

66 Stem Cell Biology

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Stem cell biology is a relatively new field that explores the characteristics and possible clinical applications of the different types of pluripotent cells that serve as the progenitors of more differentiated cell types. In addition to potential therapeutic applications (Chap. 67), patient-derived stem cells can also provide disease models and a means to test drug effectiveness.

IDENTIFICATION, ISOLATION, AND DERIVATION OF STEM CELLS

Resident Stem Cells The definition of stem cells remains elusive. Stem cells were originally postulated as *unspecified* or *undifferentiated* cells that provide a source of renewal of skin, intestine, and blood cells throughout the lifespan. These *resident stem cells* are now identified in a variety of organs, i.e., epithelia of the skin and digestive system, bone marrow, blood vessels, brain, skeletal muscle, liver, testis, and pancreas, based on their specific locations, morphology, and biochemical markers.

Isolated Stem Cells Unequivocal identification of stem cells requires the separation and purification of cells, usually based on a combination of specific cell-surface markers. These *isolated stem cells*, e.g., hematopoietic stem (HS) cells, can be studied in detail and used in clinical applications, such as bone marrow transplantation (Chap. 68). However, the lack of specific cell-surface markers for other types of stem cells has made it difficult to isolate them in large quantities. This challenge has been partially addressed in animal models by genetically marking different cell types with green fluorescence protein driven by cell-specific promoters. Alternatively, putative stem cells have been isolated from a variety of tissues as side population (SP) cells using fluorescence-activated cell sorting after staining with Hoechst 33342 dye. However, the SP phenotype should be used with caution as it may not be function for stem cells.

Cultured Stem Cells It is desirable to culture and expand stem cells in vitro to obtain a sufficient quantity for analysis and potential therapeutic use. Although the derivation of stem cells in vitro has been a major obstacle in stem cell biology, the number and types of *cultured stem cells* have increased progressively (Table 66-1). The cultured stem cells derived from resident stem cells are often called *adult stem cells* to indicate their adult origins and to distinguish them from *embryonic stem* (ES) and *embryonic germ* (EG) cells. However, considering the presence of embryo-derived tissue-specific stem cells, e.g., trophoblast stem (TS) cells, and the possible derivation of similar cells from embryo/fetus, e.g., neural stem (NS) cells, it is more appropriate to use the term, *tissue stem cells*.

Successful derivation of cultured stem cells (both embryonic and tissue stem cells) often requires the identification of necessary growth factors and culture conditions, mimicking the microenvironment or *niche* of the resident stem cells. For example, the derivation of mouse TS cells, once considered impossible, became possible by using FGF4, a ligand known to be expressed by cells adjacent to the developing trophoblast in vivo. Therefore, it may be possible to culture other resident stem cells (e.g., intestinal stem cells) or isolated stem cells (e.g., HS cells) by studying the factors that constitute their normal niche.

SELF-RENEWAL AND PROLIFERATION OF STEM CELLS

Symmetric and Asymmetric Cell Division The most widely accepted stem cell definition is a cell with a unique capacity to produce unaltered daughter cells (*self-renewal*) and to generate specialized cell types (*potency*). Self-renewal can be achieved in two ways. *Asymmetric cell division* produces one daughter cell that is identical to the parental cell

and one daughter cell that is different from the parental cell and is a progenitor or differentiated cell. *Asymmetric cell division* does not increase the number of stem cells. *Symmetric cell division* produces two identical daughter cells. For stem cells to proliferate in vitro, they must divide symmetrically. Self-renewal alone cannot define stem cells, because any established cell line, e.g., HeLa cells or NIH3T3 cells, proliferate by symmetric cell division.

Unlimited Expansion In Vitro Resident stem cells are often quiescent and divide infrequently. However, once the stem cells are successfully cultured in vitro, they often acquire the capacity to divide continuously and the ability to proliferate beyond the normal limit of passages typical of primary cultured cells (sometimes called *immortality*). These features are primarily seen in ES cells, but have also been demonstrated for NS cells, MS cells, MAPCs, maGSCs (adult-derived tissue stem cells), and USSCs (newborn-derived tissue stem cells), thereby enhancing the potential of these cells for therapeutic use (Table 66-1).

Stability of Genotype and Phenotype The capacity to actively proliferate is associated with the potential accumulation of chromosomal abnormalities and mutations. Mouse ES cells have been extensively used to produce gene-targeted animals and are known to maintain their euploid karyotype and genome integrity. In contrast, human ES cells appear to be more susceptible to mutations after long-term culture. Another limitation is the possible formation of tumors after transplanting actively dividing stem cells. Mouse ES cells can form teratomas when injected into immunosuppressed animals.

POTENCY AND DIFFERENTIATION OF STEM CELLS

Developmental Potency The term *potency* is used to indicate a cell's ability to differentiate into specialized cell types. The current lack of knowledge about the molecular nature of potency requires the experimental manipulation of stem cells to demonstrate their potency. For example, in vivo testing can be done by injecting stem cells into mouse blastocysts or immunosuppressed adult mice and determining how many different cell types are formed from the injected cells. In vitro testing can be done by differentiating cells in various culture conditions to determine how many different cell types are formed from the cells. The in vivo assays are not applicable to human stem cells. The formal demonstration of self-renewal and potency is performed by demonstrating that a single cell possesses such abilities in vitro (*clonality*). Cultured stem cells are tentatively grouped according to their potency (Fig. 66-1).

From Totipotency to Unipotency *Totipotent cells* can form an entire organism autonomously. Only a fertilized egg (zygote) possesses this feature. *Pluripotent cells* (e.g., ES cells) can form almost all the body's cell lineages (endoderm, mesoderm, and ectoderm), including germ cells. *Multipotent cells* (e.g., HS cells) can form multiple cell lineages but cannot form all of the body's cell lineages. *Oligopotent cells* (e.g., NS cells) can form more than one cell lineage but are more restricted than multipotent cells. Oligopotent cells are sometimes called *progenitor cells* or *precursor cells*; however, these terms are often more strictly used to define partially differentiated or lineage-committed cells (e.g., myeloid progenitor cells) that can divide into different cell types but lack self-renewing capacity. *Unipotent cells* or *monopotent cells*, e.g., spermatogonial stem (SS) cells, can form a single differentiated cell lineage. Terminally differentiated cells, such as fibroblast cells, also have a capacity to proliferate (which may be called self-renewal) but maintain the same cell type (e.g., no potency to form another cell type) and are not, therefore, considered unipotent cells.

Nuclear Reprogramming Development naturally progresses from totipotent fertilized eggs to pluripotent epiblast cells, to multipotent cells, and finally to terminally differentiated cells. According to Wad-

TABLE 66-1 TYPES OF CULTURED STEM CELLS

Name	Source, Derivation, Maintenance, and Properties
Embryonic stem cells (ES, ESC)	ES cells can be derived by culturing blastocysts or immuno-surgically isolated inner cell mass (ICM) from blastocysts on a feeder layer of MEFs with LIF (m) or without LIF (h). ES cells are to originate from the epiblast (m, h). ES cells grow as tightly adherent multicellular colonies with a population doubling time of ~12 h (m), maintain a stable euploid karyotype even after extensive culture and manipulation, can differentiate into a variety of cell types in vitro, and can contribute to all cell types, including functional sperm and oocytes, when injected into a blastocyst (m). ES cells form relatively flat, compact colonies with the population doubling time of 35–40 h (h).
Embryonic germ cells (EG, EGC)	EG cells can be derived by culturing primordial germ cells (PGCs) from embryos at E8.5–E12.5 on a feeder layer of MEFs with FGF2 and LIF (m). EG cells can be derived by culturing gonadal tissues from 5–11 week post-fertilization embryo/fetus on a feeder layer of MEFs with FGF2, forskolin, and LIF (h). EG cells show essentially the same pluripotency as ES cells when injected into mouse blastocysts (m). The only known difference is the imprinting status of some genes (e.g., <i>Igf2r</i>): Imprinting is normally erased during germline development, and thus, the imprinting status of in EG cells is different from that of ES cells.
Trophoblast stem cells (TS, TSC)	TS cells can be derived by culturing trophectoderm cells of E3.5 blastocysts, extraembryonic ectoderm of E6.5 embryos, and chorionic ectoderm of E7.5 embryos on a feeder layer of MEFs with FGF4 (m). TS cells can differentiate into trophoblast giant cells in vitro (m). TS can contribute exclusively to all trophoblast subtypes when injected into blastocysts (m).
Extraembryonic endoderm cells (XEN)	XEN cells can be derived by culturing the ICM in non-ES cell culture condition (m). XEN cells can contribute only to the parietal endoderm lineage when injected into a blastocyst (m).
Embryonic carcinoma cells (EC)	EC cells can be derived from teratocarcinoma—a type of cancer that most commonly develops in the testes. EC cells rarely show pluripotency in vitro, but they can contribute to all cell types when injected into blastocysts. EC cells often have an aneuploid karyotype and other genome alterations.
Mesenchymal stem cells (MS, MSC)	MS cells can be derived from bone marrow, muscle, adipose tissue, peripheral blood, and umbilical cord blood (m, h). MS cells can differentiate into mesenchymal cell types, including adipocytes, osteocytes, chondrocytes, and myocytes (m, h).
Multipotent adult stem cells (MAPC)	MAPCs can be derived by culturing bone marrow mononuclear cells, after depleting CD45 ⁺ and GlyA ⁺ cells, with FCS, EGF, and PDGF-BB (h). MAPCs are very rare cells that are present within MSC cultures from postnatal bone marrow (m, h). MAPCs can also be isolated from postnatal muscle and brain (m). MAPCs can be cultured for >120 population doublings. MAPCs can differentiate into all tissues in vivo when injected into a mouse blastocyst, and can differentiate into various cell lineages of mesodermal, ectodermal, and endodermal origin in vitro (m).
Spermatogonial stem cells (SS, SSC)	SS cells can be derived by culturing newborn testis on STS-feeder cells with GDNF (m). SS cells can reconstitute long-term spermatogenesis after transplantation into recipient testes and restore fertility.
Germline stem cells (GS, GSC)	GS cells can be derived from neonatal testis (m). GS cells can differentiate into three germlayers in vitro and contribute to a variety of tissues, including germline, when injected into blastocysts.
Multipotent adult germline stem cells (maGSC)	maGSC can be derived from adult testis (m). maGSC can differentiate into three germlayers in vitro and can contribute to a variety of tissues, including germline, when injected into blastocysts.
Neural stem cells (NS, NSC)	NS cells can be derived from fetal and adult brain (subventricular zone, ventricular zone, and hippocampus) and cultured as a heterogeneous cell population of monolayer or floating cell clusters called <i>neurospheres</i> . NS cells can differentiate into neuron and glia in vivo and in vitro. Recently, the culture of pure population of symmetrically dividing adherent NS cells became possible in the presence of FGF2 and EGF.
Unrestricted somatic stem cells (USSC)	USSCs are rare cells derived from newborn cord blood (h). USSCs can be derived by culturing the mononuclear fraction of cord blood in the presence of 30% FCS and 10 ⁻⁷ M dexamethasone. USSCs can differentiate into a variety of cell types in vitro and can contribute a variety of cell types in in vivo transplantation experiments in rat, mouse, and sheep (h). USSCs are CD45 ⁻ adherent cells and can be expanded to 10 ¹⁵ cells without losing pluripotency (h).

Note: m, mouse; h, human; FGF, fibroblast growth factor; FCS, fetal calf serum; EGF, epidermal growth factor; PDGF, platelet-derived growth factor; GDNF, glial cell line–derived neurotrophic factor; LIF, leukemia inhibitory factor; MEF, mouse embryonic fibroblast.

transplantation, or nuclear transfer (NT), procedures (often called “cloning”), where the nucleus of a differentiated cell is transferred into an enucleated oocyte. Although this is an error-prone procedure and the success rate is very low, live animals have been produced using adult somatic cells as donors in sheep, mouse, and other mammals. In mice, it has been demonstrated that ES cells derived from blastocysts made by somatic cell NT are indistinguishable from normal ES cells. NT can potentially be used to produce *patient-specific ES cells* carrying a genome identical to that of the patient. However, the successful implementation of this procedure has not been reported in humans. Setting aside technical and ethical issues, the limited supply of human oocytes will be a major problem for clinical applications of NT. Alternatively, successful nuclear reprogramming of somatic cells by fusing them with ES cells has been demonstrated in mouse and human. However, it is not yet clear how ES-derived DNA can be removed from hybrid cells. More direct nuclear reprogramming of somatic cells by transfecting specific genes or by exposing the cells to ES cell extracts is the subject of current research.

Stem Cell Plasticity or Transdifferentiation The prevailing paradigm in developmental biology is that once cells are differentiated, their phenotypes are stable. However, a number of reports have shown that tissue stem cells, which are thought to be lineage-committed multipotent cells, possess the capacity to differentiate into cell types outside their lineage restrictions (called *transdifferentiation*). For example, HS cells may be converted into neurons as well as germ cells. This feature may provide a means to use tissue stem cells derived directly from a patient for therapeutic purposes, thereby eliminating the need to use embryonic stem cells or elaborate procedures such as nuclear reprogramming a patient’s somatic cells. However, more strict criteria and rigorous validation are required to establish tissue stem cell plasticity. For example, observations of transdifferentiation may reflect cell fusion, contamination with progenitor cells from other cell lineages, or persistence of pluripotent embryonic cells in adult organs. Therefore, the assignment of potency to each cultured stem cell in Fig. 66-1 should be taken with caution. Whether transdifferentiation exists and can be used for therapeutic purposes remains to be determined conclusively.

Directed Differentiation of Stem Cells Pluripotent stem cells (e.g., ES cells) can differentiate into multiple cell types, but in culture they normally differentiate into heterogeneous cell populations in a stochastic manner. However, for therapeutic uses, it is desirable to direct

stem cells into specific cell types (e.g., insulin-secreting beta cells). This is an active area of stem cell research, and protocols are being developed to achieve this goal. In any of these directed cell differentiation systems, the cell phenotype must be evaluated critically. Interestingly, it has been reported that mouse ES cells can differentiate in vitro into

dington’s epigenetic landscape, this is analogous to a ball moving down a slope. The reversal of the terminally differentiated cells to totipotent or pluripotent cells (called *nuclear reprogramming*) can thus be seen as an uphill gradient that never occurs in normal conditions. However, nuclear reprogramming has been achieved using *nuclear*

Stage Potency	Preimplantation	Embryonic, fetal	Postnatal	Adult
Totipotent	Zygote ^{m,h}			
Pluripotent	ES ^{m,h}	EG ^{m,h}	GS ^m USSC ^h MAPC ^{m,h}	EC ^{m,h} maGSC ^m MAPC ^{m,h}
Multipotent				MS ^{m,h}
Oligopotent	TS ^m			NS ^{m,h}
Unipotent	XEN ^m			SSC ^m
Terminally differentiated cells				

FIGURE 66-1 Potency and source developmental stage of cultured stem cells. For abbreviations of stem cells, see Table 66-1. Note that stem cells are often abbreviated with or without “cells,” e.g., ES cells or ESCs for embryonic stem cells. m, mouse; h, human.

oocytes as well as sperm, which are capable of fertilizing an oocyte to produce live offspring.

MOLECULAR CHARACTERIZATION OF STEM CELLS

Genomics and Proteomics In addition to standard molecular biological approaches, genomics and proteomics have been extensively applied to the analysis of stem cells. For example, DNA microarray analyses have revealed the expression levels of essentially all genes and identified specific markers for some stem cells. Similarly, the protein profiles of stem cells have been assessed by using mass spectrophotometry. These methodologies are beginning to provide a novel means to characterize and classify various stem cells and the molecular mechanisms that give them their unique characteristics.

Stemness This term has been used to designate the essential molecular characteristics of stem cells. It is also used to indicate common genetic programs shared among ES cells and tissue stem cells (HS and NS cells). A number of common genes, such as stress-response genes, have been identified, but the lack of commonality among different studies raises concerns about the validity of this concept.

Pivotal Genes Involved in ES Cell Regulation Recent work has begun to identify genes involved in the regulation of stem cell function. For example, three genes—*Pou5f1* (Oct3/4), *Nanog*, and *Sox2*—govern key gene regulatory pathways/networks for the maintenance of self-renewal and pluripotency of mouse and human ES cells. Similarly, it has been shown that the interaction and balance among three transcription factors—*Pou5f1*, *Cdx2*, and *Gata6*—determine the fate of mouse ES cells: upregulation of *Cdx2* differentiates ES cells into trophoblast cells, whereas upregulation of *Gata6* differentiates ES cells into primitive endoderm. These types of analyses should provide molecular clues about the function of stem cells and lead to a more effective means to manipulate stem cells for future therapeutic use.

FURTHER READINGS

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