HEART DISEASE Helped With STEM CELLS

Can Stem Cells help Heart Disease and Repair a Damaged Heart? Heart attacks and congestive heart failure remain among the Nation's most prominent health challenges despite many breakthroughs in cardiovascular medicine. In fact, despite successful approaches to prevent or limit heart disease, the restoration of function to the damaged heart remains a formidable challenge. Recent research is providing early evidence that adult and embryonic stem cells may be able to replace damaged heart muscle cells and establish new blood vessels to supply them. Discussed here are some of the recent discoveries that feature stem cell replacement and muscle regeneration strategies for repairing the damaged heart and healing the heart disease.

Introduction

For those suffering from common, but deadly, heart diseases, stem cell biology represents a new medical frontier. Researchers are working toward using stem cells to replace damaged heart cells and literally restore cardiac function.

Today in the United States heart disease is at an alarming growth rate. Congestive heart failure — the ineffective pumping of the heart caused by the loss or dysfunction of heart muscle cells—afflicts 4.8 million people, with 400,000 new cases each year. One of the major contributors to the development of this condition is a heart attack, known medically as a myocardial infarction, which occurs in nearly 1.1 million Americans each year. It is easy to recognize that impairments of the heart and circulatory system represent a major cause of death and disability in the United States [5].

What leads to these devastating effects? The destruction of heart muscle cells, known as cardiomyocytes, can be the result of hypertension, chronic insufficiency in the blood supply to the heart muscle caused by coronary artery disease, or a heart attack, the sudden closing of a blood vessel supplying oxygen to the heart. Despite advances in surgical procedures, mechanical assistance devices, drug therapy, and organ transplantation, more than half of patients with congestive heart failure die within five years of initial diagnosis. Research has shown that therapies such as clot-busting medications can reestablish blood flow to the damaged regions of the heart and limit the death of cardiomyocytes. Heart disease researchers are now exploring ways to save additional lives by using replacement cells for dead or impaired cells so that the weakened heart muscle can regain its pumping power.

How might stem cells play a part in heart disease? To answer this question, researchers are building their knowledge base about how stem cells are directed to become specialized cells. One important type of cell that can be developed is the cardiomyocyte, the heart muscle cell that contracts to eject the blood out of the heart's main pumping chamber (the ventricle). Two other cell types are important to a properly functioning heart are the vascular endothelial cell, which forms the inner lining of new blood vessels, and the smooth muscle cell, which forms the wall of blood vessels. The heart has a large demand for blood flow, and these specialized cells are important for developing a new network of arteries to bring nutrients and oxygen to the cardiomyocytes after a heart has been damaged. The potential capability of both embryonic and adult stem cells to develop into these cells types in the damaged heart is now being explored as part of a strategy to restore heart function to people who have had heart attacks or have congestive heart failure. It is important that work with stem cells is not confused with recent reports that human cardiac myocytes may undergo cell division after myocardial infarction [1]. This work suggests that injured heart cells can shift from a quiescent state into active cell division. This is not different from the ability of a host of other cells in the body that begin to divide after injury. There is still no evidence that there are true stem cells in the heart which can proliferate and differentiate.

Researchers now know that under highly specific growth conditions in laboratory culture dishes, stem cells can be coaxed into developing as new cardiomyocytes and vascular endothelial cells. Scientists are interested in exploiting

this ability to provide replacement tissue for the damaged heart. This approach has immense advantages over heart transplant, particularly in light of the paucity of donor hearts available to meet current transplantation needs.

What is the evidence that such an approach to restoring cardiac function might work? In the research laboratory, investigators often use a mouse or rat model of a heart attack to study new therapies (see Figure 9.1. Rodent Model of Myocardial Infarction). To create a heart attack in a mouse or rat, a ligature is placed around a major blood vessel serving the heart muscle, thereby depriving the cardiomyocytes of their oxygen and nutrient supplies. During the past year, researchers using such models have made several key discoveries that kindled interest in the application of adult stem cells to heart muscle repair in animal models of heart disease.

Figure 9.1. Rodent Model of Myocardial Infarction. (© 2001 Terese Winslow, Lydia Kibiuk)

Recently, Orlic and colleagues [9] reported on an experimental application of hematopoietic stem cells for the regeneration of the tissues in the heart. In this study, a heart attack was induced in mice by tying off a major blood vessel, the left main coronary artery. Through the identification of unique cellular surface markers, the investigators then isolated a select group of adult primitive bone marrow cells with a high capacity to develop into cells of multiple types. When injected into the damaged wall of the ventricle, these cells led to the formation of new cardiomyocytes, vascular endothelium, and smooth muscle cells, thus generating de novo myocardium, including coronary arteries, arterioles, and capillaries. The newly formed myocardium occupied 68 percent of the damaged portion of the ventricle nine days after the bone marrow cells were transplanted, in effect replacing the dead myocardium with living, functioning tissue. The researchers found that mice that received the transplanted cells survived in greater numbers than mice with heart attacks that did not receive the mouse stem cells. Follow-up experiments are now being conducted to extend the posttransplantation analysis time to determine the longer-range effects of such therapy [8]. The partial repair of the damaged heart muscle suggests that the transplanted mouse hematopoietic stem cells responded to signals in the environment near the injured myocardium. The cells migrated to the damaged region of the ventricle, where they multiplied and became "specialized" cells that appeared to be cardiomyocytes.

A second study, by Jackson et al. [3], demonstrated that cardiac tissue can be regenerated in the mouse heart attack model through the introduction of adult stem cells from mouse bone marrow. In this model, investigators purified a "side population" of hematopoietic stem cells from a genetically altered mouse strain. These cells were then transplanted into the marrow of lethally irradiated mice approximately 10 weeks before the recipient mice were subjected to heart attack via the tying off of a different major heart blood vessel, the left anterior descending (LAD) coronary artery. At two to four weeks after the induced cardiac injury, the survival rate was 26 percent. As with the study by Orlic et al., analysis of the region surrounding the damaged tissue in surviving mice showed the presence of donor-derived cardiomyocytes and endothelial cells. Thus, the mouse hematopoietic stem cells transplanted into the bone marrow had responded to signals in the injured heart, migrated to the border region of the damaged area, and differentiated into several types of tissue needed for cardiac repair. This study suggests that mouse hematopoietic stem cells may be delivered to the heart through bone marrow transplantation as well as through direct injection into the cardiac tissue, thus providing another possible therapeutic strategy for regenerating injured cardiac tissue.

More evidence for potential stem cell-based therapies for heart disease is provided by a study that showed that human adult stem cells taken from the bone marrow are capable of giving rise to vascular endothelial cells when transplanted into rats [6]. As in the Jackson study, these researchers induced a heart attack by tying off the LAD coronary artery. They took great care to identify a population of human hematopoietic stem cells that give rise to new blood vessels. These stem cells demonstrate plasticity meaning that they become cell types that they would not normally be. The cells were used to form new blood vessels in the damaged area of the rats' hearts and to encourage proliferation of preexisting vasculature following the experimental heart attack.

Like the mouse stem cells, these human hematopoietic stem cells can be induced under the appropriate culture conditions to differentiate into numerous tissue types, including cardiac muscle [10] (see Figure 9.2. Heart Muscle Repair with Adult Stem Cells). When injected into the bloodstream leading to the damaged rat heart, these cells prevented the death of hypertrophied or thickened but otherwise viable myocardial cells and reduced progressive formation of collagen fibers and scars. Control rats that underwent surgery with an intact LAD coronary artery, as well as LAD-ligated rats injected with saline or control cells, did not demonstrate an increase in the number of blood vessels. Furthermore, the hematopoietic cells could be identified on the basis of highly specific cell markers that differentiate them from cardiomyocyte precursor cells, enabling the cells to be used alone or in conjunction with myocyte-regeneration strategies or pharmacological therapies. (For more about stem cell markers see Appendix E.i. How Do Researchers Use Markers to Identify Stem Cells?)

Figure 9.2. Heart Muscle Repair with Adult Stem Cells (© 2001 Terese Winslow, Lydia Kibiuk)

Exciting new advances in cardiomyocyte regeneration are being made in human embryonic stem cell research. Because of their ability to differentiate into any cell type in the adult body, embryonic stem cells are another possible source population for cardiac-repair cells. The first step in this application was taken by Itskovitz-Eldor et al. [2] who demonstrated that human embryonic stem cells can reproducibly differentiate in culture into embryoid bodies made up of cell types from the body's three embryonic germ layers. Among the various cell types noted were cells that had the physical appearance of cardiomyocytes, showed cellular markers consistent with heart cells, and demonstrated contractile activity similar to cardiomyocytes when observed under the microscope.

In a continuation of this early work, Kehat et al. [4] displayed structural and functional properties of early stage cardiomyocytes in the cells that develop from the embryoid bodies. The cells that have spontaneously contracting activity are positively identified by using markers with antibodies to myosin heavy chain, alpha-actinin, desmin, antinaturietic protein, and cardiac troponin—all proteins found in heart tissue. These investigators have done genetic analysis of these cells and found that the transcription-factor genes expressed are consistent with early stage cardiomyocytes. Electrical recordings from these cells, changes in calcium-ion movement within the cells, and contractile responsiveness to catecholamine hormone stimulation by the cells were similar to the recordings, changes, and responsiveness seen in early cardiomyocytes observed during mammalian development. A next step in this research is to see whether the experimental evidence of improvement in outcome from heart attack in rodents can be reproduced using embryonic stem cells.

These breakthrough discoveries in rodent models present new opportunities for using stem cells to repair damaged heart muscle. The results of the studies discussed above are growing evidence that adult stem cells may develop into more cell types than first thought. In those studies, hematopoietic stem cells appear to be able to develop not only into blood, but also into cardiac muscle and endothelial tissue. This capacity of adult stem cells, increasingly referred to as "plasticity," may make such adult stem cells a viable candidate for heart repair. But this evidence is not complete; the mouse hematopoietic stem cell populations that give rise to these replacement cells are not homogenous. Rather, they are enriched for the cells of interest through specific and selective stimulating factors that promote cell growth. Thus, the originating cell population for these injected cells has not been identified, and the possibility exists for inclusion of other cell populations that could cause the recipient to reject the transplanted cells. This is a major issue to contend with in clinical applications, but it is not as relevant in the experimental models described here because the rodents have been bred to be genetically similar.

What are the implications for extending the research on differentiated growth of replacement tissues for damaged hearts? There are some practical aspects of producing a sufficient number of cells for clinical application. The repair of one damaged human heart would likely require millions of cells. The unique capacity for embryonic stem cells to replicate in culture may give them an advantage over adult stem cells by providing large numbers of replacement cells in tissue culture for transplantation purposes. Given the current state of the science, it is unclear how adult stem cells could be used to generate sufficient heart muscle outside the body to meet patients' demand [7].

Although there is much excitement because researchers now know that adult and embryonic stem cells can repair damaged heart tissue, many questions remain to be answered before clinical applications can be made. For example, how long will the replacement cells continue to function? Do the rodent research models accurately reflect human heart conditions and transplantation responses? Do these new replacement cardiomyocytes derived from stem cells have the electrical-signal-conducting capabilities of native cardiac muscle cells?

Stem cells may well serve as the foundation upon which a future form of "cellular therapy" is constructed. In the current animal models, the time between the injury to the heart and the application of stem cells affects the degree to which regeneration takes place, and this has real implications for the patient who is rushed unprepared to the emergency room in the wake of a heart attack. In the future, could the patient's cells be harvested and expanded for use in an efficient manner? Alternatively, can at-risk patients donate their cells in advance, thus minimizing the preparation necessary for the cells' administration? Moreover, can these stem cells be genetically "programmed" to migrate directly to the site of injury and to synthesize immediately the heart proteins necessary for the regeneration process? Investigators are currently using stem cells from all sources to address these questions, thus providing a promising future for therapies for repairing or replacing the damaged heart and addressing the Nation's leading causes of death.

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