Advances in stem cell research

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In 1998, biologists Thomson and Gearhart successfully derived stem cells from human embryos. One year later, several researchers discovered that adult stem cells still retain the ability to be differentiated into unrelated types of cells. Advances in stem cell research open a promising direction for applied medical science. Moreover, it may also force scientists to reconsider the fundamental theory about how cells grow up. Stem cell research was considered by *Science* as the top of the ten breakthroughs of science of the year[1]. This paper gives a survey of recent advances in stem cell research.

1 Overview

In the 1980s, embryonic stem cell and/or embryonic germ cell line (ES cell line, EG cell line) of multifarious mammalian animals, especially those of non-human primates, had been established. In 1998, Thomson and Shamblott obtained ES, EG cell lines from human blastocysts and gonad ridges of early human embryos, respectively. Their research brought up an ethical debate about whether human embryos can be used as experimental materials. It was not appeased until 1999 when researchers discovered that stem cells from adults still retain the ability to become different kinds of tissue cells. For instance, brain cells can become blood cells[2], and cells from bone marrow can become cells in liver. Scientists believe, for a long time, that cells can only be developed from early pluripotent embryo cells; the differentiation potential of stem cells from mature tissues is restricted to only one of the cell types of the tissue where stem cells are obtained. Recent stem cell researches, however, subverted the traditional view of stem cells. These discoveries made scientists speed ahead with the work on adult stem cells, hoping to discover whether their promise will rival that of ES cells.

2 Common properties of stem cells

The definition of stem cells has been debated for more than 30 years. The prevailing definition is that stem cells are cells with unlimited or prolonged self-renewal capacity that can produce at least one type of highly differentiated descendants[3].

(i) Sources and classifications of stem cells. As shown in table 1, stem cells can be derived from two sources, i.e. embryos (e.g. ES and EG cells) or mature tissues (e.g. hematopoietic cell). ES and EG cells are also called totipotent cells since they can be transformed into all types of tissue cells. And adult cells in mature tissues are called multipotent cells due to their unlimited differentiation potential.

Table 1 Classifications of stem cells[a)]

Source	Type	Daughter tissue
Embryo or fetal tissues	embryonic(ES,EG)	all types
Fetal brain	neuronal	neurons, glia, blood cells
Adult bone marrow	hematopoietic	blood cells, glia? muscle fiber? hepatic oval cell?
	Mesenchymal	muscle, bone, tendon, cartilage

a) Modified from Vogel's table, *Science,* 1999.

(ii) Properties and biological characterization of ES cells (delegated by human ES cells). The biological properties of ES cells of primates are similar to those of mice. However, human ES cells express the same cell surface markers as undifferentiated nonhuman primate ES cells and human embryonal carcinoma cells including stage-specific embryonic antigen SSEA-3, SSEA-4, TRA-1-60, TRA-1-81 and alkaline phosphatase; whereas inner cell mass (ICM) cells, ES cells, and EC cells of mice express SSEA-1 but not SSEA-3 or SSEA-4. This suggests basic species differences between early mouse and human development.

3 Evolvement and development of stem cells

For adult organisms, in terms of the differentiation potential, cells can be classified into three types: stem cells are multipotential and self-renewing that sit at the top of the lineage hierarchy and can replenish tissue for the lifetime of the organism. A population of committed progenitors with restricted differentiation potential stay at the 2nd grade. The lowest grade is differentiated cells in adult tissue, which cannot self-renew and lose the differentiation potential. A stem cell was generally considered to undergo an asymmetric division, through an intermediate population, progenitor, to produce highly differentiated descendant (fig. 1). However, it is more reasonable to

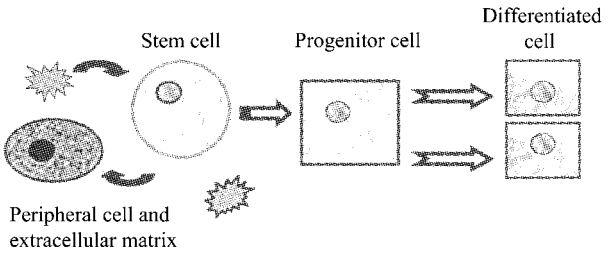

Fig. 1. Differentiation pathway of a stem cell.

think of them as appropriately differentiated for their specific tissue niches[4] than undifferentiated cells.

The formation of stem cell results from the long-time evolvement of biological circle. Although most evidence led to the point that multicellularism evolved separately in plants and animals, the homology between the *PIWI* gene in *Drosophila*, which is in charge of germ line stem cells, and the *ZWILLE* gene in *Arabidopsis*, which controls the stem cells of the shoot meristem, has led to the suggestion that stem cells may evolve from a single-celled ancestor, or a multicellular ancestor shared by both plants and animals. Moreover, the similar biochemical machinery existing in plants and animals may contribute to producing stem cells through convergent evolution[3].

Stem cells are produced and increased with the organism developing. After researching into the origin of hematopoietic stem cells (HSCs), scientists found that although aorta-gonad-mesonephros and liver hematopoietic precursors appear similar, there are discrepancies between their descendants, which was thought to be a reflection of the variation in the microenvironment from postnatal to adult life. And, there appears to be a clear increase in numbers of HSCs capable of repopulating in a lethally irradiated host, when advancing from aorta-gonad-mesonephros to fetal liver to adult bone marrow. Similarly, a huge increase was seen in the numbers of neural stem cells during the late embryogenesis in mice[5]. Hence, all the accumulating evidence points to an increasing appearance of somatic tissues in the perinatal to adult phase of the mammalian life cycle, as part of the organism's ability for repopulation and renewal. A conclusion is drawn that stem cells may not be the first cells that are present embryonically in a specific tissue to create that tissue, but rather appear later in development where they can replenish adult tissue.

4 Present research status of stem cells

Stem cells, mainly ES cells, have been widely used in studying embryogenesis and gene function. ES cell could reconstitute a chimera embryo when injecting them into mice blastocyst. Using *in vitro* cultivating system, differentiated ES cells produce embryoid bodies (EBs) which provide model systems for studying system genesis (such as cardiogenesis and hematopoiesis). Transplanting the neural cells and hematopoietic cells coaxed from ES cells into mice embryo or adult mice, they appeared integrity both in morphology and function with new host[6]. Although ES cells have been widely applied, the ICM, which contributes to ES cells, still needs to be further studied: initial formation of the ICM depends on asymmetric division of polarized cells of the compacted morula, but the genes that control these divisions are unknown. It is clear, however, that the *POU* domain transcription factor OCT4 is essential for the genesis of ICM[7]. But it remains unclear whether all cells in the ICM produce ES cells.

The breakthrough in the research of stem cells builds up a milestone in the history of cell biology. While Bjornson's article "Turning Brain into Blood…" turned over a new page in the history of stem cell research. It was reported that neural stem cells transformed themselves into different types of blood cells in lethally irradiated host[2], which is widely divergent from the former point that stem cells in mature tissue produce only one type tissue cell among several. After that, researchers found *in vitro* coaxing bone marrow cells gave rise to new muscle cells and hepatic cells, and the implant used in one experiment was purified hematopoietic cell[8]. Despite more than a dozen papers on the potential differentiation of adult stem cell, there is not yet sufficient evidence suggesting that all the stem cells get such capacity. Anyway, this is a virgin region, so promising to be explored.

5 Applications of stem cells

Bone marrow (BM) transplantation is used in patients with cancer treated by chemotherapy and radiotherapy. But the curative effect of autologous hematopoietic transplants is dissatisfied, one reason is that tumor cells have metastasized to BM before implanting. Although BM used in allogeneic transplantation is not contaminated by cancer cells, the T cells in allogeneic BM could encounter and respond to host antigens in all tissues in the body, leading to a multisystem graft-versus-host syndrome. But it was found that HSCs contained in BM were radioprotective, and HSCs were the only radioprotective element in mouse bone marrow; also as HSCs dose increased, the time to engraftment of donor-derived blood cells shortened[9]. Subsequently, the anti-programmed cell death gene *bcl-2* was found to be able to increase the frequency of HSCs in BM[10]; G-CSF alone or along with cyto-reductive drugs induced the release of G_1 HSCs to blood, which was convenient to collect for implantation. All these achievements accelerate the pace of application of HSCs in clinic.

Until now, there is no efficient way to cure denaturalization disease in the neutral system, such as multiple sclerosis, Parkinson's syndrome, etc. It seemed that Oliver Brustle's researches brought hopes to such kind of diseases. Mice ES cells were induced to form glial cells (a type of support cell in the brain encephalon which produces myelin), injecting these glial cells into the spinal cords of rats with a genetic defect in making myelin, the glia soon got to work coating the rat's neurons with myelin[6]. This experiment is the first example of an application of the stem cell transplantation to a neurological disorder. From what we know already, isolated fetus- or

adult- derived neural stem cells from mouse, rat, and human brain tissue may survive well *in vitro*. However, whether the stem cells take on the exact function of the cells they replace remains to be determined, which is the key problem to be solved for the clinical application of stem cells. Therefore, to use neural stem cells for transplantation therapy, there is a long way to go.

The most prominent problem for liver organ transplantataion is that the recent donor livers are most likely not HLA-matched to the recipient and powerful immunosuppression is required. It is reasonable to assume that if liver-repopulating stem cells are available, sibling transplants may become feasible. Diabetes melitus is another common disease, islet transplantation is supposed to be the terminal treatment. As we know, islet contains different cells difficult to expand *in vitro*. Thus, it becomes researcher's goal to search for conditions wherein islets are continuously generated from stem/progenitor cells. Moreover, the isolation of skeletal muscle satellite stem cells[8] gives hope to the therapy of intrinsic muscular dystrophies. In terms of this way, cardiac infarction following coronary artery blockage would be cured. Unfortunately, the satellite cell equivalent in the heart tissue has not yet been found.

Stem cells transplantation paves a new way for conquest of ailments, even the repulsive reaction after implantation could be alleviated after the adoption of relevant strategies, such as establishing ES cell library for tissue matching, which includes allele of multiple tissue compatible antigen complex; finding a wide donor system, with their ES cells genetically modified; recombining donor MHC gene with ES cells by transgene to obtain the ES cells containing donor genome.

6 Present research status of stem cells in China

In the mid 1980s, Chinese researchers established mice ES cell line. Using this line, differentiation of ES cell and genes which play an important role in embryogenesis had been studied[11]. Especially, the knockout technology was used to study the specific function gene in ES cell. In 1998, our domestic researchers isolated human ES cell[12], then directed it to differentiate into plasma cell[13] and HSC[14]. In 1999, mice EG cell line was established in Shanghai[15]. It was reported that human organ would be cloned in the next three to five years in Shanghai Transgene Research Center, of course with human ES cells. HSCs transplantation has been used in clinic[16], and the pertinent studies are going on.

Stem cells, as the youngest cells, have attracted the attention of the world. Stem cell researches challenged the traditional theory of cell developments. Stem cells from embryos or mature tissues greatly help the understanding of intricate embryo developments and the generation of congenital maladies. It can also be applied as the transplant donor, and to testing the safety of medicine.

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