Stem Cell Transplantation as a Mode of Regenerative Medicine

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Abstract: Advances in stem cell biology have made it possible for organ regeneration to become a reality, and this new technique is poised to enter the field of clinical medicine. The stem cells used in regenerative medicine are classified as embryonic or adult. Neurons, vascular endothelial cells, skeletal muscle cells, cardiomyocytes, osteoblasts, and chondroblasts have already been obtained from stem cells in the laboratory setting. Embryonic stem cells are amenable to mass culture and have versatile pluripotency but tend to be associated with problems in clinical application, including tumorigenesis, immunological rejection, and ethical issues. Since adult stem cells are obtained from the bone marrow of the patient, problems related to donors, ethics, rejection, and tumorigenesis do not apply. However, techniques for the isolation and in vitro amplification of adult stem cells have yet to be established, raising issues that await future solutions. For stem cells to be used in the clinical setting, regeneration at the tissue level is necessary, requiring the combined resources of tissue engineering and material science. Regenerative medicine is expected to play a leading role in 21st century medicine. However, the integration of studies from various scientific fields seems necessary for success in this area.

Key words: Embryonic stem cells; Adult stem cells; Regenerative medicine; Cell transplantation; Cardiomyocytes

Introduction

This paper outlines the current status and future prospects of regenerative medicine, particularly with regard to the use of stem cells. This new field of medicine has attracted a great deal of attention and is at the cutting edge of 21st century medicine. It is well-known that when a limb or tail of a newt or lizard is cut off, the missing part is regenerated from the stump. This occurs because cells at the cut edge can dedifferentiate into immature, pluripotent stem cells which can then differentiate into various cell types and regenerate the missing part.
cells, and then differentiate again into the target cells after cell division and proliferation. Although this regenerative phenomenon does not occur in mammals, it does not necessarily mean that humans and other mammals lack regenerative capacity. Human somatic cells also include pluripotent stem cells, which are capable of proliferating and differentiating to repair tissue when an impairment or defect has occurred that leads to dysfunction of the organ. The aim of regenerative medicine is to treat disease and injury by making use of this capability.

Heart transplantation, which has been the main treatment option for severe heart failure, provides an example. Although heart transplantation is an excellent treatment, it is not widely employed because of the need for a donor and the possibility of rejection after transplantation. In contrast, regenerative medicine uses stem cells to induce the formation of cardiomyocytes, which are then transplanted to the impaired heart of the patient.

**Fig. 1** Establishment of ES cells and their application to regenerative medicine

Stem cells used in regenerative medicine

Stem cells that can be used for regenerative medical therapies are broadly divided into two groups: embryonic stem cells (ES cells) obtained from early-stage embryos and adult stem cells that still are present in the adult body. These two types of stem cells have their own particular advantages and disadvantages. Whether one type is superior to the other remains controversial, depending on the type of tissue to which they are to be transplanted. A method of culture has already been established for ES cells, and their particular advantage is that they are capable of differentiating into any type of cell within the body.

Current status and problems of regenerative medicine using ES cells

At present, the regeneration of neurons,
On the other hand, from clinical experience with the transplantation of fetal midbrain obtained through artificial termination of pregnancy into the nigrostriatum in patients with Parkinson’s disease, it has been found that about one month of immunosuppressive therapy is sufficient for cases of transplantation of allogeneic nerve cells into the brain, because lymphocytes cannot cross the blood-brain barrier. Thus, ES cells are presumed to be superior for the regeneration of nerve cells in the central nervous system.

Application of adult stem cells to regenerative medicine

Let us now turn to the other type of stem cells, adult stem cells. To begin with, stem cells are known to be characterized by their capacity for self-replication, proliferative potency, and pluripotency. Stem cells are ranked from high to low in terms of the diversity of their ability to differentiate. For example, ES cells, which can differentiate into any type of cell, are given the highest rank, whereas hematopoietic stem cells are ranked in the middle, and cutaneous stem cells are considered low-ranking stem cells or precursor cells. Somatic tissues are formed from endoblasts, mesoblasts, or ectoblasts in the
fetal stage (Fig. 2). Skin and nerve tissues are derived from ectoblasts, and the stem cells of these tissues are therefore present in local areas. More specifically, cutaneous stem cells are present in the granular layer of the dermis, and neural stem cells are present around the cerebral ventricle of the cerebral hippocampus, where they are responsible for the regeneration of the respective tissues. Visceral organs such as the liver and pancreas are derived from endoblasts, and the stem cells of these tissues are present in the respective organs. If the liver is excised, oval cells or small hepatocytes in the remaining liver proliferate to regenerate the liver.

Various other cells of the body, such as bone, cartilage, fat, ligament, tendon, skeletal muscle, myocardium, and smooth muscle, are derived from mesoblasts. Among the stem cells of these tissues, some exist in muscle, for example, satellite cells, which are low-ranking stem cells found in the skeletal muscle. However, recent studies have revealed that the stem cells of these tissues are present in the bone marrow. As is well known, bone marrow consists mainly of hematopoietic stem cells and other cells of the blood cell series, but cells which are not blood cells are also present in bone marrow. Called bone marrow stromal cells, these cells are known to secrete various cell growth factors and cytokines that control the proliferation and differentiation of the blood cell series.

In recent years, it has become apparent that mesenchymal stem cells in the bone marrow with pluripotent capacity are present among marrow stromal cells. It had been reported by the early 1990s that mesenchymal stem cells differentiate into osteoblasts, chondroblasts, and adipocytes, and these cells began to be referred to as mesenchymal in the sense of mesoblast-derived stem cells. Since mesenchymal stem cells are stem cells for mesoblast-derived cells, we wondered whether they could differentiate to become cardiomyocytes, and we carried out studies along this line. We demonstrated that cardiomyocytes that beat regularly by themselves could be obtained from mesenchymal stem cells. It has also been reported that mesenchymal stem cells can differentiate into mesoblast-derived tissues such as tendon and ligament.

At this point, it is important to determine to what extent these stem cells in bone marrow are pluripotent and how far they can differentiate. Results were reported in the U.S. last year of an autopsy case of leukemia in a female patient who died after the transplantation of bone marrow from a male donor. In this patient, cells possessing the Y chromosome derived from the male donor were found in liver, skeletal muscle, and the intestinal tract. Thus, it became apparent that adult stem cells can differentiate not only into mesoblast- but also into endoblast-derived organs. A more recent study demonstrated that mesenchymal stem cells can differentiate into ectoblast-derived nerve cells. Therefore, the expression “mesenchymal” is no longer accurate, and these cells have been called adult stem cells because they can differentiate to become tissues derived from any germ layer.

Current status of regenerative medicine using adult stem cells

What procedures, then, are necessary for enticing these stem cells to differentiate into the target cells? The procedures naturally vary according to the cells that are desired. For instance, differentiation into osteoblasts that produce bone can be induced by adding dexamethasone, ascorbic acid, and β-glycerophosphate to the culture medium. To obtain differentiation into chondroblasts, the presence of insulin, transferrin, proline, and sodium pyruvate is required. Thus, selective differentiation can be induced by using known growth factors, biological substances, or even chemical substances in some cases. For those cells for which an established procedure does not currently exist, the use of differentiation inducers is now an option.
Although various differentiation-inducing agents are now available, we prefer a demethylating agent of DNA, 5-azacytidine. Details of its mechanisms of action, however, will not be discussed here. With this agent, it is possible to induce stem cells to differentiate into various directions through the random activation of transcription factors. From among the cells that have differentiated along various lines, those suitable for the particular purpose should be selected and utilized for the regenerative medicine procedure (Fig. 3).

Another method is to infuse stem cells directly into the target organ or tissue, to cause tissue regeneration. In each tissue, paracrine cytokines and growth factors are secreted from the surrounding cells, and tissue-specific cell adhesion factors and extracellular matrix are also expressed. Differentiation is induced by placing stem cells in such situations. Such a local environment is called a “niche”. It is expected that the niche will induce the infused stem cells to differentiate in the same direction as the surrounding cells.

Adult stem cells are advantageous in that they are present within the bone marrow and their collection causes no organ loss to the donor. Further, the already well-developed bone marrow bank system theoretically makes possible HLA-compatible transplantation. Another advantage of adult stem cells is that the ethical issues surrounding them are far fewer than those related to ES cells. In addition, if the bone marrow of the same patient is used, there is no posttransplantation rejection or any need for immunosuppressive drug therapy, providing another great advantage.

### Problems associated with adult stem cells

One problem associated with adult stem cells is that they are present in small numbers in the body, occurring at a rate of one in several hundred thousand marrow cells. The success of regenerative medicine using adult stem cells depends on whether they can be collected efficiently and proliferated under in vitro conditions while maintaining their pluripotent capacity.

Second, the problem of how to induce stem cells to differentiate into target cells is an important issue. Aside from particular cells for which the method of differentiation of stem cells is already known, further close investigation is necessary to establish methods of differentiation into various other cells present in the body. Another area of further investigation is to determine to what extent it is possible to induce differentiation in the niche by direct transplantation of stem cells in the target organ. Although the use of the niche is feasible for some organs such as myocardium and skeletal muscle, it is extremely difficult at present in the case of complexes of multiple types of cells, such as those comprising liver, lung, and kidney.
Use of tissue engineering and material science

Finally, it should be stressed that regenerative medicine has a very close relationship with tissue engineering and material science. Even if target cells can be obtained by using stem cells, they will need to possess a form consistent with their purpose when transplanted into the patient’s body. For example, even if vascular smooth muscle cells are regenerated, blood vessels are not necessarily formed. For the cells to take the form of a blood vessel, a scaffold should be created from high-molecular-weight compounds that would dissolve slowly in the body. The cells should be placed on the scaffold and incubated to achieve the form of a blood vessel. The development of good materials for such scaffolding is another important aspect of the regenerative medicine.

Conclusion

Regenerative medicine has become an important focus of the medical profession in the 21st century. However, the success of this type of medical care will require the cooperation of various fields of science, including molecular biology, developmental biology, embryology, anatomy, tissue engineering, and material science. People have high expectations of regenerative medicine. It is therefore important that basic research and translational research that applies the results of basic research continue to make progress.

REFERENCES