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Review

Stem cells: Biology and clinical potential

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Stem cell technology has developed rapidly in recent years to the point that we can now envisage its future use in a variety of therapeutic areas. This review seeks to summarize the types and sources of stem cells that may be utilized in this way, their pattern of development, their plasticity in terms of differentiation and transdifferentiation, their ability to self-renew, the privileged microenvironment in which they are housed, their cell surface markers used to track them, issues relating to their transfection, and their fate. Particular reference is made, as prime examples, to how both the function of mesenchymal and neural stem cells are being studied experimentally, and currently used clinically in certain circumstances, towards the ultimate aim of their mainstream therapeutic use.

Key words: Stem cells, apoptosis, differentiation, mesenchymal and neural stem cells, therapy.

INTRODUCTION

Stem cells are characterized by their ability to undergo symmetric cell division resulting in one undifferentiated daughter cell and one committed daughter cell. The undifferentiated daughter cell can maintain a population of stem cells by continual self renewal. Stem cells are characterized according to their plasticity, or number of different cell types they can have (Mezey et al., 2003).

Regenerative medicine applies the basic stem cell knowledge to develop specific cell or tissue to replace the original cells or tissue, which has been degenerated, injured or damaged by different processes. This is the basic concept of the promising cell and tissue based therapy that would have a potential to make many chronic diseases to be curable, such as insulin dependent diabetes mellitus (IDDM), myocardial infarction and other conditions (Seale et al., 2006).

Stem cells have been viewed as a potential source of replacement cells for any tissue due to their functional capacity to give rise to virtually any type of cell. Among stem cells, stromal stem cells can be obtained from the bone marrow and induced to undergo differentiation to a variety of types of adult tissue. These mesenchymal stem cells (MSCs) can proliferate extensively *in vitro*, and differentiate under appropriate conditions into bone, cartilage and other mesenchymal tissues (Brazelton et al., 2000). After injury to nervous tissue, MSCs migrate to the damaged brain, where they could provide an ideal cell source for repair (Li et al., 2002).

DEFINITION

Stem cells are immature, unspecialized cells that their developmental direction has not yet been determined. These cells have the remarkable potential to develop into many different cell types in the body. Serving as an internal repair system, stem cells can theoretically divide without limit to replenish native or damaged cells for as long as the person or animal is still alive. When a stem cell divides, each resulting cell has the potential to either remain a stem cell or become another type of cell with a more specialized function, such as a muscle cell, a red blood cell or a brain cell (Melton et al., 2004).

PROPERTIES OF STEM CELLS

First, stem cells undergo multiple, sequential self

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renewing cell division, a prerequisite for sustaining the population. Second, single stem cell-derived daughter cells differentiate into more than one cell type. Examples include hematopoietic stem cells (HSCs) that give rise to all hematopoietic cells, neural stem cells (NSCs) that give rise to neurons, astrocytes and oligodenrocytes, and mesenchymal stem cells that differentiate into fibroblasts, osteoblast sand chondroblasts. Some adult stem cells (ASCs) may give rise to only a single mature cell type, such as the corneal stem cell (Holm, 2002). Third, stem cells functionally repopulate the tissue of origin when transplanted into a damaged recipient. This has already been shown extensively for HSCs and, more recently, for liver progenitors and NSCs. Fourth, and less well established, is that stem cells contribute differentiated progeny *in vivo* even in the absence of tissue damage (Catherine et al., 2002; Yang et al., 2002; Caplan, 2005).

THE DIFFERENTIATION POTENTIAL OF STEM CELLS

Many of the terms used to define stem cells depend on the behavior of the cells in the intact organism (*in vivo*), whether under specific laboratory conditions (*in vitro*), or after transplantation *in vivo*, often to a tissue that is different from the one from which the stem cells were derived.

Totipotent stem cell

The fertilized egg is said to be 'totipotent'- from the Latin *totus,* meaning entire, because it has the potential to generate all the cells and tissues that make up an embryo to support its development *in utero*. The fertilized egg divides and differentiates until it produces a mature organism. Adult mammals, including humans, consist of more than 200 types of cell. These include nerve cells, muscle cells, skin cells, blood cells, bone cells and cartilage cells. Other cells, which are essential for embryonic development (but are not incorporated into the body of the embryo), include the extraembryonic tissues, placenta and umbilical cord. All these cells are generated from a single, totipotent cell- the zygote or fertilized egg.

Pluripotent stem cell

The term pluripotent was used to describe stem cells that can give rise to cells derived from all three embryonic germ layers (mesoderm, endoderm and ectoderm). These three germ layers are the embryonic source of all cells of the body. All the different kinds of specialized cells that make up the body are derived from one of these germ layers "pluri"- derived from the Latin *plures*-meaning several or many. Thus, pluripotent cells have the potential to give rise to any type of cell, a property observed in the natural course of embryonic development and under certain laboratory conditions.

Unipotent stem cell

This is a term usually applied to a cell in adult organisms, which means these cells are capable of differentiating along only one lineage. "Uni" is derived from the Latin word *unus*, which means one. Adult stem cells in many differentiated, undamaged tissues are typically unipotent and give rise to just one cell type under normal conditions. This process would allow for a steady state of self-renewal for the tissue. However, if the tissue becomes damaged and the replacement of multiple cell types is required, pluripotent stem cells may become activated to repair the damage. The embryonic stem cell is pluripotent- it can give rise to cells derived from all three germ layers. Multipotent cell or precursor cell, such as the hematopoietic cell, is one with a more limited range of differentiation and can give rise to cell types whose lineage can be traced back to only one primary germ layer (Figures 1 and 2) (Chandross and Mezey, 2001).

STEM CELL PLASTICITY

A widely accepted definition of plasticity is yet to be established but, in general, this term refers to the newly discovered ability of adult stem cells to cross lineage barriers and to adopt the expression profiles and functional phenotypes of cells unique to other tissues.

Mechanisms of stem cell plasticity

There are a number of possible mechanisms that could explain these phenomena. One possibility is trans-differentiation of a committed cell directly into another cell type as a response to environmental cues. Trans-differentiation has been shown mainly *in vitro*, though some *in vivo* data also support this mechanism. Direct transdifferentiation would clinically be limited by the number of cells that can be introduced into an organ without removal of resident cells. If bone marrow cells could on the other hand give rise to stem cells of another tissue, then they could in theory repopulate whole organs from just a few starting cells. This model of dedifferentiation is consistent with recent data from animal models. Genetic analysis of cells of donor origin *in vivo* and *in vitro* has brought to light another possible mechanism. The fusion of host and donor cells can give rise to mature tissue cells without trans- or de-differentiation. The resulting heterokaryons are able to

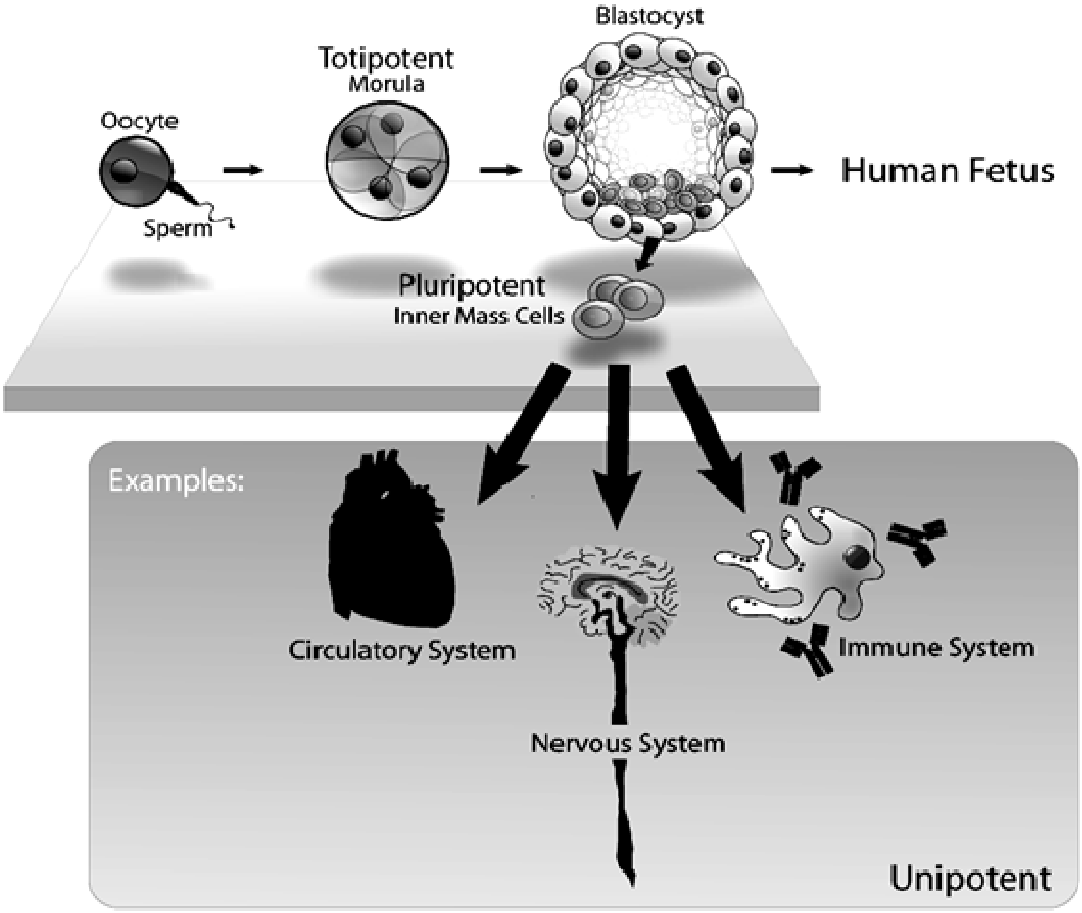

Figure 1. The differential potential of stem cells (adopted from Jones, 2006).

cure a lethal genetic defect and do not seem to be prone to giving rise to cancer (Kashofer and Bonnet, 2005).

Several mechanisms (Figure 1) may underlie this apparent plasticity. Multiple tissue-specific stem cells are present in different organs, and plasticity is the result of fusion of the donor cell with resident cells in an organ. Cells undergo de- and re-differentiation, or true multi- or pluri-potent stem cells can persist in postnatal life.

STEM CELL FATE

Stem cells may remain quiescent and simply not enter the cell cycle. This may be important in sequestering a reserve pool of cells for use in times of stress, or at later stages of development. A stem cell may undergo apoptosis and not contribute to further development; this scenario may be the norm in tissues such as the brain where turnover of differentiated cells (neurons and glial cells) is very low. Stem cells may undergo symmetric cell divisions to self renew or undergo terminal differentiation, or they may undergo asymmetric cell divisions to generate differentiated progeny as well as maintain a pool of stem cells. A dynamic balance between proliferation, survival and differentiation signals ensures that an appropriate balance between stem cells, precursor cells and differentiated cells is maintained throughout development and adult life (Rao and Mattson, 2001).

Stem cell quiescence

An important property of stem cells that is often overlooked, yet relates to the phenomenon of self-renewal is that, *in vivo*, stem cells reside in a state of quiescence. This is thought to be true for stem cells of the bone marrow (Morrison and Weissman, 2000) and the skin (Miller et al., 2002). The mechanisms by which these cells are induced to enter the cell cycle are not well understood. However, once these quiescent cells enter the cell cycle, many undergo a high rate of expansion and do not exit the cell cycle after one division but undergo continuous division. Many of these cells pass through G_1 and G_2 phase so quickly that these phases

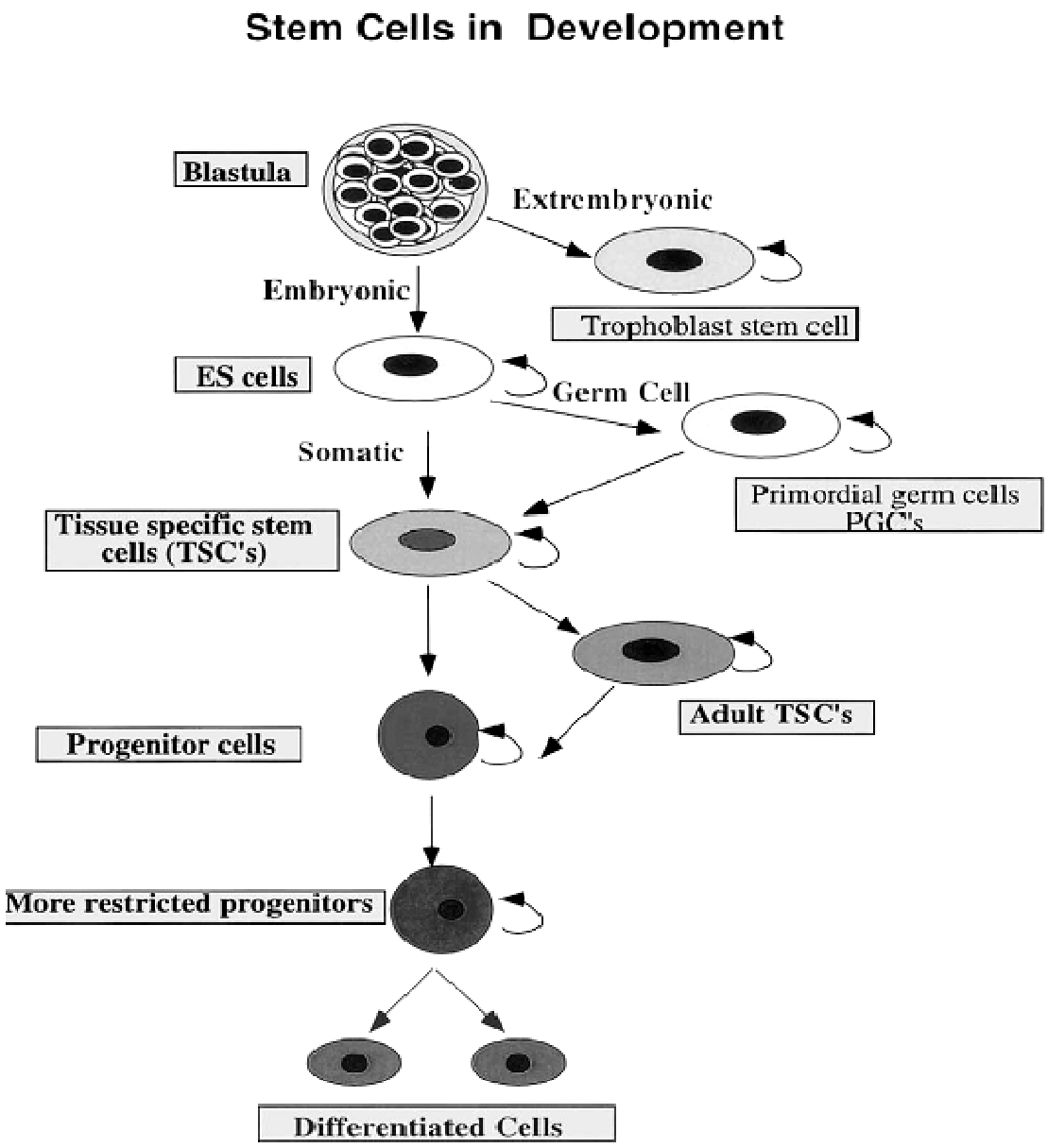

Figure 2. Outline of the progressive restriction of stem cell potentials during development (adopted from Rao and Mattson, 2001).

are virtually non-existent. Embryonic cells, skin stem cells, hematopoietic stem cells and cancer cells continuously divide without exiting the cell cycle (Sherr, 2000).

Stem cell niche (microenvironment)

Stem-cell populations are established in 'niches'- specific anatomic locations that regulate how they participate in tissue generation, maintenance and repair. The niche saves stem cells from depletion, while protecting the host from over-exuberant stem-cell proliferation. It constitutes a basic unit of tissue physiology, integrating signals that mediate the balanced response of stem cells to the needs of organisms. Yet the niche may also induce pathologies by imposing aberrant function on stem cells or other targets. The interplay between stem cells and their niche creates the dynamic system necessary for sustaining tissues, and for the ultimate design of stem-cell therapeutics (Scadden, 2006).

For simplicity, a niche may be considered as a subset of tissue comprising stem cells and extracellular substrates that can indefinitely house one or more stem cells, and also control their self-renewal and progeny

production *in vivo*. Niches also modify their regulatory properties in response to changing conditions to ensure that stem cell activity parallels the organism's needs for particular differentiated cell types (Wright, 2000).

STEM CELL MARKERS

What are stem cell markers? Coating the surface of every cell in the body are specialized proteins, called receptors, which have the capability of selectively binding or adhering to other "signaling" molecules. There are many different types of receptors that differ in their structure and affinity for the signaling molecules. Normally, cells use these receptors and the molecules that bind to them as a way of communicating with other cells and to carry out their appropriate functions in the body. These same cell surface receptors are the stem cell markers. Each cell type, for example a liver cell, has a certain combination of receptors on their surface that makes them distinguishable from other kinds of cells. Scientists have taken advantage of the biological uniqueness of stem cell receptors and chemical properties of certain compounds to tag or "mark" cells. Researchers owe much of the past success in finding and characterizing stem cells to the use of markers. Researchers use the signaling molecules that selectively adhere to the receptors on the surface of the cell as a tool that allows them to identify stem cells. Many years ago, a technique was developed and attached to the signaling molecule, another molecule (or the tag) which has the ability to fluoresce (emit light energy) when activated by an energy source such as an ultraviolet light or laser beam. At the researchers' disposal are multiple fluorescent tags with emitted light that differ in color and intensity. One approach for using markers as a research tool is with a technique known as fluorescence-activated cell sorting (FACS) (Figure 3). With this technique, a suspension of tagged cells (fluorescent tags are bound to the cell surface markers) is sent under pressure through a very narrow nozzle, so narrow that cells must pass through one at a time. Upon exiting the nozzle, cells then pass, one-by-one, through a light source, usually a laser, and then through an electric field. The fluorescent cells become negatively charged, while nonfluorescent cells become positively charged. The charge difference allows stem cells to be separated from other cells.

A second method uses stem cell markers and their fluorescent tags visually to assess cells in tissues. Often, researchers want to assess how stem cells appear in tissues and, in doing so, they use a microscope to evaluate them rather than using FACS. In this case, a thin slice of tissue is prepared, and the stem cell markers are tagged by the signaling molecule to which is attached the fluorescent tag attached. The fluorescent tags are then activated either by special light energy, or by a chemical reaction. The stem cells will emit a fluorescent light that can easily be seen under the microscope.

Genetic and molecular biology techniques are extensively used to study how cells become specialized in organ development. In doing so, researchers have identified genes and transcription factors (proteins found within cells that regulate a gene's activity) that are unique in stem cells. Scientists use techniques such as polymerase chain reaction (PCR) to detect the presence of genes that are "active" and play a role guiding the specialization of a cell. This technique allowed researchers to identify "genetic markers" that are characteristic of stem cells. For example, a gene marker called PDX-1 is specific for a transcription factor protein that initiates activation of the insulin gene. Researchers use this marker to identify cells that are able to develop islet cells in the pancreas. Recently, researchers have applied a genetic engineering approach that uses fluorescence, but is not dependent on cell surface markers. The importance of this new technique is that it allows the tracking of stem cells as they differentiate or become specialized. Scientists have inserted into a stem cell a "reporter gene" called green fluorescent protein (GFP) (Eiges et al., 2001).

SOURCES OF STEM CELLS

Embryos

Embryonic stem cells (ESCs) are derived from the cells of the inner cell mass of the blastocyst during embryonic development. Embryonic stem cells have the capacity to differentiate into any cell type and the ability to self-replicate for numerous generations. A potential disadvantage of ESCs is their ability to proliferate endlessly unless they are controlled (Thomson et al., 1998).

Fetal tissue

Embryonic germ cells (EGCs) have been derived (for research) from cells found in the gonadal region of an aborted fetus (Shamblott et al., 1998).

Amniotic fluid-derived stem cells (AFSCs)

AFSCs can be isolated from aspirates of amniocentesis during genetic screening. An increasing number of studies have demonstrated that AFSCs have the capacity for remarkable proliferation and differentiation into multiple lineages such as chondrocytes, adipocytes, osteoblasts, myocytes, endothelial cells, neuron-like cells and liver cells (Barria et al., 2004).

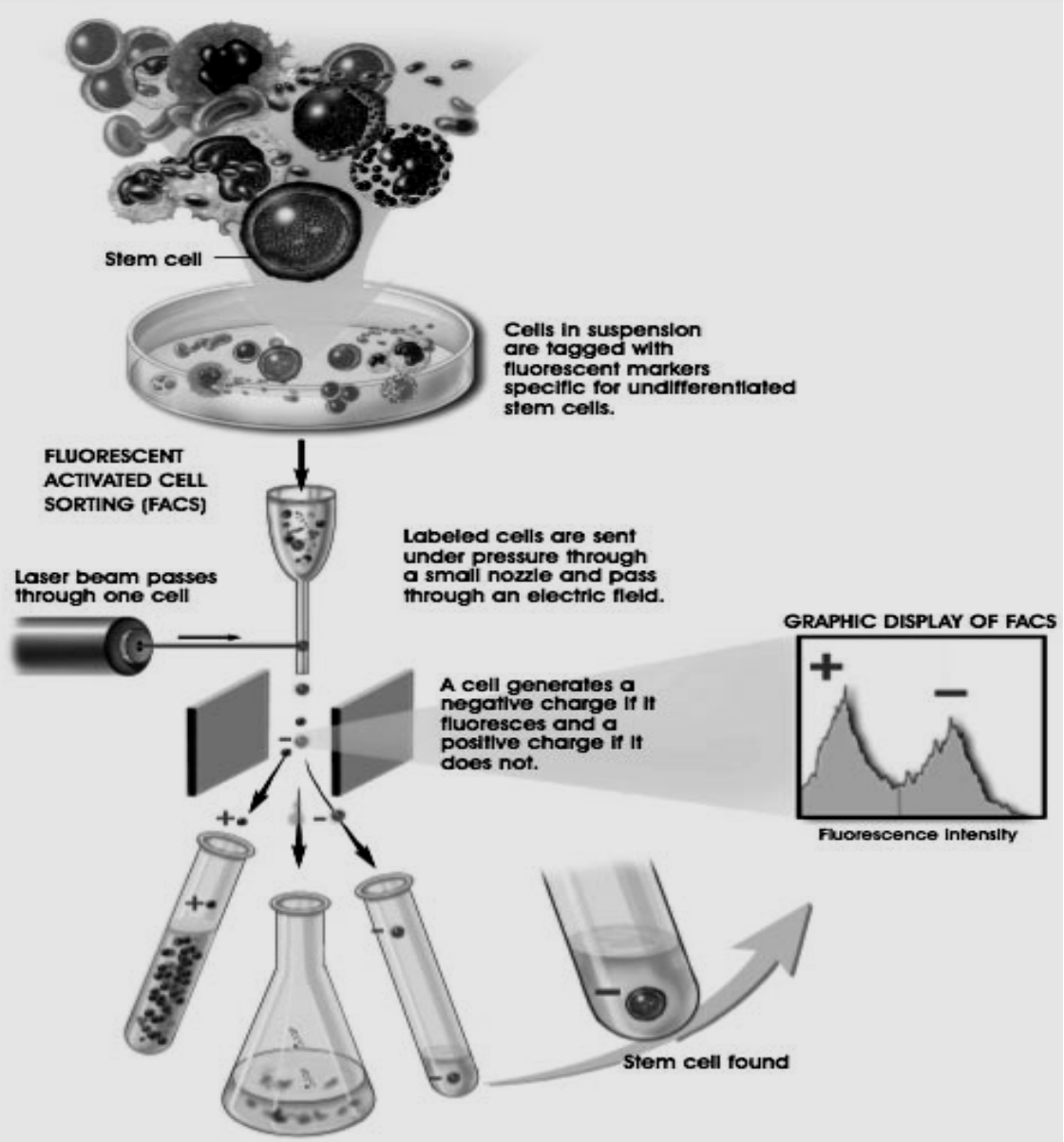

Figure 3. Fluorescence activated cell sorting (FACS) (adopted from Herzenberg and De Rosa, 2000).

Placental tissue

The placenta is a unique source of different populations of stem cells including mesenchymal, hematopoietic, trophoblastic and, possibly, more primitive stem cells (Fauza, 2004).

Umbilical cord blood stem cells (UCBSCs)

UCBSCs are derived from the blood of the umbilical cord (Laughlin, 2001). There is a growing interest in their capacity for self-replication and multilineage differentiation. Umbilical cord blood stem cells can be differentiated into several cell types like cells of the liver, skeletal muscle, neural tissue, pancreatic cells, immune cells and mesenchymal stem cells (Gang et al., 2004).

Adult stem cells (ASCs)

Sources of adult stem cells include the umbilical cord, amniotic fluid, bone marrow, adipose tissue, brain and teeth (Pittenger et al., 1999).

TYPES OF STEM CELLS

There are three major types of stem cells; embryonic, fetal and adult, each comes from different sources, and each has different properties (Brazelton et al., 2000).

Embryonic stem cells (ESCs)

When a sperm fertilizes an egg it becomes what is known as zygote, many scientists view the zygote as the ultimate stem cell because it can develop into any cell, not only cell of embryo, but also cell of surrounding tissues (such as placenta). The zygote has the highest degree of transdifferentiation; it is referred to as a totipotent stem cell meaning that totipotent cells are the first stage stem cells as the zygote develops into both embryonic and extra embryonic tissues (Papaioannou, 2001). Thirty hours after fertilization, the zygote begins to divide, and by the fifth or sixth day, the cells form a blastocyst. These cells have somewhat less potential for differentiation and more specialized than the totipotent zygote stem cell. Those on the outer surface of the blastocyst develop into placenta and other tissues that surround the fetus, while those inside, referred to as embryonic stem cells become the cells of the fetal organs and tissues. These stem cells can become any of the more than 200 types of cells in the body, and are accordingly called pluripotent stem cells (Tzukerman et al., 2003).

Sources of human ESCs

There are three sources of ESCs, which are derived from early embryos in culture: 1) embryos created by *in vitro* fertilization (IVF) for infertility treatments that were not implanted because they were no longer needed, 2) embryos created by IVF expressly for research purposes, and 3) embryos resulting from somatic cell nuclear transfer (SCNT) or other cloning techniques (Hwang et al., 2005).

Embryonic germ cells (EGCs)

Five to nine weeks after fertilization, the growing embryo, now called a fetus, develops a region known as the gonadal ridge. The gonadal ridge contains the primordial germ cells, which will eventually develop into eggs or sperm. Embryonic germ cells are isolated from these primordial germ cells of the five to nine weeks fetal tissue that resulted from elective abortions. Like ESCs, EGCs are also pluripotent (Shamblott et al., 1998). As the embryo grows, it accumulates additional embryonic stem cells in the yolk sac; as the fetus grows from weeks eight to 12, it accumulates fetal stem cells in the liver. Both embryonic and fetal stem cells generate the developing tissues and organs. At this stage, the stem cells are more tissue specific rather than generating all the body's 200 different cell types, for example, fetal stem cells in liver tend to generate liver and blood cell families. Such cells are generally designated as multipotent. Nevertheless, fetal cells may have an advantage over embryonic stem cells in that they may not form teratomas. Fetal liver tissue has been shown to be a rich source of stem cells (Rollini et al., 2004). Until week 12, fetal stem cells (as well as the ES cells which preceded them) have a very important property that they can be transplanted into an individual without being rejected, this is because they have little or no certain type of protein on their surface (Class II HLA) (O'Donoghue and Fisk, 2004). After the 12th week, fetal stem cells start to express these immune-triggering proteins; and therefore using these cells beyond this time carries the potential of possible rejection reactions. Accordingly, stem cells derived from these sources may have therapeutic potential only when given to the individual from whom they were derived (autologous transplantation) or from an immunological matched donor (allogenic transplantation) (Rollini et al., 2004).

In the U.S.A., scientists have proposed methods for preparing and cryo-preserving stem cells from fetal tissues (derived from elective abortions) for later clinical use, and recommended procedures that separate the abortion decision from the donation decision, and thus preserve confidentiality between donor and recipient. Umbilical cord blood haemopoietic stem cells have been extensively investigated and widely utilized over the last 10 to 20 years, and fetal neural tissue has already been used therapeutically in Parkinson's disease with some evidence of clinical improvement (Lindvall, 2003). Recent interest in stem cell biology and its therapeutic potential has led to the search for fetal stem cells in fetal organs obtained at termination of pregnancy, as well as for accessible sources of fetal stem cells that might be collected for autologous use in ongoing pregnancies (O'Donoghue and Fisk, 2004; Allan et al., 2010; Alenzi et al., 2009, 2010).

Adult stem cells (ASCs)

The term adult stem cell refers to the cells found in adult organs that constantly replenish the somatic cells in the tissue of their origin. A rigorous assessment of adult stem cells is needed to prospectively purify a population of cells using cell surface markers, transplant a single cell from the purified population into a syngeneic host without any intervening *in vitro* culture, and observe self-renewal and tissue, or organ regeneration for multipotency. These cells possess strong regenerative capability to replenish

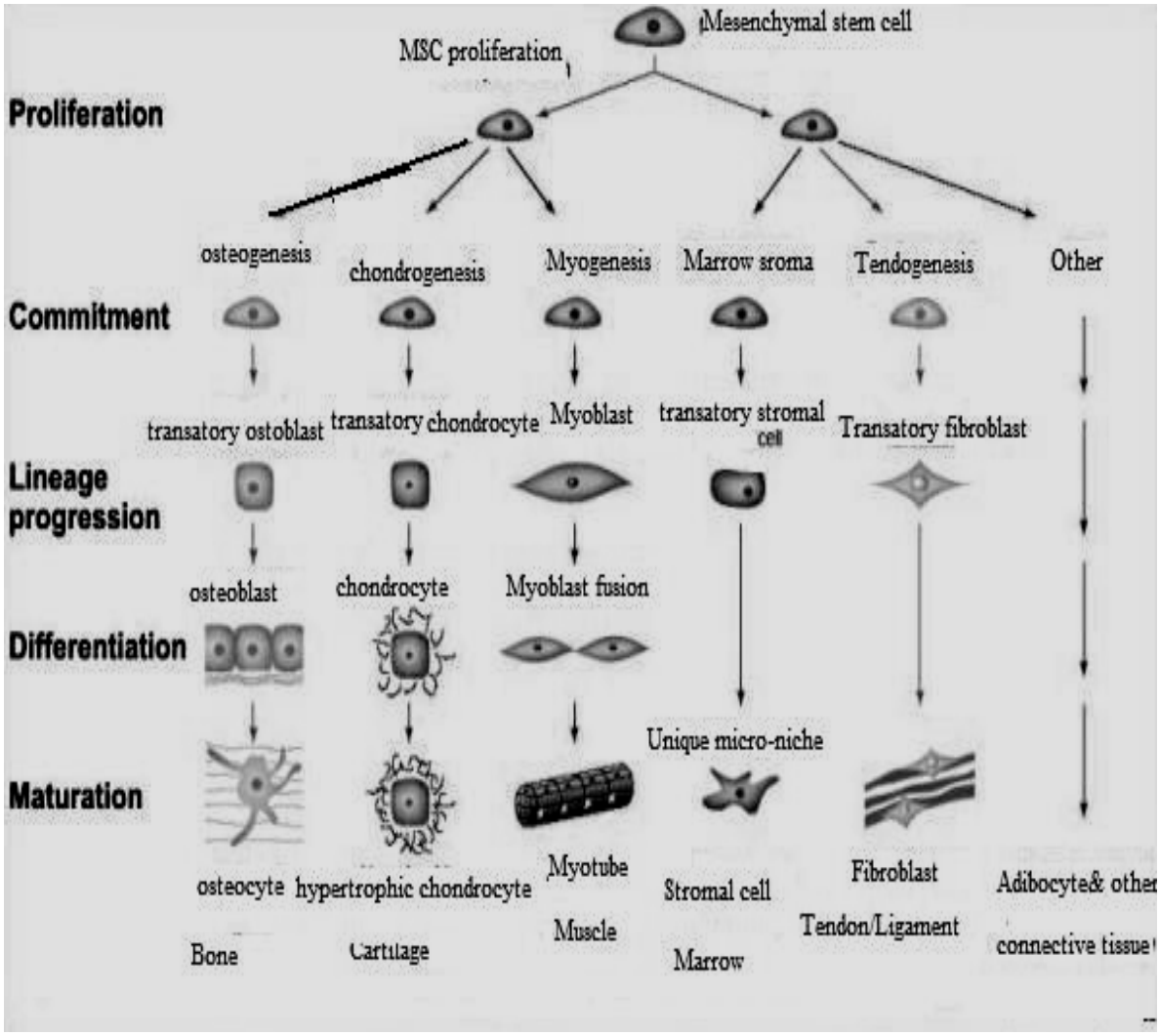

Figure 4. The differentiation processes of mesenchymal stem cells. Mesenchymal stem cells have the capacity to differentiate into bone, cartilage, muscle, marrow stoma, tendon, fat and other connective tissue (adopted from Caplan, 2005).

the senile or sick cells of the tissue in which they reside under pathological condition or injury, and the study of these stem cells appeared to be unrelated to each other (Brazelton et al., 2000).

The primary roles of ASCs in living organisms are to maintain and repair the tissue in which they are found. These cells have more limited differentiation potential so, they are called multipotent stem cells. Scientists have found ASCs in many tissues more than they thought was possible. This finding has led scientists to ask whether ASCs could be used for transplants. Certain type of ASCs seems to have the ability to differentiate into a number of different cell types, given the right conditions. If this differentiation of ASCs can be controlled in the laboratory, these cells may become the basis of therapies for many serious common diseases. These ASCs are very small in number in each tissue and reside in a special area of each tissue where they remain quiescent (non dividing) for many years until they are activated by disease or tissue injury (Caplan, 2005).

MESENCHYMAL STEM CELLS

One of the most extensively studied populations of multipotent ASCs has been MSCs from the bone marrow (Figure 4) (Pittenger et al., 1999). There are three stem cell populations that reside within the bone marrow: endothelial stem cells, HSCs and mesenchymal stem cells. HSCs give rise to all the differentiated blood lineages, whereas MSCs give rise to the stromal cells of the marrow. Mesenchymal stem cells represent 2 to 3%

of the total mononuclear cells of the marrow. They are thought to be multipotent cells that have the capacity to differentiate into a variety of connective tissue types including osteogenic (Jaiswal et al. 1997), chondrogenic or adipogenic lineages when placed in the appropriate culture conditions (Barry et al., 2001). Also, MSCs could differentiate into endodermal and epidermal cells, such as vascular endothelial cells, neurocytes, lung cells and hepatocytes (Petersen et al., 1999; Schwartz et al., 2002 and Davani et al., 2003).

In culture, MSCs can be isolated from HSCs and other cells of the bone marrow by their tendency to adhere to tissue culture plastic and their prolonged proliferative ability (Pittenger et al., 1999). Mesenchymal stem cells can be isolated using standard techniques and expanded in culture through many generations while retaining their ability to differentiate when exposed to the appropriate conditions (Barry et al., 2001). These properties represent a potential powerful tool in tissue engineering for the development of new therapeutic strategies for the repair of various tissues damaged as a result of trauma or disease (Pittenger et al., 1999).

Mesenchymal stem cell identification

The successful isolation of a homogeneous population of true MSCs capable of multilineage differentiation has remained elusive. Many groups have described similar populations of MSC using a multitude of cell surface markers, but no true marker or combination of markers has been described to isolate a homogenous population. Established methods such as micro array analysis, flow cytometric analysis and genotype finger printing had been used to begin characterization of this population at a molecular level (Hunziker, 2001).

It is probably only through this type of detailed molecular analysis that it will be possible to compare various populations of MSC that have been isolated by different methods and thus identify core “stem” genes that are common to all these MSC isolates. It is also important to examine the cells that make up the niche or microenvironment that supports the survival and differentiation of these stem cells. The characterization of the microenvironment could provide valuable information about signaling as well as cell to cell interactions that are important for *in vivo* differentiation, as well as potentially allowing more efficient *ex vivo* expansion of these mesenchymal cells (Caplan, 2005).

Mesenchymal stem cell isolation and expansion

The protocol, by centrifugation in a density gradient to separate bone marrow-derived mononucleated cells from plasma and red blood cells, is still widely used. The mononucleated cells can then be plated on tissue culture polystyrene with frequent changes of culture medium. Non-adherent cells such as hematopoietic cells are discarded upon medium change. Some of the adherent cells are MSCs (Aubin, 1998; Caplan, 2005). Bone marrow extracts contain heterogeneous cell populations. Mesenchymal stem cells represent a small fraction of total mononucleated cells within bone marrow (Barry, 2003). Further enrichment techniques have been explored, such as positive selection using cell surface markers including STRO-1, CD133 (prominin, AC133), p75LNGFR (p75, low-affinity nerve growth factor receptor), CD29, CD44, CD90, CD105, c-kit, SH2 (CD105), SH3, SH4 (CD73), CD71, CD106, CD120a, CD124 and HLA-DR (Pittenger et al., 1999; Alhadlaq and Mao, 2004). Flow cytometry is another helpful enrichment tool based on an array of cell surface markers. Negative selection is also helpful by utilizing antibody cocktails that label bone marrow derived cells that are not MSCs (Lee et al., 2004).

Potentiality of MSCs for tissue repair

Rat bone marrow stromal cells (BMSCs) and human bone marrow stromal cells intravenously injected after stroke, migrate selectively to target damaged brain, enhance neurogenesis, promote neural stem cell proliferation and improve functional recovery (Jin et al., 2001). Intravenous administration of BMSCs also increases the expression of brain-derived neurotrophic factor (BDNF) after traumatic brain injury, which possibly contributes to the improvement of functional outcome (Mahmood et al., 2001).

Neural stem cells

Although neurons do not divide, there is evidence that there are NSCs within the adult mammalian and human brain that, when provided with the proper stimulus, can be activated to replace lost or injured neurons. Some of these cells have the capacity to produce glial cells, and others to differentiate into neurons. These NSCs exhibit multi-potential ability to differentiate into the cells of the tissue into which they were introduced (Gartner and Hiatt, 2007).

The pool of NSCs may constitute a reserve of cells that, under correct stimulation, could replace lost neurons. Some regions of the brain and spinal cord of adult mammals retain stem cells that can generate astrocytes, neurons, and oligodenrocytes. It was shown that NSCs can even generate cells not related to the nerve tissue. This observation demonstrates that NSCs have a great potential for differentiation (Junqueira and Corneiro, 2005). The long standing and previously unquestioned dogma was that the brain cannot renew its cells and that

the number of neurons therefore remains constant throughout an individual's entire adult life. The concept of neurogenesis did not gain wider understanding until recently, although previous studies on cell proliferation with the use of 3H-thymidine or BrdU had confirmed neurogenesis throughout adulthood and the continuous generation of new neurons (Luskin, 1993). Neurogenesis in the adult brain takes place in its two major regions: the subventricular zone (SVZ) and the hippocampaldental gyrus (DG). The SVZ is the region of the highest neurogenic activity and the place from which the first NSCs were isolated *(*Reynolds and Weiss, 1992).

Mature neurons are formed, for example, in the olfactory bulb (OB), the region to which NSCs migrate from the SVZ along a discrete pathway called the rostral migratory stream. The SVZ contains a marrow-like structure harboring ependymal cells and astrocytes that play a role very similar to stromal cells in bone marrow. The ependymal cells and astrocytes form specific channels called glial tubes that are used by migrating neuroblasts. Neuroblasts form tight chains and migrate towards the OB, where they differentiate to periglomerular or granule neurons, changing their migration pattern from tangential to radial (Maciy-Tarnouski and Aleksander, 2006).

Astrocytes in glial tubes provide trophic support to the migrating cells and insulation from electrical and chemical signals released from the surrounding parenchyma (Maciy-Tarnouski and Aleksander, 2006). In addition to astrocytes, ependymal cells and neuroblasts, transitory amplifying progenitor (TAP) cells called type C cells are present in the SVZ. The type C cells are immature, fast-proliferating cells that do not express any characteristic feature of neuroblasts or glial cells. However, Doetsch et al. (1997) reported that TAP cells are not only progenitor cells derived from stem cells, but they also retain stem cell competence when exposed to growth factors. Moreover, depletion of mitotically active cells in the SVZ following injection with the antimitotic substance cytosine-arabinoside revealed that glial fiberally acidic protein (GFAP) positive cells repopulated the zone (Doetsch et al., 1999). Glial fiberally acidic proteins are a member of a family of intermediate filament proteins and are involved in maintaining the shape and function of astrocytes. Therefore, GFAP is considered as a specific marker of astrocytes. Astrocytes from the SVZ function as the primary precursors of rapidly dividing transit amplifying cells, and GFAP +ve astrocytes in the SVZ give rise to OB inter-neurons. In a very similar manner, the sub-granular layer of astrocytes in the hippocampus generates neurons in the dentate gyrus (Seri et al., 2001).

The main criterion distinguishing NSCs from other neural cells present in the brain is the *in vitro* formation of neurospheres by the former. The neurosphere cells proliferate and differentiate into clusters of cells with phenotypes of neurons, glia and oligodendrocytes (Reynolds and Weiss, 1992). The most unique feature of neurosphere cells is their ability to generate secondary spheres following dispersion and their renewing abilities even after several passages. All the observations suggest that the cells arise from pluripotent precursors and may reflect properties of *in vivo* progenitors. The formation of neurospheres could also be induced by the presence of growth factors, such as the epidermal growth factor (EGF*)* and the basic fibroblast growth factor (bFGF). Stem cells forming neurospheres express numerous markers such as nestin and neurofilaments (Morshead et al., 1994).

When it became apparent that NSCs really exist, that they have capabilities for self-renewal, and that it is possible to maintain them as stable cell lines, the next step was to check their plasticity. The results were very surprising and also very promising. NSCs (epidermal) out-stretched the brain boundaries such that they appeared to be able to transdifferentiate. The first to evaluate this was an elaborate work published by Bjornson et al. (2000), who reported that clonally derived neuronal stem cells, could give rise to hematopoietic cells *in vivo*. Also, similar data have been reported from a study conducted with NSCs in humans.

Transfection of stem cells

Lipofectamine 2000 (green fluorescent protein) is a proprietary formulation suitable for the transfection of nucleic acids into eukaryotic cells with green fluorescent protein. GFP is a 27-kD protein, originally discovered in jellyfish *Aequorea victoria*. Since GFP is theoretically a non-rejected protein, immunological rejection is eliminated in the transplantation between female GFP transgenic and Sprague-Dawley (SD) wild-type dogs and this model can also be regarded as a simulation of autologous transplantation (Okabe et al., 1997; Ito et al., 2001).

The fluorescent activity of GFP requires no substrates, cofactors or additional gene products, so cells expressing the intracellular GFP marker can be scored directly by fluorescent microscopy and/or flow cytometry without the requirement for antibody staining or extensive cell manipulation. Therefore, transgenic rabbits-derived cells are easily distinguished from wild-type rabbits-derived cells, thus, it was hypothesized that the use of GFP transgenic rabbits as transplantation donors would be helpful in developing autologous transplantation examinations, and in comprehending clearly the behavior of transplanted mesenchymal cells during the cellular repair processes of the defect (Persons et al., 1998).

CONCLUSION

A phenomenal expansion in our understanding of stem

cell biology in recent years, combined with a rapidly widening palette of successful technical breakthroughs and approaches, has led to the real possibility that stem cells will in future be used clinically in ever increasing numbers of diseases. This represents an especially attractive prospect in those conditions that have hitherto been extremely difficult to treat by conventional medicine. Currently, a number of previously intractable diseases represent prime targets to develop definitive approaches involving the appropriate application of stem cell technologies, either to arrest the progressive deterioration that are hallmarks of these conditions, or even to cure them

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