Regenerative Medicine for Cardiomyocytes

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Abstract: Heart transplantation is the ultimate treatment option for severe cardiac failure, but is available only for a very small fraction of cases due to a serious donor shortage. Increasing attention has become focused upon a novel therapeutic approach, regenerative medicine, to break the present impasse. Attempts to regenerate cardiomyocytes have been made by using pluripotent embryonic stem cells or marrow-derived mesenchymal stem cells (adult stem cells). Cardiomyocytes can be regenerated from embryonic stem cells as well as from adult stem cells, but the regenerated cells differ in characteristics depending on the source stem cells. These two groups of stem cells differ with respect to proliferative potency, pluripotency, method of induction of differentiation into cardiomyocytes, rejection reactions, and tumorigenic potential. Further studies to ascertain which type of stem cell will be more useful and safer for this purpose remain to be carried out. Studies in laboratory animals have reportedly demonstrated improvement of cardiac function through regenerated cardiomyocyte transplantation into the heart, encouraging the hope that a new treatment modality has been found for severe heart failure.

Key words: Embryonic stem cell; Adult stem cell; Cardiomyocyte; Regenerative medicine

Introduction

In Japan, cases of heart disease have consistently been increasing with the aging of the population and the Westernization of the diet. A wide variety of pharmacotherapies has been developed for the treatment of intractable severe heart disease, with proven efficacy, however, heart transplantation is the sole radical treatment option. There is still no increase in brain-dead donors and heart transplantation is a treatment available only for a very few cases.

To break the present impasse, therefore, a method to treat intractable cardiac failure by regenerating and transplanting cardiomyocytes is being sought. Studies of heart muscle cell regeneration have been making a steady progress, though at the level of laboratory ani-
Table 1 Comparison of ES Cells and Adult Stem Cells as Materials for Regenerative Cardiomyocytes

<table>
<thead>
<tr>
<th></th>
<th>ES Cells</th>
<th>Adult Stem Cells</th>
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<tbody>
<tr>
<td><strong>Origin</strong></td>
<td>Post-fertilization early-stage embryo (inner cell mass of blastocyst)</td>
<td>Marrow stromal cell</td>
</tr>
<tr>
<td><strong>Cell isolation technique, etc.</strong></td>
<td>Method of cell establishment is already established, and is relatively easily performed.</td>
<td>Sparse among bone marrow cells, and method of cell establishment is yet to be established.</td>
</tr>
<tr>
<td><strong>Proliferative potency</strong></td>
<td>At present, cells are considered to infinitely proliferate.</td>
<td>Proliferate to some extent but the number of divisions is unknown.</td>
</tr>
<tr>
<td><strong>Pluripotency</strong></td>
<td>Differentiate into any type of cell in vivo. The cells differentiate into early developmental stage cells <em>in vitro</em>, but it is thought to be difficult for them to differentiate into cells that appear late in the fetal stage.</td>
<td>Recognized to be able to differentiate into mesodermal cells such as osteoblasts, chondroblasts and adipocytes, but reportedly undergo differentiation into nerve cells (ectoblast-derived) and cells of the liver (entoblast-derived) as well.</td>
</tr>
<tr>
<td><strong>Differentiation into cardiomyocytes</strong></td>
<td>Differentiate relatively easily, but a method to have ES cells specifically differentiate into cardiomyocytes has not been established.</td>
<td>Demonstrated to differentiate into cardiomyocytes, but a method to have the cells specifically differentiate into cardiomyocytes has not been established.</td>
</tr>
<tr>
<td><strong>Rejection reactions</strong></td>
<td>Occur</td>
<td>No rejection reactions if the cells are autologous.</td>
</tr>
<tr>
<td><strong>Tumorigenic potential</strong></td>
<td>There is potential risk of teratomas after transplantation if undifferentiated cells remain.</td>
<td>No</td>
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Stem cells currently used for regenerative medical therapy for the myocardium are broadly divided into two groups: embryonic stem cells (ES cells) obtained from early stage embryos post *in vitro* fertilization and marrow adult stem cells obtained from the bone marrow of adults. Whether one type is superior to the other for cardiomyocyte regeneration remains to be seen. Characteristics of the two types of stem cells are summarized in Table 1. This article outlines the current status and future prospects of regenerative medicine for the myocardium.

### Differentiation from ES Cells to Cardiomyocytes

Figure 1 illustrates the outline of cardiomyocyte regeneration using ES cells. ES cells are those cells constituting the inner cell mass, destined to form the fetus, from an early embryo having reached the stage of blastocyst. These cells are known to differentiate into any type of cell *in vivo* and have been shown to differentiate *in vitro* into a variety of organic cells such as cardiomyocytes, skeletal muscle cells, vascular endothelial cells, smooth muscle cells, neurons, and hepatocytes. However, most types of cells that appear late in the fetal stage have not been demonstrated from ES cells *in vitro*.

It is generally recognized that various cell growth factors, cytokines, and cell adhesion factors are required for ES cell differentiation into those various cells. Recent studies have clarified a cascade operating for selective differentiation of ES cells into motor neurons, however, the cascade for ES cell differentiation into cardiomyocytes has not been fully elucidated to date.

A method to have ES cells form a cell mass (embryoid) has been introduced as a general means of inducing ES cell differentiation into cells capable of differentiation. The frequency with which differentiation from the embryoid
to cardiomyocytes is regarded as being about 7–8% at best. Humoral factors known to induce ES cell differentiation specifically into cardiomyocytes include bone morphogenetic protein-2 (BMP-2) and Wnt 11, which facilitate the differentiation, and Wnt 3 and Wnt 8, which act as inhibitors. Possible involvement of various other factors is presumed; the process seems to be quite intricate. Selective differentiation of ES cells into cardiomyocytes may become feasible from analysis of the pathways of the differentiation process.

**Adult Stem Cell Differentiation into Cardiomyocytes**

Bone marrow has been universally recognized to be the site of hematopoiesis with the predominance of hematopoietic stem cells. In fact, more than 99% of marrow cells take part in the production and development of blood cells. It was discovered recently that, among bone marrow cells, there are cells termed stromal cells that essentially do not represent blood cells but secrete cytokines and growth factors to support cells of the hematopoietic system. The presence of pluripotent stem cells capable of differentiating into various types of cells among the marrow stromal cells has become recognized. These marrow stromal cells with pluripotent capacity are referred to as mesenchymal stem cells on account of their ability to differentiate into such mesenchymal cells as osteoblasts, chondroblasts, and adipocytes.

In view of the mesenchymal stem cells being...
able to differentiate into mesoblast-derived organs, we wondered if they could differentiate to become cardiomyocytes as well, which are also of mesodermal origin. Our studies demonstrated that cardiomyocytes that beat regularly by themselves can be obtained from mesenchymal stem cells. Figure 2 shows an outline of the process. Mesenchymal stem cells have recently been reported to undergo differentiation into nerve cells (ectoblast-derived) and cells of the liver (entoblast-derived) as well, and are now termed adult stem cells.

**Characteristics of Regenerated Cardiomyocytes**

Cardiomyocytes derived from bone marrow show expression of fetal ventricular muscle type genes soon after their differentiation from adult stem cells, and thereafter gradually express adult type genes. Expression of genes for atrial natriuretic polypeptide and cerebral natriuretic polypeptide that are considered cardiomyocyte-specific have also been demonstrated. The cells proved to self-beat, and those differentiated from murine adult stem cells showed 120–250 beats/min. The cardiomyocytes exhibited the sinus node pattern of action potentials early after their differentiation from adult stem cells, and the pattern gradually changed to the ventricular muscle cell type.

Catecholamin $\alpha_1$ receptor (cardiac hypertrophic effect) and $\beta_1$ and $\beta_2$ receptors (positive chronotropic and positive inotropic effects) play important roles in the muscle cells of the heart. In regenerated cardiomyocytes of bone marrow origin, $\alpha_1$ receptor switched to myocardial type (mainly $\beta_{1A}$ and $\beta_{1B}$ receptors) as the differentiation proceeded into heart muscle cells, and simultaneously, $\beta_1$ and $\beta_2$ receptors that had not existed early in the course of differentiation became expressed. Stimulation of these catecholamine receptors led to activation of subreceptor signaling to produce cardiac hypertrophy, increases in heart rate and increases in myocardial contractile force.

The above findings indicate that the regenerated cardiomyocytes are endowed with practically normal characteristics of cardiomyocytes.

**Treatment of Cardiac Failure by Cell Transplantation**

Cardiomyocyte transplantation has been...
extensively investigated since the mid-1990s at the level of animal experiments. Cells harvested in primary cultures of heart muscle cells obtained from the fetus or the neonate were transplanted into the heart of sexually mature animals, and the transplantation was shown to improve post-infarction cardiac function. Clinical experience with the transplantation of fetal midbrain obtained through artificial termination of pregnancy into patients with Parkinson’s disease has yielded some gratifying therapeutic results. The amount of cell transplants required is apparently greater in the case of cardiomyocyte transplantation, so that it is not practical to use aborted fetuses as the source.

Regenerated cardiomyocytes derived from ES cells, when transplanted, reportedly proved to electrically bound to recipient myocardium and contract synchronously with surrounding cells, thus fueling hopes for the use of regenerated cardiomyocytes. Our experience with regenerated cardiomyocyte transplantation into the hearts of adult patients showed long-term engraftment with gratifying outcomes. However, there have been reports demonstrating that the number of cardiomyocytes taken as compared to that of cardiomyocytes transplanted diminished due to cellular necrosis during the course of engraftment. Further study is needed, including assessments of transplantation methods.

Problems Associated with Myocardial Regeneration and Future Prospects

To bring regenerative cardiomyocyte transplantation to realization requires securing regenerated cardiomyocytes and supplying those cells safely and at moderate expense. When adult stem cells and ES cells are compared, this author’s view is that the latter will come into use earlier in the future. Supply of regenerated cardiomyocytes derived from ES cells will become a reality within several years. Major problems are rejection reactions and method of transplantation. To avoid rejection, it is essential to transplant the nucleus of a somatic cell into an egg cell, as shown in Fig. 1. The nuclear transplantation eventually has a close bearing upon the matter of human cloning, therefore it is important to hold nationwide discussion of this ethical problem.

REFERENCES

