Review Articles

Stem Cells

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Abstract

Stem cell, a specialized cell, that has a unique capability to renew itself and differentiated in to many kinds of specific cell types such as bone, cartilage and muscle cells. It can be distinguished in to two kinds based on degree of differentiation. Embryonic stem cell, the cell which derived from a group of cells called the inner cell mass and adult stem cell, the undifferentiated cell which derived from a specialized tissue in the adult e.g. blood and bone. Embryonic stem cell, although can be able to generate a broad range of cell types but it can induce the formation of the teratomas and has a problem about the ethic. Whereas adult stem cell, although has limited in a proliferation and generated a narrow range of cell types, but it has many advantages as it can be easily received, has no ethical problem and immunological rejection moreover it also can reduce the risk of tumor formation. Form the above reasons; therefore recently the utilization of adult stem cell is markedly increased.

Keywords: Stem cells, Stem cells plasticity

Stem Cells, Precursor Cells and Progenitor Cells

A stem cell, by definition, is an undifferentiated cell that can produce daughter cells which either remain a stem cell (a process called self-renewal) or commit to a pathway leading to differentiation. The pathway to differentiation usually involves the daughter becoming a precursor cell, which proliferates before it differentiation. Because the proliferation amplifies the number of differentiated cells eventually produced, the precursors are often called transit amplifying cells. The terms precursor cell and progenitor cell are usually used interchangeably, but some use progenitor cell to refer to a cell with greater developmental potential than a precursor cell (Fig.1) (Martin 2003; Körbling et al. 2003; Catherine 2002; Amy and Irving 2004; Sharon et al. 2003; Claudius and Ralf 2005; Kirschtein and Skiboll 2001).

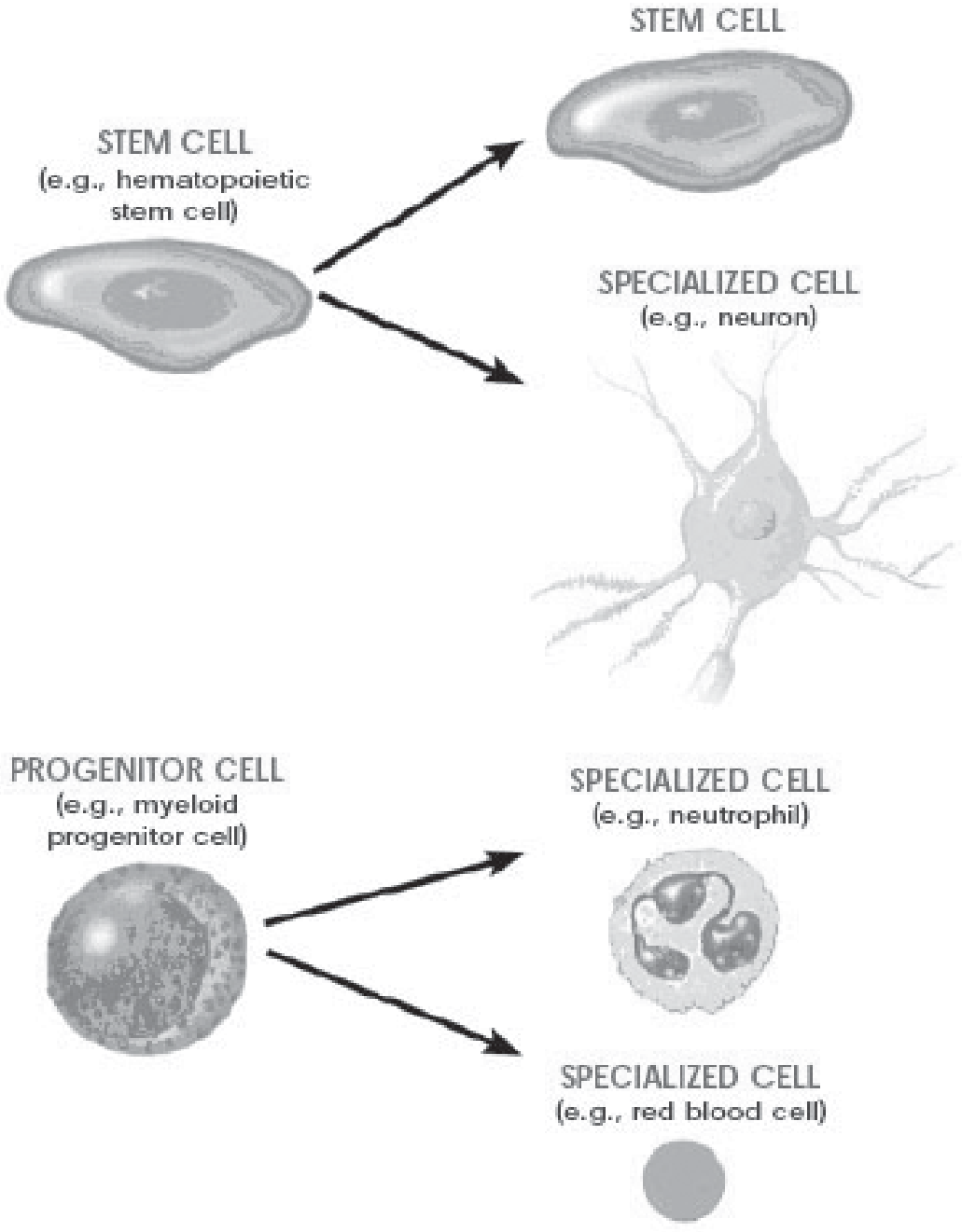

Figure 1 Distinguishing Features of Progenitor/Precursor Cells and Stem Cells (Kirschtein and Skiboll 2001).

Type of Stem Cells

Two types of stem cell can be distinguished in term of the degree of differentiation: embryonic stem (ES) cell which can only be derived from pre-implantation embryos and have a proven ability to form cells of all tissues of the adult organism (termed "pluripotent") and adult stem cell which is found in a variety of tissues in the fetus and after birth and is under normal conditions, more specialized ("multipotent") with an important function in tissue replacement and repair (Guido and Christine 2003).

1. Embryonic Stem Cells: ES Cells

1.1 The Characteristics of Human ES (hES) Cells

The embryonic stem cells (ES cells) are isolated from mouse in 1981 by Evans and Kaufman together with Martin while the human embryonic stem cells (hES cells) are first isolated by Thomson and his colleague in 1998. hES cells are derived from the inner cell mass of blastocysts and can proliferate indefinitely *in vitro*. Although hES cells can form all somatic tissues, they cannot form all of the other extraembryonic tissues necessary for complete development, such as the placenta and membranes, so that they cannot give rise to a complete new individual. For the reason above, they are classified to pluripotent stem cells. hES cells are immortal due to they express high levels of telomerase. The expression of telomerase, a ribonucleoprotein that adds telomere repeats to chromosome ends, thereby maintaining their length, is highly correlated with immortality in human cell lines. Most diploid somatic cells do not express high levels of telomerase and enter replicate senescence after a finite proliferative life span in tissue culture, usually after 50-80 population doublings (Martin 2003; Catherine 2002; Alan and Martin 2001; Anna 2001; Peter and Andras 2001; Jon et al. 2001).

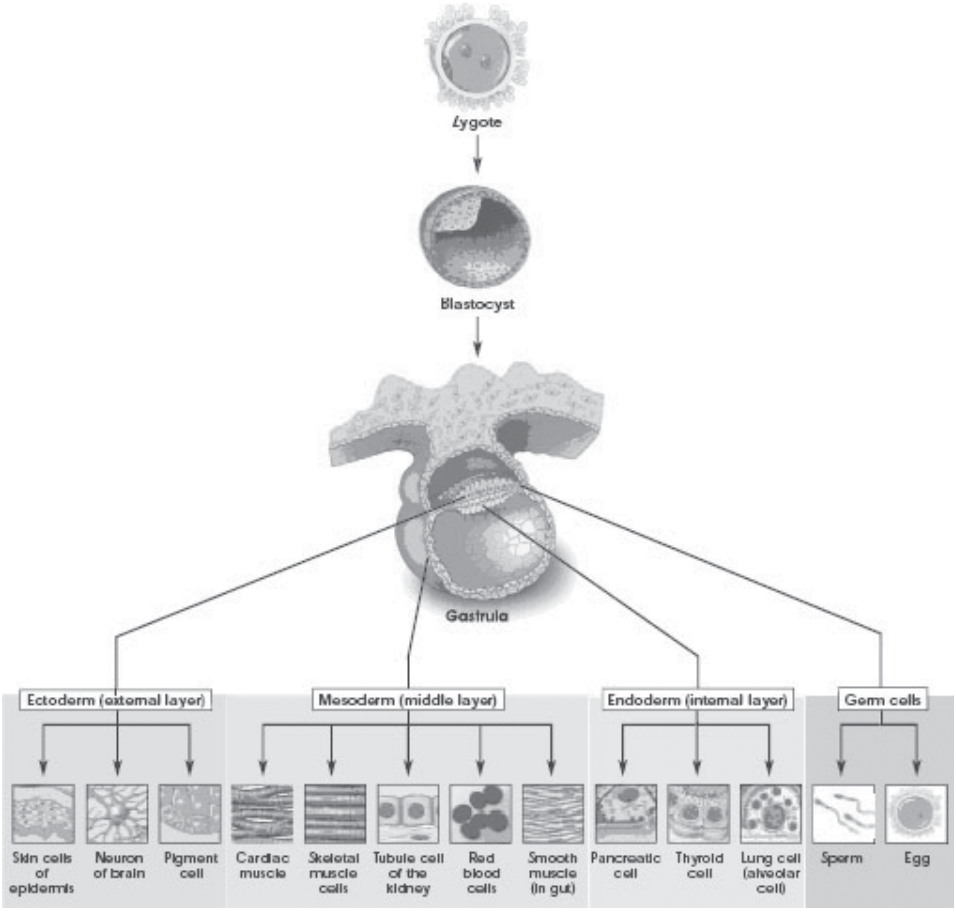

Figure 2 Differentiations of Embryonic Stem Cells (Kirschtein and Skiboll 2001).

The others characteristics of hES cells are described. They can give rise to differentiated cell types that are derived from all three primary germ layers of the embryo (endoderm, mesoderm and ectoderm as shown in Fig 2). They have a capacity to integrating into all fetal tissues during development. They express the transcription factor Oct-4 which then activates or inhibits a host of target genes and maintains ES cells in a proliferative, non-differentiating state. In addition, they also expressed many cell surface markers including stage-specific embryonic antigen (SSEA)-3, SSEA-4, TRA-1-60, TRA-1-81 and alkaline phosphatase (Amy and Irving 2004; Kirschtein and Skiboll 2001; Anna 2001; Tsuyoshi et al. 2004; Rachel and Nissim 2002; James et al. 1998).

1.2 The Advantages and Disadvantages of hES Cells

Many applications have been proposed for hES cells. The most-often discussion is their potential use in transplant therapy for replace or restore tissue that has been damaged by disease or injury. Diseases that might be treated by transplanting hES-derived cells include Parkinson's disease, diabetes and Duchenne's muscular dystrophy. However, treatments for any of these diseases require that human ES cells be directed to differentiate into specific cell types prior to transplant. The research is occurring in several laboratories, but is limited because so few laboratories have access to human ES cells. Thus, at this stage, any therapies based on the use of hES cells are still hypothetical and highly experimental (Kirschtein and Skiboll 2001).

One of the current advantages of using ES cells as compared to adult stem cells is that ES cells have an unlimited ability to proliferate *in vitro*, and are more likely to be able to generate a broad range of cell types through directed differentiation. The differentiation of hES cells in culture is responded to cytokines, hormones and growth conditions. For example, Nicole et al. found that hES cells can differentiation into osteoblastic cells under the influence of ascorbic acid, β-glycerophosphate and 1α, 25 dihydroxy vitamin D3. (Nicole and Hans 2003).

The potential disadvantages of the use of hES cells for transplant therapy include the propensity of undifferentiated ES cells to induce the formation of tumors (teratomas), which are typically benign. Because it is the undifferentiated cells---rather than their differentiated progeny---that have been shown to induce teratomas, tumor formation might be avoided by devising methods for removing any undifferentiated ES cells prior to transplant. Also, it should be possible to devise a fail -safe mechanism---i.e., to insert into transplanted ES-derived cells suicide genes that can trigger the death of the cells should they become tumorigenic (Kirschtein and Skiboll 2001; Benjamin 2004; Nicole and Hans 2003).

2. Adult Stem Cells

Adult stem cells are undifferentiated (unspecialized) cells that reside among differentiated (specialized) cells in a tissue or organ and have the capability for self-renewal (producing identical copies of itself) throughout theirs lifetime. They are also capable of producing the more mature (specialized) cell types of the specific tissue or organ in which they reside through differentiation. They are found in adult tissues and have the primary function in maintaining the steady state functioning of a cell---called homeostasis---and, with limitations, to replace cells that die because of injury or disease. They represented in a small fraction of the total number of cells in each tissue; therefore they are rare in the native tissue and hardly to identify. For example, in the human adult bone marrow they are found at a frequency of 1 in 10,000 to 1 in 100,000 or more of total blood cells. They are identified mainly by two major characteristics: cellular morphology (physical shape) and specific marker proteins linking them to the tissue or organ in which they are found. Reported adult tissues in which adult stem cells have been identified as shown in Table 1 (Sharon et al. 2003; Nicole and Hans 2003; Malcolm et al. 2002; Haynesworth et al. 1998; Rocky et al. 2002; Robert and Christine 2003; Henry and Asa 2004).

Several tissues are known to contain more than one adult stem cell population. For instance, in the bone marrow, they contains at least three stem cell populations that are hematopoietic stem cells (HSCs) which form all of the blood cells in the body, bone marrow mesenchymal stem cells (also referred to as bone marrow stromal stem cells); a mixed cell population that generates bone, cartilage, fat and fibrous connective tissue and the side population cells.

Table 1 Adult Human Stem Cells (Nicole and Hans 2003; Henry and Asa 2004).

Adult Stem Cell	Location (Source)	Cells or Tissues Produced
Hematopoietic stem cells	Bone marrow, peripheral blood, umbilical cord blood	Blood, endothelial, hepatic (oval) and muscle cells
Mesenchymal stem cells	Bone marrow, peripheral blood	Bone, cartilage, tendon, adipose tissue, muscle, marrow stroma, neural cells, cardiomyocytes and thymic stromal cells
Neural stem cells	Ependymal cells, astrocytes (subventricular zone) of the central nervous system	Neurons, astrocytes, oligodendrocytes, blood cells and muscle cells
Circulatory skeletal	Peripheral blood	Adipocytes and osteocytes
Endothelial precursor (angioblast)	Bone marrow	Mature endothelia and newly formed blood vessels
Stromal vascular cell fraction of processed lipoaspirate	Fat	Adipocyte, osteocyte, chondrocyte and myocyte precursors

2.1 Transdifferentiation, Plasticity of Adult Stem Cells

Embryogenesis is sequentially events results in the formation of the differentiated cells, tissues and organs. During developmental process, the major event is the specification of the three embryonic germ layers: ectoderm, mesoderm and endoderm. Ectoderm is believed to give rise to skin and neural lineages, mesoderm for blood, bone, muscle, cartilage and fat and endoderm for tissues of the respiratory and digestive tracts.

The specification of embryonic germ layers subsequently arising cells, including mature cells, progenitor cells and stem cells of each of the resulting tissue lineages. This specification is irreversible and maintain throughout adulthood. Moreover, homeostatic cell replacement and tissue regeneration in the adult have been considered to maintain tissue specificity. These specified tissues and organs retain rare tissue resident stem cells that generate only those mature cell types corresponding to their tissue origin and do not cross tissue (or germ layer) boundaries to generate cell types of different lineages. Nevertheless, recent experimental reports have challenged the new termed that are transdifferentiation.

Transdifferentiation has been defined as the process of the conversion of a cell of one tissue lineage into a cell of an entirely distinct lineage, with concomitant loss of the tissue specific markers and function of the original cell type. Transdifferentiation of adult stem cells give rise to the concept of stem cell plasticity that changes the knowledge which differentiated stem cells cannot redifferentiate to stem cells and transform to another one lineage. Lineage conversion, theoretically, occur via dedifferentiation of a tissue-specific cell to a more

primitive, multipotent cell and subsequent redifferentiation along a new lineage pathway (Amy and Irving 2004).

The first report about transdifferentiation was discovered by Ferrari et al. in 1998. They found that mouse bone marrow could give rise to skeletal muscle cells when transplanted into a mouse muscle that had been damaged by an injection of a muscle toxin (Ferrari et al. 1998). Later, there are many reports that supported the transdifferentiation events, for example, transdifferentiation of bone marrow cells to hepatocytes (Petersen et al. 1999; Theise et al. 2000), endothelial and myocardial cells (Lin et al. 2000; Orlic et al. 2001) and CNS neurons and glial cells (Brazelton et al. 20002; Mezey et al. 2000). Furthermore, they have reported that enriched hematopoietic stem cells (HSCs) could produce cardiac myocytes and endothelial cells (Jackson et al. 2001), purified HSCs could produce functional hepatocytes (Lagasse et al. 2000) and single HSCs could produce epithelial cells of the liver, gut, lung and skin (Krause et al. 2001).

2.2 The Advantages and Disadvantages of Adult Stem Cells

The use of adult stem cells for therapy has many advantages such as it is an alternative way for avoiding the ethical problem. In addition, the cells can be isolated from the patient requiring treatment then it can avoid the problem of immunological rejection and it can reduce the risk of tumor formation which occurs with high frequency when using ES cells (Martin 1980, Smith 2001). However, adult stem cells also have the limitation about the replicative senescence and they will be loss in number and multipotentiality with age (Martin 2003; Rocky et al. 2002; Christine and Günter 2005).

3. Mesenchymal Stem Cells: MSCs

Mesenchymal stem cells (MSCs) were discovered by Friedenstein and his associates more than 30 years ago. They are described as multipotent because of their ability even as clonally isolated cells, to exhibit the potential for differentiation into a variety of different cells/tissue lineages. Some researchers have used the term "marrow stromal cells" interchangeably with "mesenchymal stem cells". But in fact, studies examining and comparing the morphology, phenotype and *in vitro* function of between these two cells shown that MSCs have more homogeneous and fibroblastoid shape than marrow-derived stromal cells. In addition, marrow-derived stromal cells presented hematopoietic characteristics in varying degrees. Furthermore, although both cells types were able to support hematopoiesis, the undifferentiated MSCs were not as efficient, and while the cells displayed similar

Table 2 Example of Human MSCs Frequency and Phenotypic Properties (Baksh et al. 2004).

Study	Cell Fraction Isolated	Frequency	Major Cell Properties
Pittenger *et al*.	70% Percoll (1.073 g/ml)	1 in 1 x 10^5	Adherent fibroblastic-like cells $SH2^+$, $SH3^+$, $CD29^+$, $CD44^+$, $CD71^+$, $CD90^+$, $CD106^+$, $CD120a^+$, $CD124^+$
Koc *et al*.	Percoll (1.073 g/ml) 5.9-23.4 ml BM	0.7-1.4 in 1 x $10^{5(a)}$	Adherent fusiform fibroblastic-like cells $SH2^+$, $SH3^+$, $SH4^+$, $CD45^-$, CD14, $CD34^-$
Reyes *et al*.	Ficoll-Paque (1.077 g/ml)	1 in 1 x 10^6	Clusters of small adherent cells $CD34^-$, $CD44^{low}$, $CD45^-$, $CD117^-$, class I-HLA^-, class II-HLA-DR, CD45-GlyA cells

[(a)]A mean of 0.7-1.4 x 10^5 MSCs are recovered at the first passage from 1 x 10^6 inputs BM MNC.

mRNA and cytokine profiles, their individual responses to Interleukin-1 (IL-1) treatment differed. For the reason above, it can conclude that the stromal cells are actually early differentiated progeny of the MSCs (Rocky et al. 2002; Stuart et al. 2002; Roufosse et al. 2004).

Firstly, Human MSCs can be isolated from bone marrow. But, in the later time, researchers discovered that many source of tissues can be found MSCs, for example, periosteum, trabecular bone, adipose tissue, synovium, sketetal muscle and deciduous teeth. MSCs can be isolated by using cell density gradient centrifugation. The example of human MSCs (hMSCs) frequency and phenotypic properties are shown in Table 2 (Rocky et al. 2002; Christine and Günter 2005; Baksh et al. 2004).

3.1 Characteristics of MSCs

By light or phase contrast microscopy, MSCs culture shows a rather homogenous population of fibroblast-like cells and in phenotypically, they display a number of nonspecific markers. It is generally accepted that MSCs are not display the hematopoietic and endothelial markers, including CD11b, CD31 and CD45. The expression of CD34 in MSCs is demonstrated in variation. Moreover, MSCs often express a great deal of surface adhesion molecules such as CD44, CD49e and CD62. In addition, they are typically positive for MHC class I and Sca-1. Because MSCs have heterogeneous in population between species and within cultures, variable expression of CD90 (Thy1.1), CD117 (c-kit), SH2 (CD105 or endoglin), SH3, SH4 (CD73) and STRO-1 are often observed. These discrepancies arise due to the differences in isolation method, tissue and species of origin and culture conditions (JoŚe et al. 2001; Baksh et al. 2004; Elisabeth et al. 2004; Brenton et al. 2003; Fibbe 2002).

Extended cytokines and growth factors receptors of MSCs have been described as shown in Table 3

Table 3 Main Characteristics of MSCs (JoŚe et al. 2001).

Expression of specific antigens, cytokine receptors, adhesion molecules and production of cytokines and matrix molecules

Marker Type	Designation
Specific antigens	SH2, SH3, SH4, STRO-1, α-smooth muscle actin, MAB1740
Cytokines and growth factors	Interleukins: 1α, 6, 7, 8, 11, 12, 14 and 15 LIF, SCF, Flt-3 ligand, GM-CSF, G-CSF, M-CSF
Cytokine and growth factor receptors	IL-1R, IL-3R, IL-4R, IL-6R, IL-7R, LIFR, SCFR, G-CSFR, IFNγR, TNFIR, TNFIIR, TGFβIR, TGF?IIR, bFGFR, PDGFR, EGFR
Adhesion molecules	Integrins: αVβ3, αVβ5 Integrin chains: α1, α2, α3, α4, α5, αV, β1, β3, β4 ICAM-1, ICAM-2, VCAM-1, ALCAM-1, LFA-3, L-selectin, endoglin, CD44
Extracellular matrix	Collagen type I, III, IV, V and VI Fibronectin, laminin, hyaluronan, proteoglycans

3.2 Plasticity of MSCs

MSCs can proliferate as undifferentiated cells and have the capability to differentiate into several different lineages of mesodermal origin, for instant, cartilage, bone, fat, tendon, muscle, myocardium and marrow stroma, both *in vivo* and *in vitro*, upon culture with appropriate combination of growth factors and chemicals. A summary of MSCs plasticity in each stimuli condition is shown in Table 4. This information is supported by *in vivo* studies, demonstrates that MSCs develop into terminally differentiated phenotypes, like those forming bone, cartilage, tendon, muscle, neural, adipose tissues and hematopoietic-supporting stroma (JoŚe et al. 2001; Stuart et al. 2002; Angelo and Laura 2003).

Table 4 Differentiation Potential of MSCs *In Vitro*: Stimuli, Molecular and Cellular Markers (JoŚe et al. 2001).

Differentiation to:	Stimuli	Terminal Phenotype Identification Markers	
		Molecular	Cellular
Adipocytes	Dexamethasone + Isobutilmethyxanthine	PPARγ2, C/EBPβ, adipsin, leptin, lipoprotein lipase	Cytoplasmic lipid droplet accumulation
Chondrocytes	TGFβ3 + ascorbic acid TGFβ1 + ascorbic acid	Cbfa-1, collagen type II and IX, aggrecan	Matrix enriched in proteoglycans and collagen type II and IX

Table 5 Differentiation Potential of MSCs *In Vitro*: Stimuli, Molecular and Cellular Markers (JoŚe et al. 2001).

Differentiation to:	Stimuli	Terminal Phenotype Identification Markers	
		Molecular	Cellular
Osteoblasts	Dexamethasone + β-glycerophosphate + ascorbic acid	Cbfa-1, alkaline phosphatase, bone sialoprotein, osteopontin, osteocalcin, collagen type I	Mineralized matrix formation
Tenocytes	BMP-12	Collagen type II, proteoglycans	Improved biomechanical properties of implanted tendon
Skeletal muscle cells	5-Azacytidine	MyoD, Myf 5 and 6, Myogenin, myosin, MRF-4	Multinucleated contractile cells
Smooth muscle cells	PDGF-BB	ASMA, metavinculin, calponin, h-caldesmon, later smooth muscle actin	

Conclusion

A particular cell which can be both proliferates and differentiates is called stem cell. It can be classified into subtype by degree of differentiation. It has a unique characteristic such as cell surface markers which can be distinguished it from other cell types. Beside of differentiation degree, the resource of cell is different. Embryonic stem cell is found from the inner cell mass of blastocyst whereas adult stem cell is found in many tissues e.g. blood, bone and neural tissue. In the process of differentiation, it require the appropriate milieu, for example in osteoblastic differentiation, it need ascorbic acid, β-glycerophosphate and 1α, 25 dihydroxy vitamin D3 while chondrocyte differentiation require TGFβ-1/ TGFβ-3 and ascorbic acid. From its ability, stem cell is used in many purposes of generative medicine.

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