Review Article

The plasticity of adult stem cells and their application in myocardial regenerative medicine

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Abstract Current therapies for myocardial infarction and congestive heart failure are limited in efficacy or in applicability. The plasticity of adult stem cells and cellular transplantation offer a novel therapeutic approach to improve cardiac function. This review describes the latest progress in research, summarizes recent studies of adult stem cells and their application in myocardial regenerative medicine in China and abroad, and discusses the future directions of cell transplantation as a new therapy to repair injured hearts. (J Geriatr Cardiol 2004;1(2):77-82.)

Key Words mesenchymal stem cells; regeneration; transplantation; cellular therapy; cardiomyocyte

Introduction

Cardiovascular diseases are the most frequent cause of death in the world. In China, patients with coronary heart disease are about 43 million, with 350,000 new cases each year. In the United States, congestive heart failure, the ineffective pumping of the heart caused by the loss or dysfunction of cardiomyocytes, afflicts 4.8 million people, with 400,000 new cases each year. The main cause for the development of this condition is myocardial infarction (MI), affecting nearly 1.1 million Americans each year. End-stage heart failure still has a bad prognosis due to the fact that only 50% of these patients survive the following years.1 Lots of studies showed that mature cardiomyocytes could not regenerate after injury, and that soon after birth the mammalian heart loses its capability of an effective proliferative response to various injuries.2-4 Although Oh H et al5 found that endogenous cardiac progenitors reside in the adult heart, regenerate cardiomyocytes functionally, and integrate into the existing heart circuitry, cardiomyocyte DNA syntheses in normal (0.0005%) and injured (0.01%) adult mammalian hearts are still extremely rare when myocyte nuclei can be reliably identified.6,7

Oclusion of a coronary artery and the resultant myocardial ischemia rapidly result in myocardial necrosis followed by scar tissue formation. Sometimes death of cardiomyocytes promotes an inflammatory cascade that results in heart failure.8 Clinically, there is no radical treatment for promoting regeneration or rebuilding the infarcted myocardium. Cardiac transplantation might be a choice for the treatment of end stage heart failure. However, its application is limited by the availability of donor organs. Another major obstacle in using this measure is that the recipient's immune response requires life-long immunosuppressive therapy.9 Presumably, identification of the cellular and molecular mechanisms that mediates adult mammalian cardiomyocyte re-entering the cell cycle would be the most desirable future goal in myocardial regeneration. Research in this line will benefit therapeutic strategies that force surviving cardiomyocytes to de-differentiate, proliferate, and re-differentiate after myocardial damage.10 However, another innovative approach, probably within nearer reach, is the delivery of exogenous cells at lower differentiated levels to the damaged heart. The delivered lower differentiated cells retaining their capability of proliferation, consequently may colonize in the scar and differentiate into cells with contractile properties.11 Recently, cellular replacement therapy and myocardial regeneration have become ideal treatments in this area. They prompted interest in identifying candidate cells for replenishing the injured myocardium with healthy cells and augmenting heart function.

Embryonic stem cells and application studies

Over the past decade, cardiac cellular transplantation
techniques have made significant advances, and a variety of cell types have been proposed as useful candidates, such as embryonic/neonatal cardiomyocytes and embryonic stem cells, neural stem cells (NSC), endothelial stem cells (EnSC), hepatic stem cells (HepSC), cardiac stem cells (CSC), skeletal myoblasts, bone marrow hematopoietic side-population stem cells (SP), and mesenchymal stem cells (MSCs). The optimal cells for transplantation and their optimal sources are of important consideration. The recent establishment of the human pluripotent embryonic stem (ES) cell lines may present a novel solution for this cell-sourcing problem. The ES lines were derived from human blastocysts and were shown to be capable of continuous undifferentiated proliferation, in vitro, while retaining the capability to form derivatives of all three germ layers, and recently a reproducible cardiomyocyte differentiation system was established using these unique cells. But, transplantation of embryonic stem cells into infarcted myocardium is not feasible in clinical practice because: (1) unresolved ethical issues need to be clarified in advance, (2) immature cardiomyocytes are not always available for therapeutic purpose in humans. Furthermore, an appropriate immunosuppressive regime would have to be established. In fact, the transplanted allogenic cells can only survive for a short time in the recipient heart because of immunorejection; therefore autologous cell transplantation would be the ideal choice.

The plasticity of adult stem cells and myocardial regeneration

In various species, such as dogs, rabbits and rats, engraftments of skeletal myoblasts are successfully colonized in injured cardiac tissues. The possibility of amplifying satellite cells in vitro and potentially continuing proliferation after transplantation, as well as a higher tolerance of ischemia, are not desirable in skeletal muscle grafting. Moreover, autologous skeletal muscle biopsies might not yield sufficient cells to repair the myocardium. The transplanted skeletal myoblasts did not express N-cadherin and connexin-43, while the host myocardium expressed both. Another important issue which must be investigated carefully is the effect of transplanted myoblasts on the electrical stability of the heart. The transplanted myoblasts did not form normal electrical junctions with the host cells in some animal models, suggesting that this could result in a significantly reduced arrhythmic threshold.

It has been claimed that stem cells derived from bone marrow could differentiate into hepatocytes, muscle cells, astrocytes, neurons, or primitive mesenchymal cells, and muscle and neural stem cells into blood cells. It has been proposed that this trans-tissue, even trans-germ layer differentiation be named “plasticity.” However, recent in vitro studies reported the occurrence of spontaneous cell fusion of putative stem cells, such as bone marrow or neural stem cells, with embryonic stem cells in co-cultivation. Nygren JM used various approaches to induce acute myocardial injury and delivered transgenically marked bone marrow cells to the injured myocardium. Their results showed that unfractured bone marrow cells and a purified population of hematopoietic stem and progenitor cells efficiently engrafted within the infarcted myocardium. Engraftment was transient, however, and hematopoietic in nature. In contrast, bone marrow-derived cardiomyocytes are observed outside the infarcted myocardium at a low frequency and are derived exclusively through cell fusion. This challenges the concept of transdifferentiation and requires additional investigation. However, data supporting stem cell plasticity are extensive and cannot be easily dismissed. Myocardial regeneration is perhaps the most widely studied and debated example of stem cell plasticity.

More and more studies indicate that adult bone marrow MSCs have multi-differentiation potential that can differentiate into skeletal muscle, cartilage, bone, liver, lung, brain, spleen, tendon and cardiac muscle cells. The discovery of the plasticity of stem cells has questioned the previous germ layer-limited theory in embryo development in spite of lack of known mechanisms. Autologous marrow MSCs have much greater advantage over other cells because they are easy to obtain, they can be easily propagated, they induce no immunorejection, they have moral support, and they have no risk of tumor growth. After 5-azacytidine treatments for the cultured murine bone marrow cells, Makino et al was able to select cardiomyocyte-like cells on the basis of spontaneous beating. Also, the same group reported the expression of β-adrenergic and muscarinic receptors after induction of differentiation by 5-azacytidine, while α-adrenergic receptors seemed to be present even before differentiation. Toma et al reported that human MSCs were able to differentiate into cardiomyocyte phenotype cells after injection into the left ventricle of adult mice.

MSCs differentiation and animal experiments in our research

In recent years, we have done some research work on the plasticity of adult MSCs and their application in myocardial regenerative medicine, including differentiation of MSCs into cardiomyocytes in vitro and in vivo. We hope this research could be of help for the future use of human adult stem cells in therapeutic cardiologyplasty.

Firstly, we investigated the potential of the adult rat
or human bone marrow MSCs in cardiomyogenic differentiation in vitro.46 MSCs were cultured in a medium supplemented with 5-azacytidine for cardiomyocyte differentiation. Immunohistochemical analysis revealed that the induced cells expressed desmin, GATA4, cardiac troponin I (cTnI) and connexin-43. Myosin heavy chain (MyHC) gene was also detected by RT-PCR technique. Electron microscopy showed a centrally positioned nucleus and cytoplasmic myofilaments. Such induced muscle cells exhibited cardiac-like electrophysiological properties including voltage-sensitive ionic current $I_{Na}$ and $I_{K}$. These findings suggest that the purified human MSCs (hMSCs) from adult bone marrow can be induced to differentiate into functional cardiac-like muscle cells in vitro and might be an alternative source of undifferentiated cells for cell therapy in myocardial regeneration.

On the basis of in vitro experimental data, we demonstrated the possibility of survival, migration and differentiation of rat MSCs (rMSCs) in host myocardium. The implanted rMSCs improved the function of the infarcted heart. The models for injured heart were induced by left coronary artery ligation. rMSCs (10⁶ cells) were transplanted into each host heart one hour after myocardial infarction and 10 weeks later the cellular transplanted hearts were harvested. Results revealed that the DAPI-labelled rMSCs formed island-like structures. However, the implanted cells with oval nucleus were broadly distributed. Their organization was comparable to the host myocardium fibers of the infarcted host heart. These cells stained positively for cardiac specific protein.45 Transplantation of rMSCs was associated with a significant decrease in the left ventricle end-diastolic volume and an increase in the left ventricular end-systolic pressure. The ratio of left ventricular pressure rise to left ventricular pressure decline ($\pm dp/dt$) was increased when compared with the media injected control hearts. The number of blood vessels at the border of the infarction site was increased. The size of the scar area was decreased significantly. These data suggest that the transplanted adult MSCs are subjected to local differentiation environmental signals. Evidently the purified exogenous rMSCs can survive in host hearts and take part in myocardium regeneration and have beneficial effects on the recovery of the heart function.47

The public attitude towards xenotransplantation is reportedly more favorable to receiving cells or tissues than a whole organ, but many scientific obstacles need to be overcome before the utilization of xenogeneic cells for cardiac repair in patients with heart disease becomes applicable to clinical practice.48 Recently, Grimono KH’s study49 showed different results. They found that adult human MSCs did not induce xenoreactivity in vitro in previously unexposed immunocompetent Sprague-Dawley rats. However, although MSCs were transplantable across allo- geneic barriers, transplant rejection might occur in a xenogenic model. When transplanted into an immunocompetent host, adult human MSCs showed persistent engraftment. Therefore, whether adult human MSCs survive and engraft in experimentally induced ischemic myocardium in rat needs intensive study.

**Microenvironment affecting cell differentiation**

Entering a new microenvironment, adult stem cells might be affected by the adjacent differentiating cells to process genetic reprogramming, which might result in generating different cell types. This process is known as “milieu-influenced” differentiation.50 It might be true that the microenvironment has important implications for the fate of stem and precursor cells. As we all know, once MI develops there is a rapid onset of contraction band necrosis and an intense inflammatory cascade. Different inflammatory microenvironments are critical to the effects of cellular transplantation. By means of transplanting DAPI-labelled MSCs into the injured hearts at different times after MI, Li RK51 and associates intensively investigated the optimal time for transplantation from aspects of cell biology, histology, immunohistochemistry and functional analysis. They found that few engrafted cells survived when grafted 1 week after MI, which might be due to inflammatory reaction, while the cells transplanted 2-4 weeks after MI were viable in large numbers. Consequently, MSCs’ transplantation at 2-4 weeks after MI thickened the scars, prevented left ventricle (LV) dyskinesis and improved LV function. These findings might suggest that excessive inflammatory reaction is not suitable for engrafted cells while the subsequent released growth factors, within or around the injured myocardium, may contribute to the graft survival or proliferation. Currently, this theory remains speculative.

Another proposed effect of transplanting bone marrow-derived stem cells seems to involve augmentation of angiogenesis. Kamiyama H et al52 found that an increase in angiogenesis after intramyocardial injection of bone marrow-derived mononuclear cells in coronary artery ligation induced MI. Similarly, Davani S et al53 found that some engrafted mesenchymal progenitor cells appeared to differentiate into endothelial cells. This revealed that bone marrow-derived MSCs could not only induce angiogenesis, but also become a part of the vasculature and thus directly contribute to the angiogenic process.

**Myocardial regeneration and gene therapy**

Recent findings in cardiovascular research provide the possibility that gene therapy and stem cell therapy
could have supportive effects on myocyte regeneration and myocardial revascularization in the damaged heart. 54 Miyagawa S et al 55 combined cell therapy with gene therapy using transfection of the gene for human hepatocyte growth factor (hHGF) to overcome the shortcomings and facilitate the myocardial regeneration. These results indicated that hHGF gene transfection enhanced the cellular cardiomyoplasty possibly by stimulating angiogenesis, restoring the impaired extracellular matrix, and promoting the integration of the dissociated grafted myocytes. The combined effects might have led to the improved cardiac performance. This strategy showed a superior effect to one using adenovirus-mediated gene transfer alone. The functional effects and safety of this strategy are integrated from both sides: the stem cell therapy and gene therapy. They not only provide a novel technical platform, but also propose a reformative concept, ex vivo gene therapy. 56 Their specific biological characteristics have established stem cells as the most desirable vector for exogenic gene transfer. In addition, with the development of stem cell enrichment technique, autologous stem cells can be utilized in ex vivo gene therapy to reduce the risk of immune rejection response without causing ethical disputes. Even more significant is the fact that in some pathological circumstances the stem cells, as vectors, can be either mobilized or homed, and therefore the problem of therapeutic targeting, which is difficult with other vectors, is well managed. 57

Techniques for delivering cells into target tissues

The techniques used for transplantation are also important to the effect of cellular therapy. If the cells were injected into the myocardial scar tissue, the graft remained largely separate from the host by collagen and connective tissue. Thompson et al delivered cultured swine bone marrow cells via a special catheter and transvenous needle (TransAccess), which was advanced to the interventricular vein via the coronary sinus. 58 Smits P et al reported the procedural and six-month results of the first percutaneous and stand-alone study on myocardial repair with autologous skeletal myoblasts. This pilot study was the first to demonstrate the potentiality and feasibility of percutaneous skeletal myoblast delivery as a stand-alone procedure for myocardial repair in patients with post-infarction heart failure. Similarly, a trans-endocardial approach, using a special device (NOGA-system), was employed to transplant cultured human bone marrow cells 59 into pigs, and recently for the first time in patients. 60 Stamm C initiated a phase-I study of autologous stem cell transplantation in patients undergoing coronary artery bypass grafting (CABG). Stem cells were isolated from bone marrow using a ferrite-conjugated AC133 anti-body, and were injected in the border zone of the infarcted focus during the CABG operation. The results indicated that bone marrow stem cell transplantation for myocardial regeneration can be safely performed in human and there is evidence of improved revascularization and contractility of infarcted areas. Future clinical controlled studies are needed to establish the relative efficiency of this new approach. Valliat PR et al 63 investigated whether bone marrow stem cells (mesenchymal stromal cells) could be safely injected into the coronary circulation in dogs. They injected about 0.5 million cells per kg body weight of early passage of autologous MSCs into the left circumflex coronary artery of anesthetized dogs. During administration, they noted ST segment elevation and T wave changes in acute myocardial ischaemia. Seven days later, macroscopic and microscopic evidence of MI was noted. Histological sections of myocardium showed scattered regions of dense fibroplasia accompanied by macrophage infiltrates only in areas where the MSCs were observed. Their results showed a potential complication of injecting MSCs, or probably any similarly sized cells, into the coronary circulation. Although differences between canine and human coronary circulation exist, and different cell types and sizes have been used for selected cytotherapeutic applications, this potential complication should be thoroughly investigated before MSCs are routinely injected into the arterial circulation of patients.

Summary

In summary, cellular cardiomyoplasty is a new strategy for provision of surrogated cardiomyocytes and for treatment of various diseases including MI and heart failure. Preliminary data, obtained in patients with acute MI, suggest that the observation obtained in the experimental animal may be transferred to the clinical arena in the near future. 64 The first clinical experience was reported in November 2000 by Menasche and associates 65 of Paris using myoblasts by way of coronary bypass surgery. Strauer BE et al delivered autologous bone marrow stem cells via percutaneous coronary angiography and stents for myocardial regeneration following MI in a 46-year-old man. 66 This report of autologous intracoronary bone marrow transplantation in patients with myocardial infarction demonstrated the need for noninvasive techniques to monitor the MSCs delivery. Britten and colleagues 67 also reported intracoronary arterial injection of bone marrow and progenitor cells in patients with myocardial infarction and found that cell therapy is one of the ideal methods for myocardial regeneration. However, many problems still remain unresolved and need to be addressed in the future research before a clinical application is appropriate. The effects on the transition of heart failure, the best thera-
apeutic approach, and different techniques to optimize transplantation remain to be determined. Controlled studies are needed to clarify the role of cell transplantation in myocardial regeneration. Also, the genetic and cellular mechanisms that initiate differentiation or cell fusion must be defined. Only then will we be able to adopt this process and begin to realize the full potential of adult stem cells in regenerative medicine.

Acknowledgements: We would like to thank the State 863 High Technology R&D Project of China (2002AA205051 and 2003AA205160) and the National Key Project for Basic Research of China (2001CB51F900) for the support of our work. We would like to express sincere thanks to Mr. Churnian HAN and Dr. Dongmei WANG for providing generous help.

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