

Physiology and Regenerative Potential of Cardiomyocyte Renewal

Kosit Sribhen, M.D.,* Choosri Sribhen, Dr.sci.agr.**

*Department of Clinical Pathology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700,**Department of Physiology, Faculty of Veterinary Medicine, Kasetsart University, Bangkok 10900, Thailand.

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Until the past decade, it was generally believed that the mammalian heart, unlike other organ systems, is a terminally differentiated organ that exerts virtually no capability for cardiomyocyte renewal. This limits the ability of the myocardium to restore function after any significant injury and renders the heart to the development of organ dysfunction and failure. However, accumulating evidence in recent years have indicated that myocyte renewal and regeneration exist in the myocardial tissue. Thus, the dogma that the heart is a postmitotic nonregenerating organ has been challenged. The present review focuses on the physiology of cardiac myocyte renewal and turnover, and discusses the regenerative potential of cardiac stem/progenitor cells in physiological and pathological states, as well as the therapeutic strategies that have been developed for cardiac repair.

Traditional concept of cardiomyocyte renewal

In the mammalian heart, cardiac myocytes increase rapidly through proliferation during early fetal life, but lose their ability to proliferate soon after birth. Myocardial cell growth is initiated by activation of insulin-like growth factor (IGF)-I or fibroblast growth factor (FGF)-2 and is under the regulation of several transcription factors (e.g. c-Myc, E2Fs) and cell cycle inhibitors (e.g. p38MAPK, TSC2) (Fig 1). In the perinatal period, the myocytes undergo an additional round of DNA synthesis and nuclear mitosis without cytokinesis (acytokinetic mitosis), leaving the majority of adult cardiomyocytes binucleated: the percentage of binucleated cells ranges from 25 to 57% in humans and is up to 90% in mice and rats.¹ According to the traditional concept of myocardial cell renewal, further increases in cardiac mass are achieved through an increase in cell size (hypertrophy) and not in cell number (hyperplasia). These terminally differentiated cells do not reenter the cell cycle in response to mitogens or normal physiological stress.

In other words, the age of individuals should coincide with the age of their myocytes. Since myocardial cells do not live indefinitely, the old heart is characterized by a reduction in cell number and hyper-

trophy of the remaining myocytes.² Thus, according to the previously accepted paradigm, cardiac homostasis is very static because of the absence of myocyte renewal, and it is dependent on the ability of cardiomyocytes to be as long-lived as the individual. This also means that in the absence of any significant turnover of the myocytes and the fact that myocyte death occurs throughout the lifespan of an individual independently from cardiac disease, the normal heart would lose most of its mass in a few decades, and the old and diseased heart would disappear in several months to few years.

New concept of cardiomyocyte renewal

The traditional concept that the heart is a terminally differentiated postmitotic organ was challenged several years ago by the finding that cardiac myocytes were capable of reentering the replicative phase of the cell cycle. Accordingly, a new concept of the heart as a dynamic, potentially self-renewing organ emerged. Several investigators have independently demonstrated the presence of cardiac progenitor cells or stem cells in adult myocardium with the capacity to differentiate into cardiac myocytes, smooth muscle, and endothelial cells.³⁻⁵ The stem cells are present in the myocardium

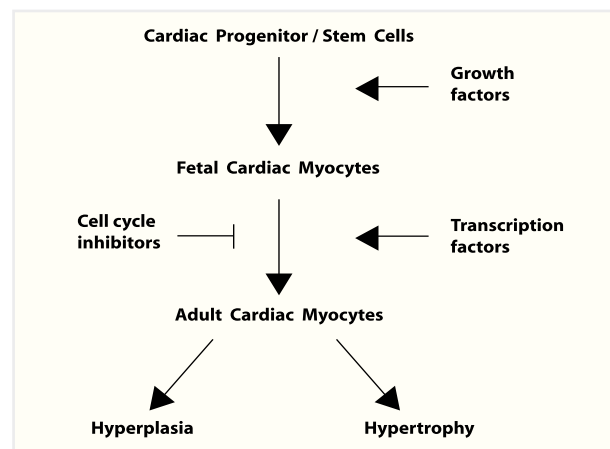


Fig 1. Schematic description of myocardial cell cycle control. (Modified from reference 1)

either as a resident population of embryonic origin or as a blood-born population that continuously seeds the myocardial tissue.

Critical factors that determine the proliferative capacity of cardiomyocytes seem to be the nuclear state and cellular size: smaller, mononucleated myocytes display higher potential to reenter into the cell cycle than the fully differentiated, multinucleated larger myocytes. Recently, Rota et al.⁶ reported on the functional characteristics of two cell types in the mouse that differed by age and size, and found an inverse correlation between cell volume and telomere length. The old, large and multinucleated myocytes tended to have short telomeres which is associated with a loss of telomerase function, whereas the young, small and mononucleated myocytes showed long telomeres and signs of mitosis. Results from this study are in accordance with those from Chen et al.,⁷ who have demonstrated a population of small, proliferative cardiac myocytes with immature physiological properties in the adolescent feline heart.

The concept that myocyte replication occurs under pathological conditions of the heart has been described both in the animal model and humans. Previous studies have shown that cardiac myocytes express growth-related genes after infarction and heart failure, with increases in quantities of cyclins and their associated kinase activities, and high levels of DNA replication, karyokinesis and cytokinesis have been identified.⁸⁻¹⁰ This concept has been strengthened by the finding of a marked increase in the activity of telomerase in dogs with heart failure.¹¹ In the human heart, the number of dividing myocytes is several fold higher in the acute phase of infarction⁸ than in chronic MI and heart failure.¹¹

Physical exercise and myocardial cell turnover

Physical activity has been shown to be associated with lower all-cause mortality rates both in healthy individuals and in those with chronic diseases, the specific mechanisms of which are multifactorial and extend beyond the favorable influence on established cardiovascular risk factors such as blood pressure reduction, increased insulin sensitivity and improved lipid profile. These include the beneficial effect on endothelial function by increasing nitric oxide production as well as in favorably modulating the balance between sympathetic and parasympathetic tone.¹²

In the vast majority of healthy individuals without signs and symptoms of cardiovascular diseases, measurable concentrations of cardiac specific protein marker troponins were detected,¹³ which may reflect the physiological myocardial cell turnover.¹⁴ On the other hand, it has been reported that a significant number of healthy athletes exhibited minimal transient increases in cardiac troponin levels above the upper limit of the reference range after endurance exercise events.¹⁵ This may be related to the toxic effect of free radicals, generated during endurance exercise, on myocyte membranes causing releases of the cytosolic troponins into the circulation. Since physical exercise has also been demonstrated to be associated with increases in endothelial progenitor cells,¹² it is possible that an increase in cardiac myocyte turnover can be achieved through exercise-mediated activation of resident cardiac progenitor cells. In addition, a number of studies in recent

years have provided evidence that exercise and physical activity improve the function and regeneration of the cardiovascular system by activating mobilizing resident stem cells or by recruiting blood-circulating stem or progenitor cells.¹⁶ Nevertheless, the molecular mechanisms by which these cells are activated are at present not fully understood. A better understanding of these mechanisms will make physical activity a useful tool as an adjunctive strategy to stem cell therapy in cardiovascular disease.

Therapeutic strategies of cardiomyocyte renewal

Theoretically, the resident cardiac stem cells (CSCs) and bone-marrow-derived stem cells (BMCs) or those of other origins (embryonic, mesenchymal), all have the potential of regenerating myocardium and improving cardiac function. However, non-cardiac-derived stem cells have to re-programme themselves to give rise to progeny differentiating into cardiac lineages, a process that can be avoided during activation and migration of CSCs to the site of injury. In addition, the time in reaching functional competence and the structural characteristics of mature myocytes and vessels may be faster for CSCs than BMCs.²

A novel approach to the use of stem cells for cardiac repair is to reactivate the proliferative potential of CSCs by genetically manipulating key cell cycle regulators to promote cell cycle progression.¹ It has been demonstrated that elevated DNA synthesis and mitotic index were achieved in cyclin A2 (a cell cycle mediator) overexpressing transgenic mice, and post-infarct delivery of this mediator expressing adenoviral vector induced myocardial regeneration and enhanced cardiac function postinjury.¹⁷ Similar results of inducing mitosis in cardiac myocytes and improving cardiac function were obtained in another study after treatment with FGF-1 (a growth factor) in combination with p38 MAPK (a cell cycle inhibitor).¹⁸

Another approach of myocardial regenerative therapy is the direct cell implantation of a purified population of putative cardiomyocyte progenitors (CMPs) into the infarcted hearts. As mentioned above, the CMPs have several advantages as donor cells for myocardial regeneration. They are already committed to cardiomyocyte lineages, and thus may not be affected in their differentiation by an adverse environment. In addition, due to the autologous nature of the implanted cells, there is no need for immunosuppression. By utilizing this approach, Yang et al.,¹⁹ reported that CMPs were able to be retained and proliferate within the ischemic heart of the rat. The progeny of implanted cells migrated along the infarcted scar, reconstituted regenerated cardiomyocytes and inhibited cardiac remodeling with decreased scar formation. Recently, Matsuura et al.,²⁰ have demonstrated in a similar experimental model in mice that transplantation of sheets of clonally expanded CMPs resulted in a differentiation of these cells into cardiac myocytes and vascular cells, and restored cardiac function and angiogenic activity after myocardial infarction.

CONCLUSION

Adult mammalian myocardium contains a sub-population of progenitor or stem-like cells that have the capability of entering the cell cycle and the potential

of cardiomyocyte self-renewal and angiogenesis, possibly throughout the lifespan of an individual. This provides the basis for an increase in myocardial mass in response to physical or pathological demands on the heart. Recently, Bergmann et al.,²¹ have provided the definitive evidence that human cardiomyocytes are renewed during postnatal life. They measured the incorporation of carbon-14 (¹⁴C), generated by nuclear-bomb tests during the 1950s, into genomic DNA in order to calculate the rates of turnover in these cells. Since DNA is stable after a cell has gone through its last cell division, the concentration of ¹⁴C in DNA serves as a date mark for when a cell was born and can be used to retrospectively determine the birth date of cells in humans. The authors reported that cardiac myocytes renew throughout life at a very low rate, with a gradual decrease from approximately 1% turning over annually at the age of 25 years to 0.45% at the age of 75 years. During a normal life span, fewer than 50% of cardiomyocytes renew.

The results from the study by Bergmann et al, although substantiating the new concept of myocardial cell renewal, have raised several questions of clinical and practical concern. Due to the limited potential of cardiomyocyte self-renewal, it is at present largely unknown, as to whether the resident cardiac progenitor/stem cells, as compared to the bone-marrow-derived stem cells, the embryonic stem cells or mesenchymal progenitor cells, represent the optimal sources for cardiac regenerative therapy. Likewise, it is still not known, whether autologous transplantation strategies or pharmacologic strategies to stimulate and enhance the regenerative process of resident progenitor/stem cells are the best approach. These represent topics of research interest and remain to be fully elucidated in future investigations.

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