny of other stem cells that, throughout life, home to the myocardium through the systemic circulation.

It remains a general belief in biology that tissue stem cells may come from remnants of the development stage. The heart is composed of several different cell types, including cardiac myocytes, endocardial endothelial cells, fibroblasts, vascular smooth muscle, vascular endothelial cells, and epicardial epithelium. These cell types have not only different functions but also distinct responses to disease and injury. Moreover, they have distinct embryonic origins. Myocardial and endocardial cells arise from the lateral splanchnic mesoderm[45,46] and are the only resident cells in the heart until the looping phase of cardiac development.[47] At this phase, the proepicardial organ (PEO), an epithelial structure derived from the septum transversum, migrates to and over the surface of the myocardium to form the epicardium and pericardium. A subgroup of PEO cells then undergo transformation, invade into the actively proliferating myocardium and give rise to cardiac fibroblasts and coronary vasculature.[48]

The potential for these embryologic remnants to contain stem cells and recapitulate their developmental program after injury and disease is an unexplored area of research. Evidence demonstrates that isolated epicardial mesothelial cells, in fact, can mimic at least some components of epicardial differentiation and coronary vasculogenesis.[49] Notably, evolving data suggest that, at least *in vitro*, epicardial cells may also be able to differentiate along a cardiomyocyte lineage as well as the vascular path.[50] Whether such behavior occurs *in vivo* during remodeling change after injury or disease is to be determined. In summary, the identification, localization and purification of these puzzling cells, and the characterization of their biology, will shed the light on the mystery of cardiac development and regeneration.

Could primitive cells with the ability to regenerate myocardium come from outside the heart? The cases of sex-mismatched cardiac transplants in humans where a female heart is transplanted into a male host offered an ideal setting to further explore this hypothesis.[18] The colonization and differentiation of host cells homed to the transplanted heart can be identified by the presence of the Y chromosome in the host-derived cells. These differentiated host cells in the donor heart are an irrefutable proof of new myocyte formation in the adult heart and presuppose the existence of mobile stem cells outside the heart able to differentiate into the three main cardiac cell types including cardiomyocytes, endothelial cells and smooth muscle cells.

Where are these mobile stem cells from? The experimental and clinical data published in the last several years revealed the new pathophysiological roles of bone marrow derived cells. These data demonstrate that in animals and humans, bone marrow-derived cells mobilize and recruit tissue-specific karyotypically diploid stem cells, including skeletal muscle,[51] brain,[52] liver[53] and heart,[51,54,55] both anatomically and functionally, and that these cells can proliferate as progenitors and participate in normal regenerative processes of these tissues in response to injuries.[56] We and others, in experimental animal studies, showed that labeled bone marrow-derived cells traffic through the blood circulation[51,76] or are delivered directly[54,87] to reach the injured myocardium. They undergo in situ differentiation and express various phenotypes, including those of cardiomyocytes, vascular endothelial and smooth muscle cells, and myofibroblasts.

In summary, the heart should not be considered a post-mitoic organ. The proposed cardiac homeostasis in normal and diseased hearts is illustrated in Figure 1. This evidence, however, still falls short of establishing a precursor-product relationship between these primitive cells and the fully differentiated cardiac cells. As we map out the intersecting molecular genetic pathways that regulate their migration, homing and differentiation, we may ultimately find their origin can be clinically manipulated in order to regenerate a wide array of impaired structures and improve function in the ailing heart.

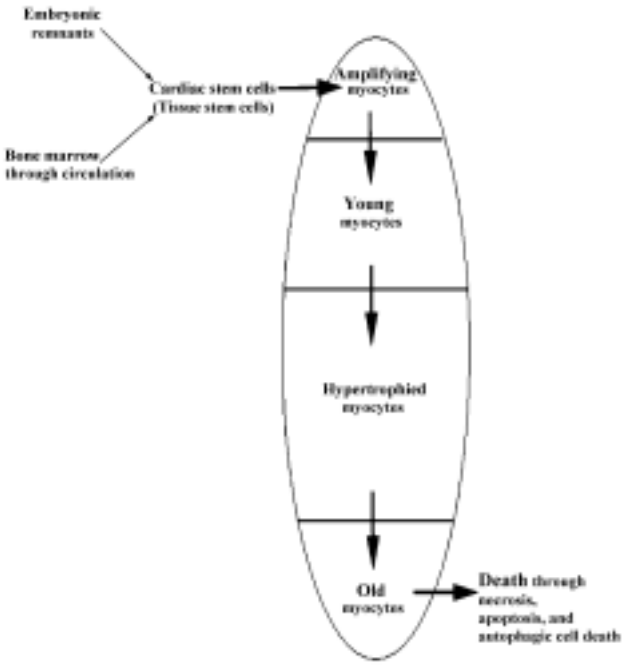

Figure 1. *Proposed cardiac homeostasis in normal and diseased heart. Oval block and its compartments represent whole myocyte mass in the heart and subgroups of various myocytes. Arrows represent the direction of myocyte turnover.*

CELL THERAPY FOR MYOCARDIAL REGENERATION

The idea of using living cells to treat disease is as old as blood transfusion, but recent progress in molecular and cellular biology has offered fresh perspectives on this venerable concept. Introducing useful cells at relevant sites of disease may help to reverse pathology and maintain the integrity of organs before further degeneration occurs. The delivery of genes into the cells can also help to promote the survival of surrounding tissues.[57] The advances of cell therapy have provided insight into the underlying disease process and reveale opportunities for new therapies for conditions previously untreatable. The aim of cell therapy is to replace, repair or enhance the biological function of damaged tissue or organs. This can be achieved by the transplantation of isolated and characterized cells to a target organ in a sufficient number and quality for them to survive long enough to restore function. Likewise, a promising future treatment of heart disease has emerged through cell therapy for myocardial regeneration, in which one delivers donor cells into injured myocardium or stimulates proliferation and migration of endogenous stem cells. It represents a novel means of augmenting myocyte population and contractile function of a diseased heart. Current data derived from animal models and clinical trials have demonstrated the feasibility of this approach as mentioned below.

RATIONALE OF CELL THERAPY FOR MYOCARDIAL REGENERATION

The rationale to consider cell therapy for myocardial regeneration is that progressive ventricular remodeling, in which the balance between myocyte death and regeneration is impaired, characterizes the common pathway of cardiomyopathies of different etiologies.[58] Until recently, myocardial regeneration following injury was thought to be severely limited by the inability of post-embryonic heart tissue to recruit muscle progenitor cells. As we mentioned before, the dogma that adult heart has no regenerative capacity has recently been challenged by observations of mitotic division of cardiomyocyte in the adult heart.[39] However, it is assumed that the rate of proliferation cannot meet the demand needed for tissue regeneration. In this sense, cell therapy, which provides proliferating and myogenic cells to diseased heart, can be seen as an enhancement of the natural healing process of the myocardium. In the

Table 1. Major concerns about cell source in clinical application of cell therapy for myocardial regeneration

Cell source	Ethical problem	Acquisition concern	Rejection	Potential oncogenicity
Adult cardiomyocytes	No	Yes	No	No
Fetal cardiomyocytes	Yes	Yes	Yes	No
Skeletal myoblasts	No	Yes	No	No
Embryonic stem cells	Yes	Yes	Yes	Yes
Bone marrow cells	No	No	No	No

Acquisition concern includes logistic and other difficulties in getting donor cells, potentially irreversible injury to recipient/donor, and the difficulty in cultivating and expanding cells *in vitro*.

following, we will discuss the various potential cell sources for myocardial regeneration. Major concerns about the cell source in clinical application are summarized in Table 1.

ADULT CARDIOMYOCYTES

Cardiomyocytes isolated from the heart of the patient is the most obvious source of cells. One of the principal advantages of this approach is that it represents an autologous graft, and as such, will not induce the immune response. However, isolated adult human cardiomyocytes have no ability to divide and withdraw from cell cycle permanently.[39,43] This biological characteristic would need a large number of myocytes from the donor heart to repair the injured heart. Besides, isolating myocytes from a diseased heart also has obvious drawbacks.

FETAL CARDIOMYOCYTE

Fetal cardiomyocytes are an attractive donor source for cell therapy for myocardial regeneration. They are capable of proliferation in utero and have the potential to mature into cardiomyocytes. Due to this biological feature, they are used widely for research.[59,60] As with adult cardiomyocytes, there are ethical and logistic difficulties in obtaining sufficient fetal hearts as the source for donor cells, when this is to be applied therapeutically for patients with heart failure. The other major problem is rejection. Fetal cardiomyocyte implantation is in fact allografting and the recipient requires immunosuppression similar to patients who receive organ transplantation from another individual. Although fetal cardiomyocyte implantation is of considerably biological interest, its clinical applicability is limited.

SKELETAL MYOBLAST

Skeletal muscles of human and mammals contain satellite cells. These cells are now known to be skeletal muscle myoblasts capable of responding to injury signals, upon which they can migrate, proliferate and differentiate to repair the damaged skeletal muscle fibers.[61] During the last decade, studies confirmed that myoblasts implanted within a myocardial scar could survive, differentiate into striated muscle fibers[62,63] and ameliorate the depressed cardiac function.[64] A Phase I clinical trial was initiated in France in the year 2000,[65] and more recently in the United States and elsewhere, as will be discussed further below. However, there is an ongoing debate as to whether these myofibers derived from myoblast implantation into the myocardium express skeletal myofiber phenotype or cardiomyogenic phenotype.[66-68]

EMBRYONIC STEM CELL

Embryonic stem cells (ES) derived from the inner cell mass of blastocyst-stage embryos are pluripotent cells, meaning that under different stimuli their progeny can differentiate into any cell types specified at later stages of development.[69] The availability of human ES cells[70] and the possibility of generating autologous ES cells by nuclear transfer[71] provide exciting perspectives for therapeutic cloning, which creates the patient own ES cells for clinical application. Thus, ES cell technology may become the most important basis for new cell replacement therapies. Unfortunately, embryonic stem cells also have a dark side. The full potential of recent discoveries on embryonic stem cells will be realized only if society deems this research worthy of support. Besides this ethical concern, the limiting factor in this therapeutic approach lies in reliably isolating and inducing stem cell populations to divide *in vitro* and subsequently differentiate into desired tissues. Rigorous purification of such cells will be required to safeguard the recipients to avoid cells spreading inappropriately or forming unwanted tissue, for example, teratoma. However, with the rapid pace of advancement in the stem cell field, this approach may become technically possible in the future.

BONE MARROW-DERIVED CELLS

Another potential source for myocardial regeneration is bone marrow-derived stem cells. These cells possess the properties of stem cells in addition to being blood-forming progenitors.[72,73] When treated with

5-azacytidine, a cardiomyogenic cell line could be isolated from murine bone marrow mesenchymal stem cells by repeated screening of spontaneously beating cells.[74] We and others have demonstrated that bone marrow mesenchymal stem cells can differentiate into cells expressing phenotypes of cardiomyocytes including cardiac-specific contractile protein and gap junction proteins.[75-77] They are theoretically the ideal cell source for myocardial regeneration because they can be isolated repeatedly from the patient's bone marrow and expanded in culture. Most recently, data demonstrate that a subpopulation of bone marrow stem cells were capable of generating new myocardial tissue comprising proliferating myocytes and vascular structures in the infarcted scar with improvement of cardiac function.[78] These findings proved a preliminary indication of the wide variety of possibilities in the use of bone marrow-derived cells in myocardial regeneration.

CLINICAL TRIALS

Implantation of autologous myoblast

Autologous skeletal myoblast implantation was initiated in the year 2000 at several institutions in Europe[65,79,80,81] and recently in the USA, mostly by epicardial injections during cardiac surgery but in some cases/studies by intracoronary infusion or endocardial punctures using catheters. It is indicated by PET scan that implanted cells within the scar survived for many months after implantation, and echocardiographic data showed some improvement in segmental contractions. When the concomitant procedures were carried out, such as with coronary artery bypass operation, it was difficult to evaluate the safety or the efficacy, because of the confounding factors introduced. Another issue of concern is the occurrence of post-operative arrhythmia in some patients which required implantation of automatic defibrillator devices.[80] Nevertheless, it is difficult to be determined whether such arrhythmias were caused by cell implantation, since many patients are prone to arrhythmia even without cell therapy.

Implantation of autologous bone marrow-derived cells

Bone marrow-derived cells have also been used as donor cells in some clinical trials worldwide.[82-85] A couple of those clinical implantations employed autologous bone marrow aspirates without cell selection, with the hope of simultaneously inducing angiogenesis and myogenesis. Again, it was reported that echocardiography showed improved function in the area of the scarred myocardium where the bone marrow cells were injected. However, conflicting data from large animals were also reported.[86]

Limitations

This cell therapy for myocardial regeneration still face significant hurdles before it can become a established clinical therapy. Many preclinical studies reported so far have shown that implantation of not only stem cells and progenitor cells but even differentiated smooth muscle cells[87] and fibroblasts in a scar could improve global and segmental regional contractile functions but a sound physiological explanation is lacking. Further functional studies to verify this phenomenon are important.[88,89]

Some basic biological problems need to be settled before a wider clinical application. In our earlier *in vivo* studies,[90] we noted that upon implantation of marrow mesenchymal stem cells into the myocardium, the stem cells surrounded by scar tissue appeared to differentiate poorly, while those in direct contact with native cardiomyocytes more readily expressed morphology and phenotypic molecular markers of cardiomyocytes. Such findings led us to speculate that direct cell-to-cell contact could be an important signaling mechanism for *in situ* differentiation of the adult stem cells. Further studies to understand the micro-environmental signals should be undertaken. On the other side, culture expansion of donor stem cells prior to implantation offers the opportunity to intervene and manipulate their fate following implantation, by modulating their gene expressions while under culture.[91] Even after the differentiation of the implanted adult stem cells into various phenotypes of cells constituting the myocardium, if they are arranged in random and not integrated with the native structure of the tissue, they will be functionally ineffective. In the case of neocardiomyocytes, we have shown that their contact with native cardiomyocytes and fibers can induce the formation of gap junctions, and eventually lead to the full integration of these cells into the existing cardiac myofibers.[92]

Applying the experience from tissue engineering using bioreactors,[93] in which biomechanical forces are applied to align cellular orientations, we proposed the hypothesis that gap junction formation allowed the shear stress generated by contacting native cardiomyofibers to induce self-assembly of neocardiomyocytes, presumably through cell surface mechanoreceptors and re-organization of the cytoskeletal system.[94]

Many other clinically relevant issues also need to be examined before a sound therapeutic regimen can be designed for wide use. We speculate that adult cell therapy can be expected to be useful not only in myocardial infarction, a pathological condition so far studied almost exclusively in preclinical and early clinical studies, but also in myocardial injury due to end-stage valvular diseases, as well as cardiomyopathies of various etiologies. Thus, studies using appropriate models of diffuse cardiomyopathy and heart failure to examine the optimal cell delivery strategies need to be pursued. Moreover, it is difficult to standardize the donor cell preparation, which in turn can make the comparison of the data obtained from one study to the other difficult. Sound techniques need to be developed to enable quantitative study of cells retained upon implantation, then survived and differentiated into a specific phenotype.

Future of cell therapy for cardiac diseases

Up to now, the cell therapy of myocardial regeneration is focused on changing infarcted scar tissue into muscle by local delivery of potent cells. However, the better way to sprout new tissues is still to rely on the body own biochemical wisdom; the appropriate cells are mobilized and homed to the desired site, and growth unfolds within the injured tissues. Cytokine administration regimen, the amplification of a naturally occurring phenomenon, used for BMC mobilization produces high levels of circulating bone marrow-derived multipotent cells.[54] It has been proved that these circulating cells continuously colonize the myocardium and contribute to myocyte renewal. The widespread application of cell therapy in cardiovascular disease, therefore, will likely be based on similar pharmacological approaches to enhance the capacity of endogenous potent cells to provide for the regeneration of diseased myocardium.

In order to achieve this result, we need to answer two important biological questions. First, how to mobilize potent cells? Second, how to home these cells to the desired repair area? The molecular signals for mobilizing bone marrow stem cells and homing of these cells into the targeted site of myocardial injury are currently under active investigation. Inflammatory cytokines released at the injury site may provide the signals for recruitment of marrow stem cells.[95,96] Identifying the signaling molecules will have therapeutic potential, since their administration may enhance the mobilization of existing marrow stem cells of the patients. On the other hand, the homing signals for stem cells to localize themselves at the injured site are not well understood. In the case of bone marrow cell transplantation to restore damaged bone marrow, various cellular adhesion and cytokine receptors, such as stroma derived factor-1 (SDF-1), intergrins and CD 44 are thought to play central roles in their homing mechanisms.[97,98] We speculate that the first step of circulating adult stem cells homing into the injured site may also be related to their interaction with local endothelial cells. These cells trapped within the microvasculature had been proved to leave the vascular space and migrate through the interstitial space toward their destination.

Another possibility is to reactivate and amplify the potential of cardiac residual stem cells for myocardial regeneration. Cardiac stem cells might be more effective than marrow stem cells in rebuilding injuried tissue. Marrow-derived cells have to re-program themselves to give rise to a progeny differentiating into cardiac lineages; by contrast, activation and migration of cardiac stem cells to the site of injury would avoid this intermediate phase. Moreover, cardiac stem cells may be faster than marrow stem cells in reaching functional competence and structural characteristics of mature myocytes and vessels. Together, recruitment and expansion of cardiac stem cells may promote a pool of young recycling myocytes and neovascularization for myocardial regeneration. Moreover, we speculate that the total re-remodeling of diseased heart may be achieved by apoptosis of scar tissue, reactive fibrosis and over-hypertrophied and aging myocytes in combination with the regeneration of new myocardium by the amplifying myocytes from cardiac stem cells in a temporally and spatially organized manner. Better understanding and manipulating this cardiac homeostasis may become a clinical reality for treating cardiovascular disease in the near future.

CONCLUSIONS

There is a tremendous impetus to proceed rapidly toward advanced cell therapy for heart disease. Many cardiac patients who cannot be salvaged with conventional therapy are eagerly waiting for experimental exposure to become clinically available. Living cells provide the starting material for a cornucopia of new strategies for cardiovascular disease, despite several biological and technical obstacles. Clinical doctors are clearly highly motivated to take this from the laboratory to the patients bedside. Nevertheless, we have to temper this excitement with caution lest we repeat the tragedies associated with gene therapy in the last few years. A aggressive and hasty attitude leading to errors could set back the progress of this highly promising therapy for years. Continued active preclinical research to address issues discussed above will help us to select optimal strategies to regenerate new myocardium for heart failure.

In this review, we provide an introductory background and the current state of basic investigation of cell therapy for myocardial regeneration, and attempt to extrapolate from the laboratory bench to the bedside. By analyzing the native regenerative power and tissue stem cells in our body, we hope to better understand how the tissues or organs including the heart maintain their homeostasis. With such an understanding, we will develope methods to deliver cells or direct cells to form functional myocardium for heart failure. Cardiologists and cardiac surgeons have often successfully utilized new advances in basic science and clinical cardiovascular medicine to improve the treatment result of cardiac diseases. It will be interesting to watch how the concepts and principles described here are transformed into a clinical reality.

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Basic science discoveries elucidating the molecular and cellular biology of the T cell have led to new strategies in this fight, includi â€¦Â 2 Clinical Genomics Program, Division of Intramural Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, USA. 3 Molecular Development of the Immune System Section, Laboratory of Immune System Biology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, USA. lenardo@nih.gov. 4 Clinical Genomics Program, Division of Intramural Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, USA. lenardo@nih.gov. Cell therapy (also called cellular therapy, cell transplantation, or cytotherapy) is a therapy in which viable cells are injected, grafted or implanted into a patient in order to effectuate a medicinal effect,[1] for example, by transplanting T-cells capable of fighting cancer cells via cell-mediated immunity in the course of immunotherapy, or grafting stem cells to regenerate diseased tissues.Â Despite being one of the fast growing areas within Life Sciences,[51] the manufacturing of cell therapy products is largely hindered by small scale batches and labour-intensive processes.[52].Â "Cartilage Repair With Autologous Bone Marrow Mesenchymal Stem Cell Transplantation: Review of Preclinical and Clinical Studies". Cartilage.Â "Therapeutic approaches for cardiac regeneration and repair". Cell therapy for myocardial repair is emerging from preclinical studies and clinical trials as a potentially viable option in the treatment of heart disease. The results so far have been exciting, but caution must be maintained. For cell therapy to reach its potentialÂ To achieve these goals will require the use of cutting-edge technologies, such as tissue engineering, new imaging modalities, and molecular biology. Clinical trials must be developed to better test the safety and efficacy of cell therapy in side-by-side comparisons in a variety of myocardial injuries, from acute myocardial infarction to end-stage heart failure. We have the opportunity to create a new era in the treatment of heart disease.