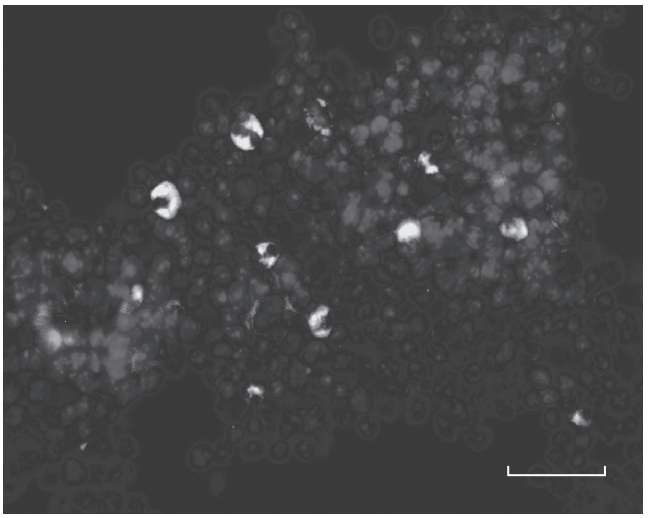
9

REGENERATION OF ADULT CELLS, TISSUES, AND ORGANS



Presence of smooth muscle α-actin-positive (green) and CD34-positive (red) cells in the bone marrow. When cultured on elastic lamina-dominant arterial matrix scaffolds, these cells can transform to smooth-muscle-like cells with α-actin filaments. These cells may serve as progenitor cells for the regeneration of vascular smooth muscle cells. Blue: cell nuclei. Scale: 10μm. See color insert.

Bioregenerative Engineering: Principles and Applications, by Shu Q. Liu

THE STEM CELL CONCEPT

Stem cells are cell types that can self-renew indefinitely and can differentiate into specified cell types. These features are critical to sustained generation and regeneration of cells, tissues, and organs during development and remodeling. Based on the function, stem cells can be classified into two subtypes: uncommitted and committed stem cells. *Uncommitted stem cells* can differentiate into all specified cell types. These cells are also known as *pluripotent stem cells* or *embryonic stem cells*, and are found in the embryo before the formation of the endodermal, mesodermal, and ectodermal layers. Uncommitted stem cells are usually obtained from the inner cell mass of the blastocyst, primordial germ cells, and epiblast. *Committed cells* are cells found in later stages of development compared to the blastocyst stage and are committed to form cells in a specified tissue, organ, or system. All committed stem cells are originated from the inner cell mass of the blastocyst. Typical examples for committed stem cells include the stem cells in the endoderm, the mesoderm, and the ectoderm. Stem cells in the fetal tissues and organs are also considered committed stem cells. In each tissue, organ, or system, there are cell types that can differentiate into specified cells, but cannot self-renew. These cell types are referred to as *progenitor* or *precursor cells.*

Embryonic Stem Cells [9.1]

Embryonic stem cells are pluripotent embryonic cells that can self-renew and self-expand indefinitely and can differentiate into all specified cell types under appropriate growth conditions. Even under cell culture conditions in vitro, embryonic stem cells exhibit these features. There are several potential sources for embryonic stem cells: the inner cell mass, primordial germ cells, and epiblast (also known as *primitive ectoderm*). The *inner cell mass* is a structure of the early embryo within the blastocyst and consists of about 30 cells. The inner cell mass cells can give rise to all cell types in the body. These cells can be collected, expanded in culture, and used for the repair and replacement of malfunctioned adult cells (Fig. 9.1). The collection and preparation of embryonic stem cells are demonstrated in Fig. 9.2. Expanded stem cells can be then delivered to a target organ, where the stem cells can differentiate into specified cell types under appropriate local environment. It should be noted that a special condition for culturing embryonic stem cells is the presence of feeder cells. Fibroblasts can serve as a feeder cell type. The feeder cells provide structural support as well as soluble factors necessary for the survival and expansion of the embryonic stem cells.

The *primordial germ cells* are a group of embryonic cells that develop into reproductive cells, including sperm and ovum (see Chapter 7). These cells arise from the proximal epiblast, a structure developed from the inner cell mass, pass through the primitive streak, migrate to the genital ridge, and differentiate into germ progenitor cells and extraembryonic mesodermal cells. The germ cells are collectively called the *embryonic germ cells* during the early embryonic stages. These cells exhibit phenotypes that are similar to those found in the inner cell mass cells and can serve as embryonic stem cells that give rise to specified cell types in all ectodermal, mesodermal, and endodermal layers.

The *epiblast* is a structure directly derived from the inner cell mass of the embryo. This structure gives rise to the three embryonic layers (ectoderm, mesoderm, and endoderm) and the amnionic ectoderm, which forms the amnionic sac. Isolated epiblast cells can be cultured and expanded in vitro. These cells demonstrate features of embryonic

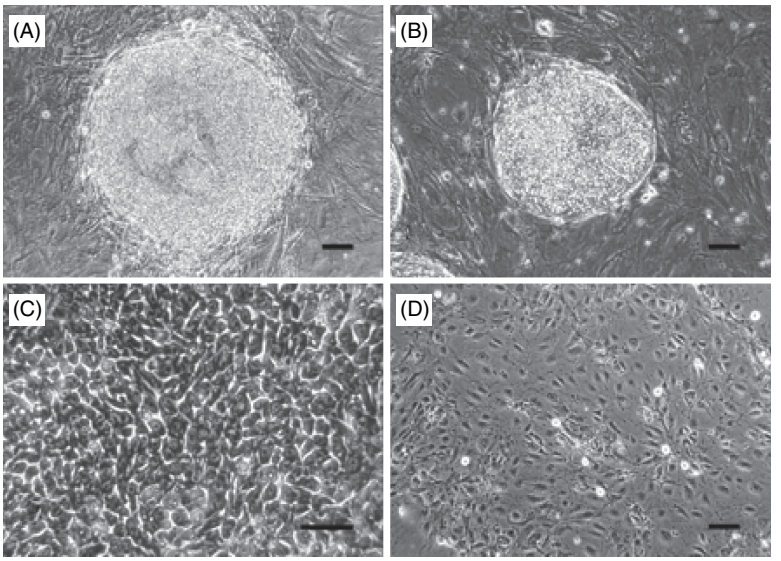


Figure 9.1. Human embryonic stem cells (cell line H9) in culture. (A) Human inner cell mass-derived cells attached to mouse embryonic fibroblast feeder layer after 8 days of culture, 24 h before first dissociation. Scale bar: 100 μm. (B) H9 colony. Scale bar: 100 μm. (C) H9 cells. Scale bar: 50 μm. (D) Differentiated H9 cells, cultured for 5 days in the absence of mouse embryonic fibroblasts, but in the presence of human LIF (20 ng/mL). Scale bar: 100 μm. (Reprinted with permission from Thomson JA et al: Embryonic stem cell lines derived from human blastocysts. *Science* 282:1145–7, copyright 1998 AAAS.)

stem cells and can differentiate into specified cell types found in all tissues and organs. Thus, epiblast cells are potential stem cells that can be used for stem cell-based therapeutic purposes.

In addition to the sources described above, stem cells can be generated by using the *somatic cell nuclear transfer* (SCNT) technology. For this technology, the nucleus of an adult cell, such as a fibroblast, from a patient is collected and transferred into an unfertilized egg. The transferred egg can be induced to give rise to stem cells with the genetic characteristics of the donor somatic cell. Because the somatic nucleus carries the genome of the patient, the derived stem cells are partially autologous to the patient, thus reducing immune rejections. The transferred stem cells may retain their pluripotent nature and can be stored for multiple transplantations. This is a potential approach for the generation of therapeutic stem cells.

To date, the developmental features of embryonic stem cells have been investigated in several mammalian species including the mouse, primate, and human. In particular, the successful preparation, expansion, and induction of differentiation of human embryonic stem cells have greatly promoted research activities in stem cell-based regenerative medicine. It is now well established that human embryonic stem cells can differentiate into numbers of functional adult cell types, including neurons, glial cells, cardiomyocytes, liver cells, vascular smooth muscle cells, pancreatic β cells, blood cells, skeletal muscle cells, and osteoblasts. Typical examples are shown in Figs. 9.3–9.5. These preliminary studies have demonstrated the clinical potential of using stem cells for regenerative therapies.

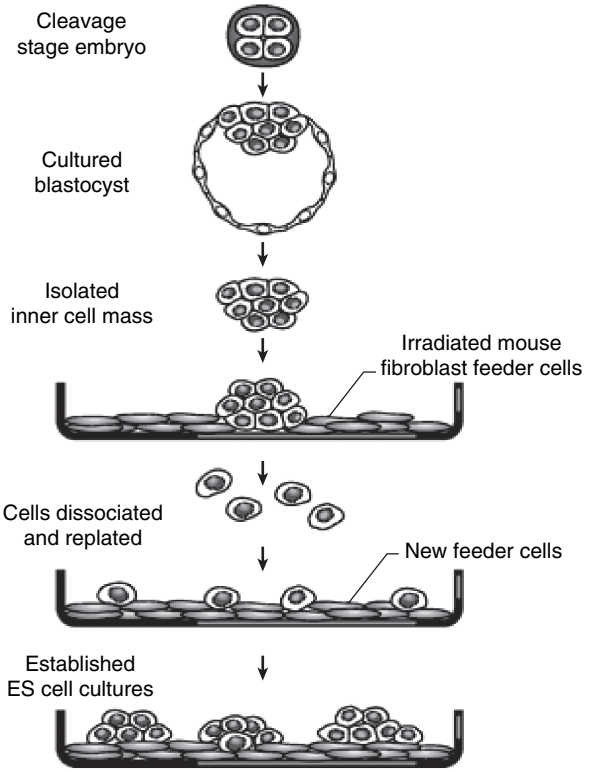


Figure 9.2. Preparation of human embryonic stem cells. Human blastocysts were grown from cleavage-stage embryos produced by in vitro fertilization. The inner cell mass (ICM) cells were collected and plated onto a fibroblast feeder substratum in medium containing fetal calf serum. Colonies were sequentially expanded and cloned. (Reprinted from Odorico JS et al: *Stem Cells* 19:193–204, 2001 by permission).

Fetal Stem and Progenitor Cells [9.1]

Fetal stem and progenitor cells are those found in the fetus. Investigations by using animal models have demonstrated the presence of various stem and progenitor cells in the fetus. Certain fetal stem cells can differentiate into specified cell types across differentiation barriers between the three developmental layers: ectoderm, mesoderm, and endoderm. However, because of ethical concerns, the therapeutic potential of human fetal stem cells has not been investigated extensively. One type of fetal stem/progenitor cells, the blood-borne fetal mesenchymal stem/progenitor cells, may be considered for clinical application. The fetal blood has been shown to contain circulating fetal mesenchymal stem/progenitor cells. Blood samples can be collected from the fetus, and circulating stem and progenitor cells can be enriched, expanded, and stored for treating disorders, if any, of the same fetus. Since these cells are from the fetal stage of development, they can potentially differentiate into various types of specialized cells.

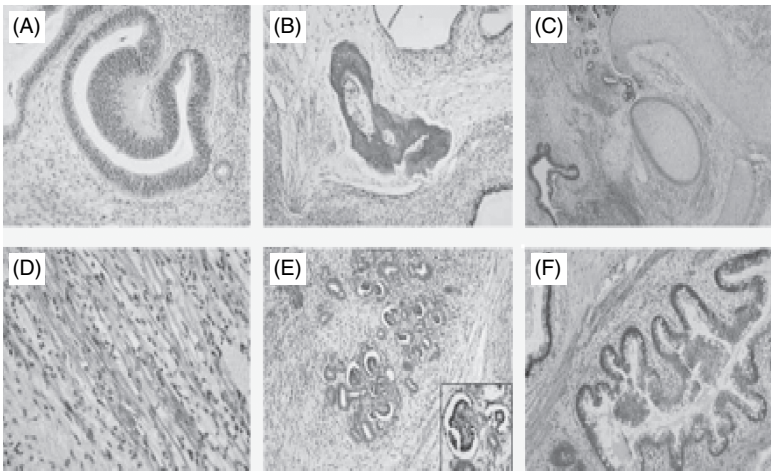


Figure 9.3. Specified cell types derived from human embryonic stem cells transplanted into the mouse. Human embryonic stem cells were prepared and transplanted into immunocompromised mice. The implanted human embryonic stem cells can form benign teratomas. Various cell and tissue types can be found from the teratomas, including neural epithelium (panel A, 100×), bone (panel B, 100×), cartilage (panel C, 40×), striated muscle (panel D, 200×), and glomeruli and renal tubules (panel E, 100×; inset 200×), and gut (panel F, 40×). All photomicrographs are of hematoxylin- and eosin-stained sections. (Reprinted from Odorico JS et al: *Stem Cells* 19:193–204, 2001 by permission.)

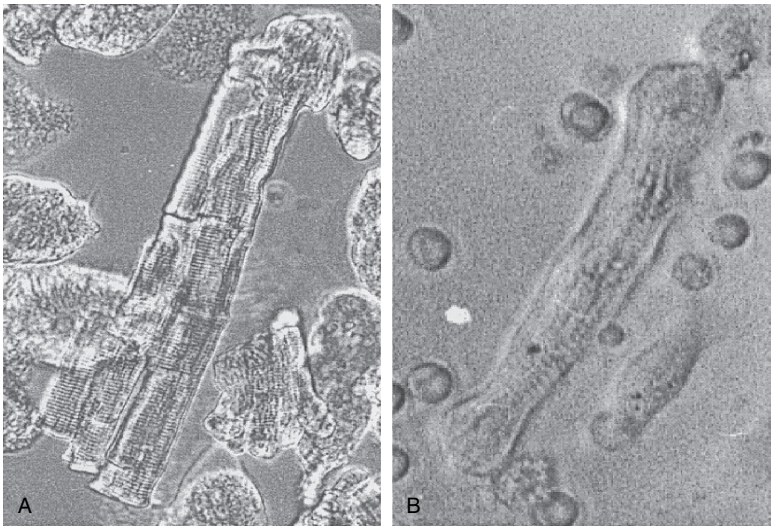


Figure 9.4. Cardiomyocytes derived from mouse embryonic stem cells. (A) Phase contrast micrograph showing a cluster of 2-day cardiomyocytes cells after isolation and fixation. (B) Micrograph showing a single cell, digitally magnified 2× compared with panel A. (Reprinted from Mummery C et al: *J Anat* 200:233–42, 2002 by permission of Blackwell Publishing.)

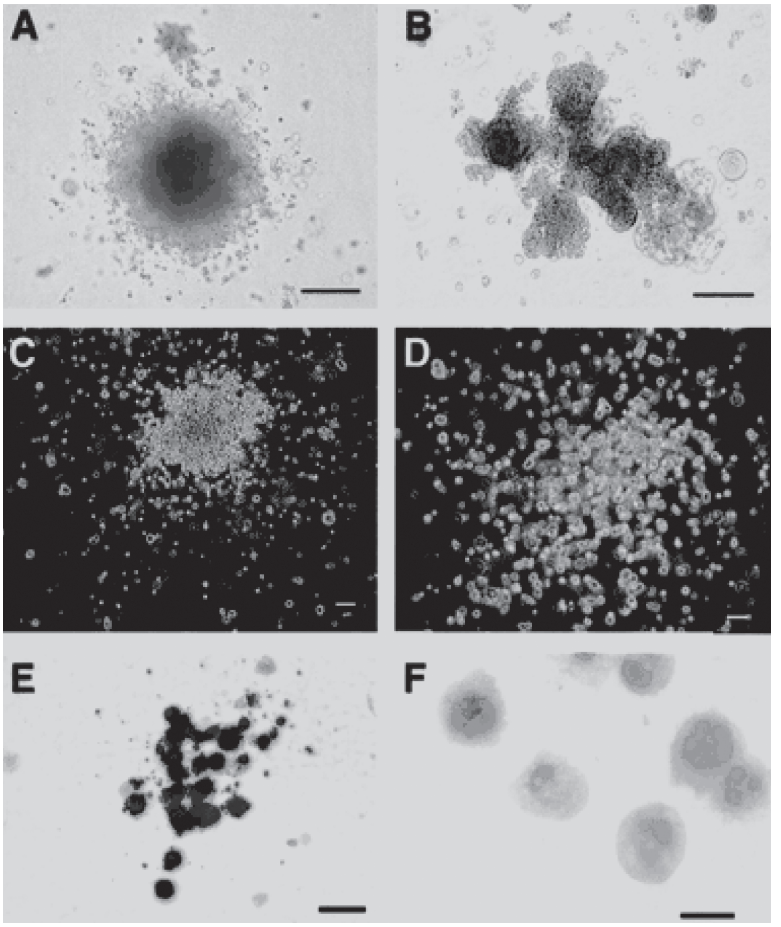


Figure 9.5. Hematopoietic cells derived from human embryonic stem cells. H1 human embryonic stem cells were allowed to differentiate on S17 cells for ~17 days. The H1 cells were harvested and cultures in semisolid media for 14 days before scoring colony phenotypes. (A) Colony of mixed erythroid and myeloid cells, incldluing CFU–granulocyte, erythroid, macrophage, and megakaryocyte (CFU-GEMM). (B) Large unstained erythroid colony (red, hemoglobin). (C) Unstained myeloid colony, CFU-GM. (D) Unstained myeloid colony, CFU-M, which was less dense than the CFU-GM colony. (E) Colony of CFU-Mk cells stained with platelet/megakaryocyte-specific antibody against CD41 (GPIIb/IIIa) with alkaline phosphatase-conjugated secondary antibody and Fast Red/naphthol reagent to provide red stain. (F) Cytospin of CFU-GM cells demonstrating granulocytes with esterase-positive red granules. Scale bars: A–D, 100 μm; E, 40 μm; F, 20 μM. (Reprinted by permission from Kaufman DS et al: *Proc Natl Acad Sci USA* 98:10716–21, copyright 2001, National Academy of Science USA.)

Adult Stem Cells

There exist various types of stem cells in adult tissues and organs. These cells can self-renew and differentiate into specified cell types. Adult stem cells can be found in the bone marrow (e.g., hematopoietic stem cells) (see chapter-opening figure), nervous system (e.g., neuroepithelial cells and olfactory cells) (Fig. 9.6), epidermis (e.g., basal epidermal

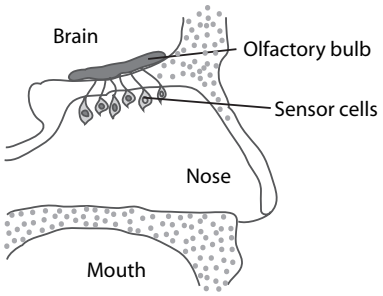


Figure 9.6. Schematic representation of the olfactory cells. Based on bibliography 9.1.

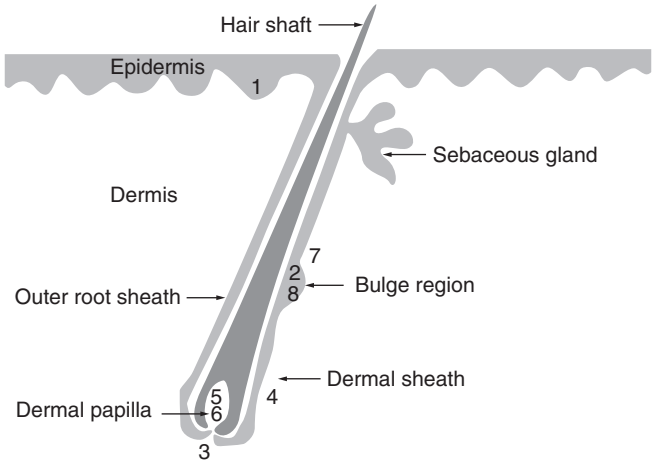


Figure 9.7. Schematic representation of skin stem cells in the hair follicle, epidermis, and dermis: (1) epidermal stem cell; (2) follicle multipotent stem cell; (3) mesenchymal stem cell; (4) dermal sheath stem cells; (5) neural crest stem cell; (6) hematopoietic stem cell; (7) endothelial stem cell; (8) melanocyte stem cell. (Reprinted from Shi C et al: *Trends Biotechnol* 24:48–52, copyright 2006 with permission from Elsevier.)

cells) (Fig. 9.7), dermis (Fig. 9.8), and intestines (e.g., epithelial stem cells) (Fig. 9.9). The presence of stem cells is the basis for cell regeneration in response to cell injury and death in these tissues. The adult stem cells have been traditionally considered committed tissue-specific stem cells, which can differentiate only into cells specific to the tissues and organs in which the stem cells reside. However, recent investigations have demonstrated that certain types of adult stem cells from one tissue type can break the developmental restriction and differentiate into specified cell types found in another tissue type. For instance, hematopoietic stem cells from the bone marrow can differentiate into not only hematopoietic cells but also mature cells in tissues other than the hematopoietic system. When hematopoietic stem cells are transplanted into the brain, heart, and liver, these cells can transform into neurons, cardiomyocytes, and hepatocytes, respectively. The property of the cross-lineage or cross-tissue transformation of adult stem cells is referred to as *plasticity*. In this section, the types and features of adult stem cells are briefly discussed.

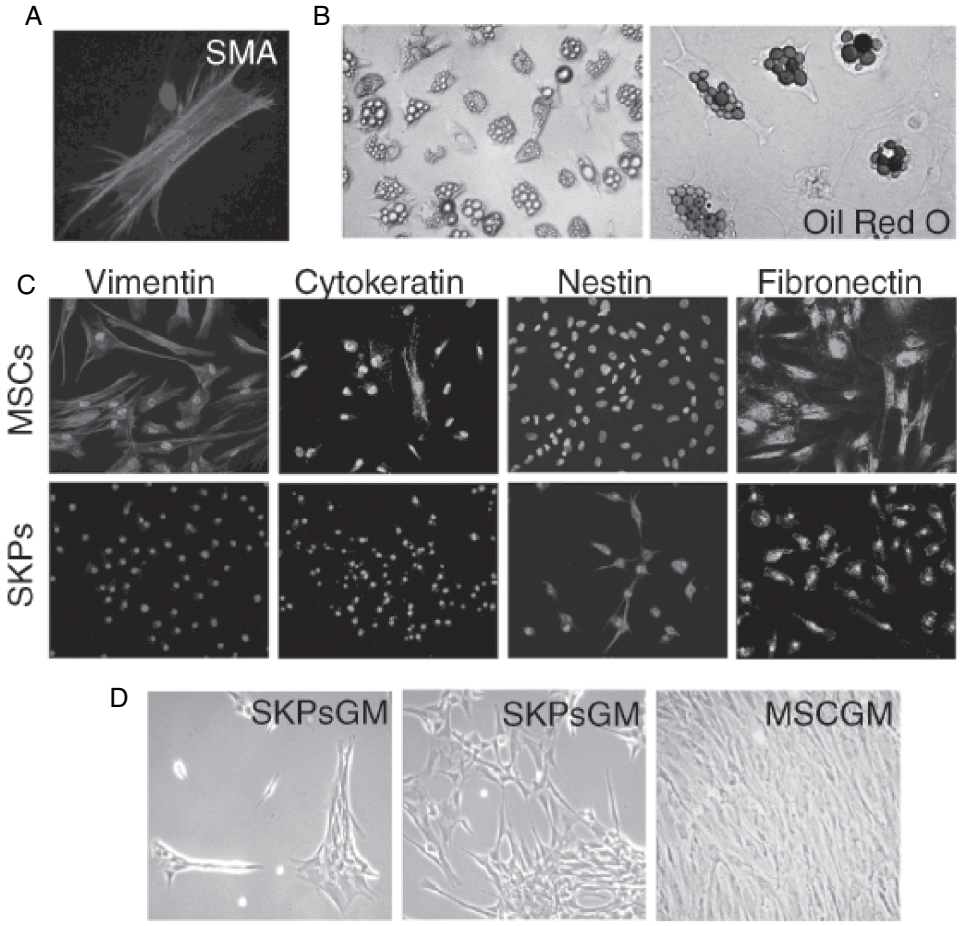


Figure 9.8. Dermis-derived precursors (SKPs) can differentiate into smooth muscle cells and adipocytes. (a) A SMA-positive smooth muscle cell differentiated from juvenile SKPs that were passaged for 3 months. (b) In culture medium with 10% FBS without added growth factors, adult SKPs differentiate into cells with the morphological characteristics of adipocytes. Left, phase-contrast micrograph; right, brightfield picture of a culture stained with Oil Red O, which stains lipid droplets. (c) Immunocytochemical analysis of mesenchymal stem cells (MSCs; top) and SKPs (bottom) for vimentin, cytokeratin, nestin, and fibronectin. SKPs differ from mesenchymal stem cells in their production of the intermediate filament proteins vimentin and nestin. In both cases, cells were dissociated and plated onto poly-D-lysine/laminin-coated slides overnight before immunocytochemistry. Mesenchymal stem cells produce high levels of vimentin and no nestin, whereas SKPs produce nestin but not vimentin. A subpopulation of mesenchymal stem cells also produce cytokeratin. Note the difference in morphology of the two cell types: SKPs are clearly much smaller and less flattened than mesenchymal stem cells. (d) Mesenchymal stem cells do not proliferate in suspension when grown under SKP conditions. Phase micrographs of mesenchymal stem cells grown on uncoated tissue culture plastic for 2 weeks in SKPs medium and growth factors (SKPsGM; left and center) or in mesenchymal stem cell medium (MSCGM; right). Note that the mesenchymal stem cells adhere to uncoated plastic and survive but do not proliferate in the SKPs medium, whereas they rapidly proliferate to reach confluence in the mesenchymal stem cell medium. (Reprinted by permission from Macmillan Publishers Ltd.: Toma JG et al: Isolation of multipotent adult stem cells from the dermis of mammalian skin, *Nature Cell Biol* 3:778–84, copyright 2001.)

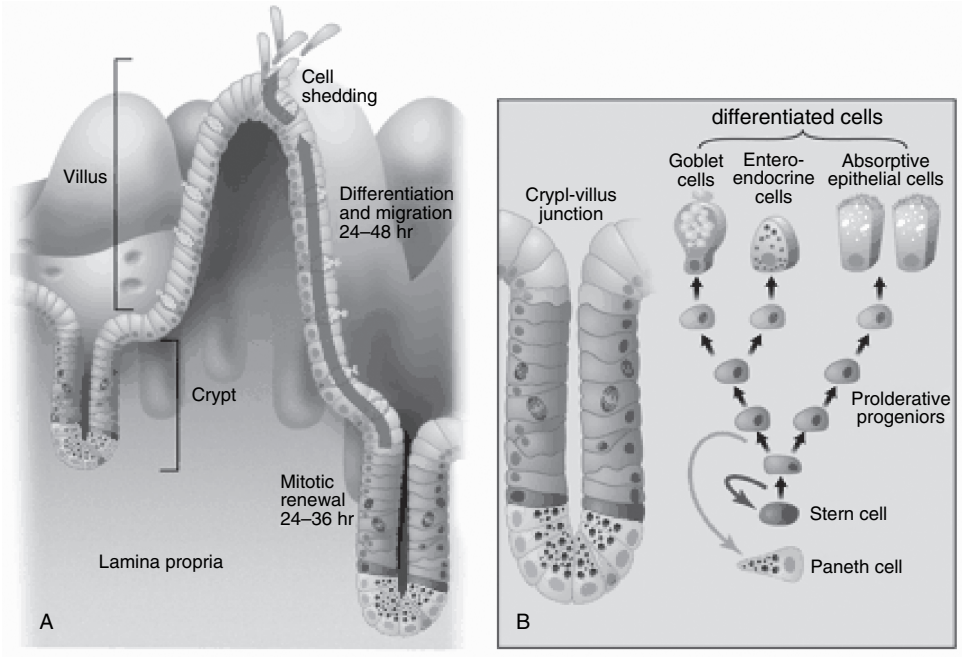


Figure 9.9. Distribution and differentiation of stem cells in the intestine. (A) Distribution of intestinal mature and stem cells. (B) Differentiation pathways of intestinal stem cells. (Reprinted with permission from Radtke F, Clevers H: *Science* 307:1904–9, copyright 2005 AAAS.)

Bone Marrow-Derived Stem Cells [9.2]. The bone marrow contains adult stem cells, including the hematopoietic stem cells and marrow stromal stem cells, which can self-renew and differentiate into specified cell types. The *hematopoietic stem cells* can differentiate into all types of mature hematopoietic cell, including erythrocytes, leukocytes, and platelets. The hematopoietic stem cells can be identified and purified based on protein makers expressed on the cell surface. Protein markers found in these cells include CD34, c-Kit, Sca-1, and Thy1.1. These markers are relatively unique to the bone marrow-derived hematopoietic stem cells. Thus, $CD34^{+}c\text{-}kt^{+}Sca\text{-}1^{+}Thy\text{-}1^{+}$ bone marrow cells are often considered hematopoietic stem cells. These markers can be identified by immunohistochemistry with specific antibodies.

The bone marrow also contains specified hematopoietic progenitor cells and mature blood cells. Since the presence of these cell lineages obscures the identification of the hematopoietic stem cells, these cells are usually identified and depleted by using lineage-specific antibodies before the hematopoietic stem cells are isolated. For instance, T cells and their immediate progenitor cells express uniquely CD3, CD4, and CD8; B cells express B220; monocytes and granulocytes express CD11b; and erythrocytes express Ter119. Blood cells with these surface proteins can be removed by using magnetic beads coated with antibodies specific to these surface proteins. Alternatively, fluorochrome-conjugated antibodies can be used to label specified cell surface markers. Antibody-labeled cells can be identified and removed by fluorescence-activated cell sorting (FACS). The remaining cells after immune depletion are referred to as *lineage-depleted cells* or *Lin^{-} cells*.

Based on the features of cell self-renewal and differentiation, hematopoietic stem cells can be divided into three groups: long-term and short-term hematopoietic stem cells, and progenitor cells. The long-term hematopoietic stem cells can self-renew indefinitely, the short-term hematopoietic stem cells can self-renew for 1–2 months, and the progenitor cells cannot self-renew.

The hematopoietic stem cells demonstrate several features that are useful for the application of these cells to the regeneration of nonhematopoietic cells and tissues:

1. A small number of hematopoietic stem cells can repopulate the entire hematopoietic system after a complete ablation of the blood cells. Indeed, this is the most convincing evidence that bone marrow contains hematopoietic stem cells.
2. Hematopoietic stem cells, when delivered to the vascular system, can engraft to the intima of blood vessels, pass through the vessel wall, and engraft to various organs, including the brain, liver, intestines, kidney, and connective tissue. Under organ-specific conditions, the bone marrow stem cells can differentiate into specified cell types. For instance, bone marrow stem cells can transform to hepatocytes in the liver (Fig. 9.10) and renal cells in the kidney (Fig. 9.11).
3. Hematopoietic stem cells can be used to transplant into allogenic recipients with or without myeloablation, and can home to the bone marrow and replenish the hematopoietic system permanently without significant immune rejection responses.
4. The transplantation of hematopoietic stem cells derived from healthy donors into allogenic recipients with autoimmune disorders (e.g., type I diabetes) can potentially reduce the autoimmune response of the recipients.

These features provide a foundation for the use of hematopoietic stem cells for treating human disorders.

Another population of bone marrow cells that include stem cells is the *marrow stromal cells*. These cells provide a stroma that supports the growth and development of the hematopoietic cells. Furthermore, the marrow stromal cells produce and release soluble mediators that are necessary for the differentiation and specification of the hematopoietic stem cells. The marrow stromal cell population contains mesenchymal stem cells that can differentiate into osteoblasts, chondrocytes, fibroblasts, adipocytes, endothelial cells, and smooth muscle-like cells. The marrow stromal cells are characterized by a unique property: the ability to adhere to a glass, plastic, or extracellular matrix substrate. Hematopoietic cells do not possess such a property. This property has been used for the identification and isolation of marrow stromal cells.

The marrow stromal cells undergo a dynamic change in phenotype and cell surface markers when cultured in vitro. During the early phase of culture when the marrow stromal cells are engaged only in adhesion, the adherent cell population contains $CD34^+$, c-Kit^+, Sca-1^+, Thy1.1^+, $CD11b^+$, $CD45^+$, smooth muscle α-$actin^+$, $calponin^+$, and smooth muscle $myosin^+$ (SM1). Certain markers are coexpressed in the adherent cells. For instance, CD34 and c-Kit are found in the majority of smooth muscle α-$actin^+$ bone marrow cells (see chapter-opening figure). Calponin and SM1, which are markers for mature smooth muscle cells, are found in a large fraction of smooth muscle α actin cells in culture (Fig. 9.12). With the progression of cell culture, stem cell markers including CD34, c-Kit, Sca-1, and Thy1.1 are gradually diminished, while the expression of CD11b, CD45, smooth muscle α-actin, calponin, and SM1 remains. Up to this stage, cells can be divided into two major groups: smooth muscle α-$actin^+$ and smooth muscle α-$actin^-$ cells. The smooth

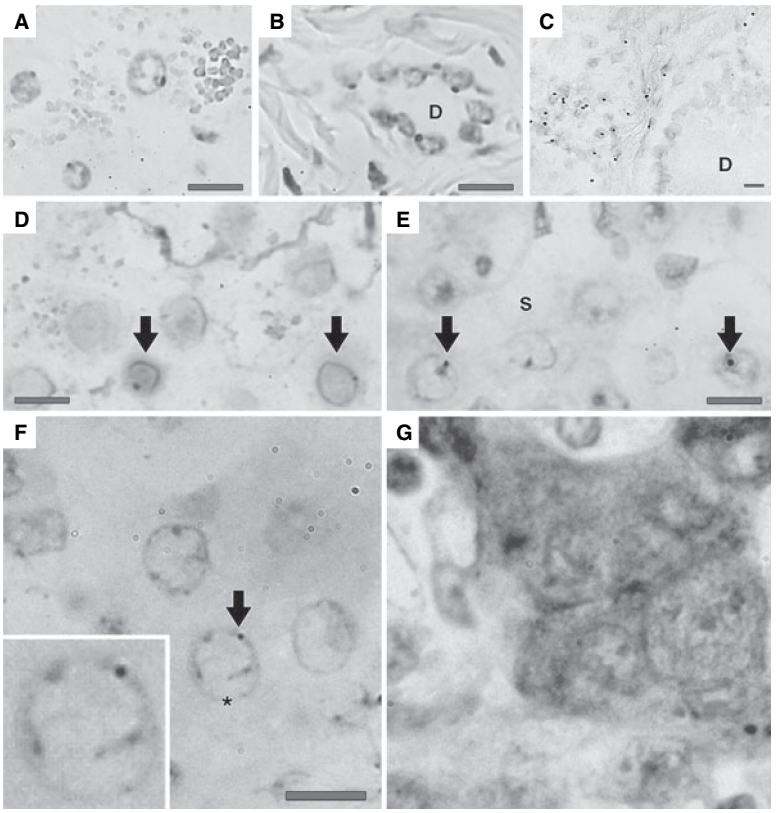


Figure 9.10. Hepatocytes derived from bone marrow cells. Adult bone marrow cells prepared from male human donors were transplanted into female patients. The presence of the Y chromosome in the female liver indicates the engraftment of the transplanted male bone marrow cells. The Y chromosome can be detected by immunolocalization of a fluorescein isothiocyanate (FITC)-labeled Y-chromosome probe using an anti-fluorescein antibody conjugated to horseradish peroxidase, and visualized using diaminobenzidine as a brown chromogen. For this preparation, the presence of the Y chromosome is indicated by a distinct brown dot, typically located at the nuclear periphery. (A) Hepatocytes were identified by their large round nuclei and cytoplasmic granules and were Y-chromosome-positive in male controls. (B) The Y chromosome was also detected in the bile ducts (D) in male controls. (C) A Y-chromosome-negative bile duct surrounded by numerous Y-positive inflammatory cells in a female liver transplanted into a male. (D) Immunodetection of two Y-chromosome-positive cells (arrows) located in a hepatocyte plate delineated by cytokeratin-8 immunostaining from a female patient who had received male bone marrow. (E–G) Y-chromosome detection in female livers transplanted into male recipients; (E) two Y-chromosome-positive hepatocytes (arrows) in a hepatocyte plate bordering a sinusoid (S); note the brown dot, demonstrating that the Y chromosome is readily distinguishable from the blue nucleolus; (F, G) consecutive sections of a group of four hepatocyte nuclei, showing (F) one Y-chromosome-positive hepatocyte (arrow, and inset at 2-fold magnification), and (G) their cytokeratin-8 immunoreactivity. Scale bars: 10 μm. (Reprinted by permission from Macmillan Publishers Ltd.: Alison MR et al: *Nature* 406:257, copyright 2000.)

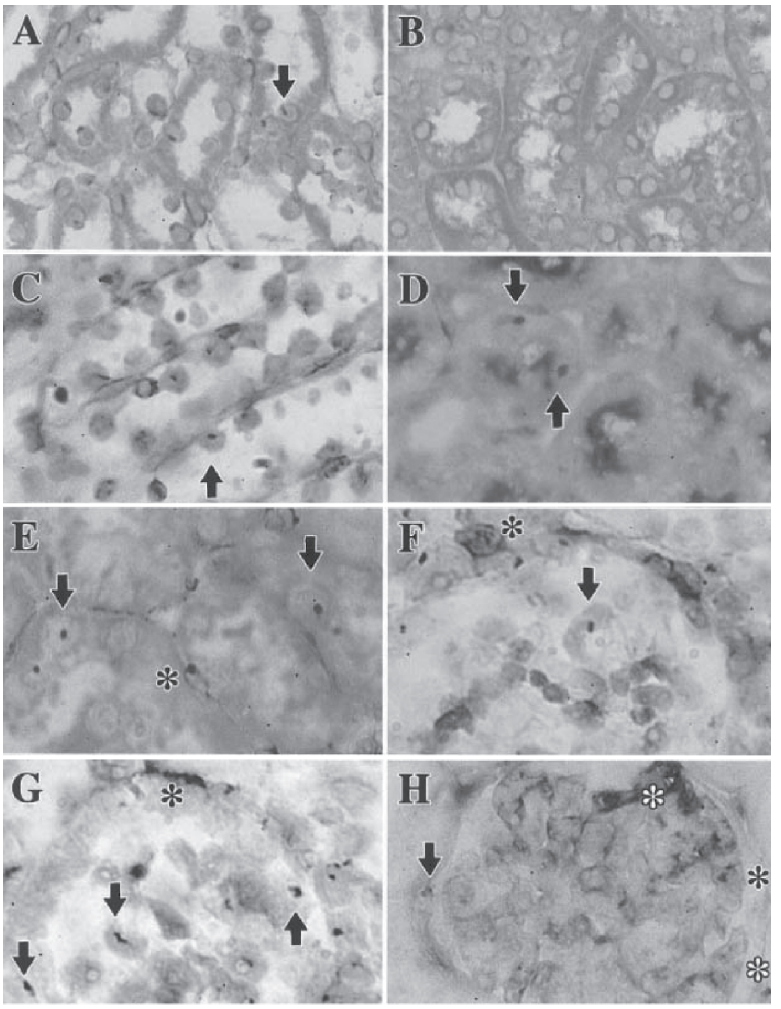


Figure 9.11. Engraftment of bone marrow cells into the kidney. Bone marrow cells were prepared from male donor mice and transplanted into female recipient mice. The Y chromosomes in the female kidney were detected by immunolabeling with the anti-CYP1A2 antibody. (A) A male control showing positive Y chromosome (arrow) in tubular epithelial cells immunostained with the anti-CYP1A2 antibody. (B) A female control showing the lack of Y chromosome within tubular epithelial cells immunostained with the anti-CYP1A2 antibody. Micrographs C–H were prepared from a female mouse 13 weeks following male whole bone marrow transplantation. (C) Y-chromosome-positive proximal tubular epithelial cells (arrow) adherent to a PAS-positive basement membrane. (D) Y-chromosome-positive tubular epithelial cells (arrows) that are reactive to RCA lectin; (E) Y-chromosome-positive proximal tubular epithelial cells (arrows) that are CYP1A2-immunoreactive. A Y-chromosome-positive interstitial cell can also be seen (*). (F) Y-chromosome-positive cells associated with the glomerulus: Y-chromosome-positive/CD45-negative cells within a glomerulus (arrow) and Y-chromosome-positive/CD45-positive cells apposed closely to the renal corpuscle (*). (G) Y-chromosome-positive F4/80-negative cells (arrows) within a glomerulus and a Y-chromosome-positive F4/80-positive cell apposed closely to the renal corpuscle (*). (H) A Y-chromosome-positive/vimentin-positive cell (black asterisk) within a glomerulus. A Y-chromosome-positive/vimentin-positive cell lining the renal corpuscle (arrow) is apparent along with several other Y-chromosome-positive cells (white asterisks) of undetermined phenotype. (Reprinted with permission from Poulsom R et al: *J Pathol* 195:229–35, 2001. Copyright Pathological Society of Great Britain and Ireland. Permission is granted by John Wiley & Sons Ltd on behalf of PathSoc.)

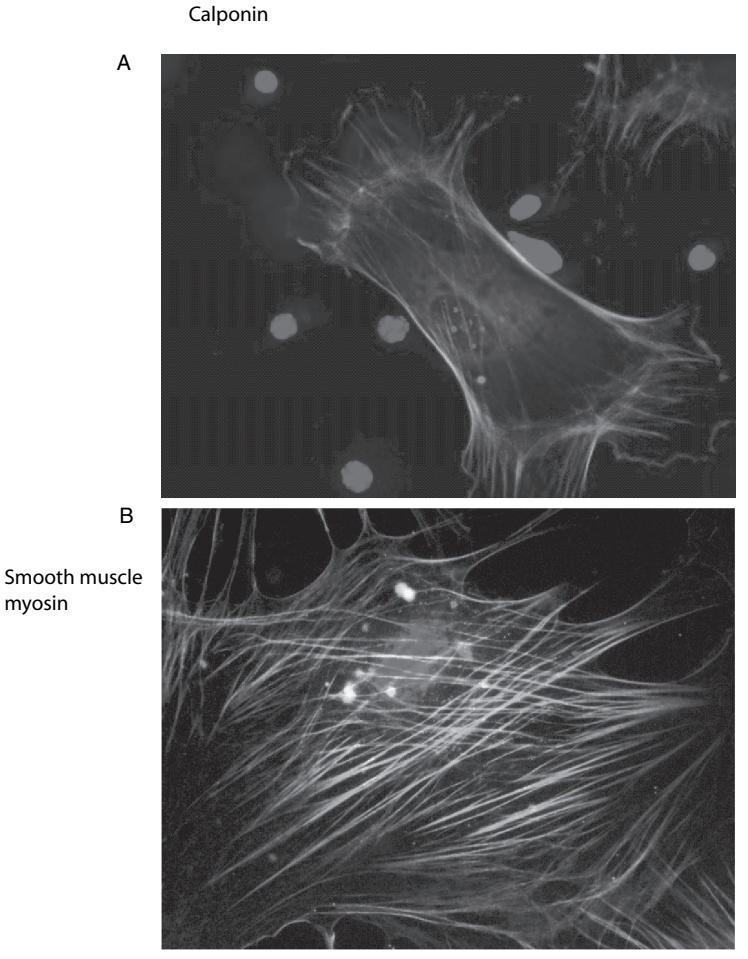


Figure 9.12. Transformation of CD34-positive cells derived from the mouse bone marrow to calponin- and smooth muscle myosin heavy chain-cells in vitro.

muscle α-actin$^+$ cells seldom express CD11b, whereas the smooth muscle α-actin$^-$ express CD11b. Both smooth muscle α-actin$^+$ and smooth muscle α-actin$^-$ cells express CD45. The smooth muscle α-actin$^+$ cells can form smooth muscle-like cells in elastic lamina-containing matrix.

Bone marrow-derived stem cells have been used to regenerate nonhematopoietic cells, including neurons, cardiomyocytes, endothelial cells, vascular smooth muscle cells, and hepatocytes. One of the major discoveries in recent studies is the transformation of hematopoietic stem cells to *hepatocytes* in an injured or transplanted liver in the mouse, rat, and human. The hematopoietic stem cells are originated from the mesoderm, whereas the hepatocytes are formed from the endoderm. The formation of hepatocytes from the hematopoietic stem cells suggests that adult stem cells can transdifferentiate across lineage barriers. Often, regional injury or disorder is necessary for the induction of stem cell transformation into a specified cell type. For instance, in a transgenic mouse model lacking

the fumarylacetoacetate hydrolase, the animal experiences progressive liver injury, dysfunction, and failure due to the accumulation of tyrosine metabolites. This injury demands and stimulates the regeneration of liver cells. When allogenic $Lin^{-}c\text{-}kit^{+}Sca\text{-}1^{+}Thy1^{+}$ hematopoietic stem cells are transplanted into myeloablated mice with liver injury, the transplanted cells can differentiate into functional hepatocytes and rescue the injured liver. The hematopoietic stem cells possess a high capacity of cell regeneration. A number of ~50 $Lin^{-}c\text{-}kit^{+}Sca\text{-}1^{+}Thy1^{+}$ hematopoietic stem cells, when transplanted into a mouse, can repopulate the hepatocyte family that is necessary for the maintenance of the liver function.

The hematopoietic stem cells can be induced to regenerate cardiomyocytes in experimental cardiac injury and in human cardiac infarction. In an experimental model, $CD34^{low}c\text{-}Kit^{+}Sca\text{-}1^{+}$ bone marrow cells, which are also known as *side population cells* (so defined because these cells are found outside the main population of bone marrow cells in a flow cytometry test), can be selected and transplanted into the heart of myeloablated mice with cardiac infarction induced by coronary arterial ligation. Following cardiac injury, the transplanted bone marrow cells can engraft to the injured cardiac tissue and regenerate cardiomyocytes and vascular endothelial cells. The newly regenerated cardiomyocytes can partially compensate for the lost cardiac function, and the regenerated endothelial cells can initiate angiogenesis, a process that improves blood circulation in the injured area and facilitates the recovery from cardiac ischemia. Furthermore, the regenerated cells can produce and release various cytokines and growth factors that initiate and enhance cardiac repair. Bone marrow-derived stem cells can also be injected directly into the injury sites of the animal and human heart, ensuring a focused delivery of therapeutic cells.

Bone marrow-derived stem cells can be used to regenerate nervous cells. Bone marrow cells originate from the mesoderm, whereas the nervous system is from the ectoderm. The regeneration of ectodermal cells from mesodermal stem cells indicates that adult stem cells can differentiate into specified cells across developmental barriers, a phenomenon previously considered impossible. This discovery provides fundamental information for the clinical application of adult stem cells to the treatment of degenerative disorders in the nervous system, which possesses a relatively low capacity of regeneration. Several investigations have demonstrated that donor bone marrow cells, when injected into the central venous system of myeloablated recipient animals, can form cells that express neuronal protein markers such as NeuN and class 3b-tubulin in the brain. These observations suggest that adult bone marrow stem cells can be potentially used to treat degenerative neural disorders such as Alzheimer's and Parkinson's diseases. Bone marrow cells can also differentiate into glial cells. When bone marrow cells are delivered to a demyelinated spinal cord, these cells can transform into oligodendrocytes that express myelin protein and induce remyelination. Bone marrow cells have been used to treat spinal cord injury, resulting in functional improvement of the injured spinal cord. However, several aspects remain to be determined. First, the morphology of the neural cells derived from bone marrow cells has not been thoroughly studied: it remains poorly understood whether bone marrow-derived cells can form axon. Second, the function of bone marrow-derived cells has not been systematically characterized. It is not clear whether bone marrow cells can develop into fully functional neurons or glial cells.

Hematopoietic stem cells have also been used for the treatment of cancer in humans following radiation-induced myeloablation. For instance, $CD34^{+}Thy\text{-}1^{+}$ autologous hematopoietic stem cells can be collected from the bone marrow and delivered to the vascular

system of patients with breast cancer and multiple myeloma. Such a treatment was effective in about 41% cancer patients. The minimal dose required to achieve an effective treatment (judged with respect to absolute neutrophil count) is $2–4 \times 10^5$ cells. These studies have demonstrated the potential of using hematopoietic stem cells for the treatment of cancers.

The pluripotent features of the hematopoietic stem cells have been described by using two models: the plasticity and heterogeneity models. The *plasticity model* suggests that there exist one or more types of pluripotent hematopoietic stem cells. These cells can cross the mesodermal lineage barrier to transdifferentiate (note that transdifferentiation is the differentiation of a stem or progenitor cell across lineage barriers) into ectodermal and endodermal cells under appropriate local conditions. These cells either possess features of the embryonic stem cells or can dedifferentiate (note that dedifferentiation is a process by which a cell changes from a mature differentiation state to a more primitive differentiation state so that the cell regains stem cell features and can differentiate into different cell types) and return to a more primitive state of specification and differentiation. In contrast, the *heterogeneity model* suggests that in the bone marrow there exist different types of stem cells, including not only the hematopoietic stem cells but also other types of committed stem cells that can develop into specified cells within a system. For instance, the bone marrow stromal cell population may contain mesenchymal stem cells that can differentiate into vascular endothelial and smooth muscle cells. Even within the hematopoietic stem cell population, there may be different subpopulations that are committed to the differentiation into specified cell types. It is important to point out that the two models are hypothetical in nature. Further investigations are necessary to test the hypotheses proposed in these models and explore cell surface markers that specify unique types of stem cells.

While bone marrow stem cells can maintain their capabilities of self-replication and differentiation throughout their lifespans in vivo, in vitro culture and expansion usually diminish these capabilities. For instance, the proliferation rate of bone marrow stem cells is reduced significantly after the first confluence in culture. The formation of specialized mesenchymal cells from in vitro expanded bone marrow stromal cells is reduced compared to freshly harvested bone marrow stromal cells. In fact, the differentiation ability of bone marrow stem cells is gradually lost when the cells are cultured for a number of passages. Such phenotypic changes are possibly due to the lack of physiological environment and necessary mediators in culture. These features should be taken into account when bone marrow stem cells are used for cellular therapies.

In vitro culture and expansion of bone marrow stem cells require the presence of growth factors, such as fibroblast growth factor, epidermal growth factor, and platelet-derived growth factor. The capability of cell proliferation is impaired in the absence of growth factors. Among the common growth factors, fibroblast growth factor 2 has been shown to effectively enhance the proliferation of bone marrow stem cells and maintains their immature stem cell phenotypes. Thus, appropriate growth factors should be used for in vitro culture and expansion of bone marrow stem cells.

In summary, bone marrow-derived stem cells can be used to regenerate a variety of cell types originally derived from the ectoderm (e.g., epidermal cells and neurons), mesoderm (e.g., skeletal muscle and kidney cells), and endoderm (e.g., pulmonary, gastrointestinal, and pancreatic cells). During the past decade, extensive investigations have been conducted to study the differentiation capacity of the adult stem cells. These investigations have established a foundation for understanding the mechanisms of adult stem cell

differentiation and applying adult stem cells to the treatment of human degenerative disorders.

Neural Stem Cells [9.3]. The brain and the spinal cord have long been considered a system containing only terminally differentiated cells that cannot differentiate and regenerate in the event of cell loss. However, recent studies have demonstrated that the adult nerve system contains neural progenitor and stem cells, which can proliferate and differentiate into neurons and glial cells in response to nerve injury and cell death. This discovery challenges the previous terminal differentiation theory and suggests possibilities for the establishment of stem cell-based therapies for degenerative nerve disorders.

In the adult brain, neurogenesis has been found since the 1960s in two major regions: the olfactory bulb and the hippocampal dentate gyrus. The olfactory bulb is the bulb-like region of the olfactory tract where the olfactory nerves enter (Fig. 9.6). It is located on the undersurface of the frontal lobe of each cerebral hemisphere. The hippocampal dentate gyrus is an archicortex that develops along the edge of the hippocampal fissure. These areas demonstrate cell division activities in the adult brain as detected by autoradiography. However, because of difficulties in identifying newborn neurons during the 1960s, these discoveries were not well accepted. The phenomenon of neurogenesis was not widely recognized until neurons were generated in vitro from adult rodent brain in 1992. The generation of neuronal cells has led to extensive investigations on neurogenesis during the past decade.

It is well known now that several regions in the adult brain contain neural stem and progenitor cells. These include the caudal portion of the subventricular zone, olfactory bulb, hippocampus, striatum, optic nerve, corpus callosum, spinal cord, cortex, retina, and hypothalamus. In vitro investigations have shown that cells collected from these regions can be induced to differentiate to neurons, astrocytes, and oligodendrocytes. Stem and progenitor cells can be identified from these regions based on immunocytochemical markers on the cell surface. For instance, the NeuN protein has been used as an identification marker for neurons, and GFAP and S100β are markers for glial cells. Neural stem cells can be confirmed by the presence of common stem cell features, such as capability of self-renewal, expansion, and differentiation into specified mature cells. Cell renewal and expansion can be tested by using the BrdU incorporation assay, designed on the basis of the fact that BrdU can be taken up only by dividing cells. As for other tissues, neural stem and progenitor cells can be expanded in vitro and transplanted into a target region in the brain to regenerate injured and lost cells in degenerative nerve disorders.

There are several features for neural stem and progenitor cells in culture. First, these cells can adhere to the base of culture dishes. The proliferation and differentiation of neural stem and progenitor cells can be modulated by altering the substrate of cell culture. Second, neural stem and progenitor cells can form aggregates in cell suspension. Such a property has been used as a criterion for identifying neural stem and progenitor cells. The culture of neural stem and progenitor cells requires the presence of growth factors. In particular, epidermal growth factor and fibroblast growth factor 2 play a critical role in regulating the survival and differentiation of neural stem and progenitor cells in vitro. The lack of these growth factors may significantly influence the expansion and differentiation of neural stem and progenitor cells. These growth factors have also been shown to participate in the regulation of neurogenesis in vivo.

Several issues remain to be clarified in neurogenesis. One of the issues is that the neural stem and progenitor cells have not been characterized in terms of structure and function.

These cells may be highly heterogeneous in function and capacity of regeneration. The formation of functional adult neurons from the adult stem or progenitor cells may be dependent on specific locations and environment. Furthermore, while the in vitro formation of neural cells provides useful information for neurogenesis, the mechanisms and the final products of cell transformation in vitro may be different from those in vivo. It is necessary to establish in vivo experimental models for the investigation of neurogenesis.

Other Adult Stem Cells [9.4]. There are other types of stem and progenitor cells in mammalian tissues and organs, including stem cells from the epidermal tissue (basal cells), intestinal tissue (crypt epithelial cells), connective tissue (adipocytes), and skeletal muscle (myoblasts). These cells can be identified and characterized by using methods and criteria described for the bone marrow and neural stem and progenitor cells. Recent studies have demonstrated that stem cells from these systems can differentiate into specified mature cells within the system and can also cross the developmental barriers to form specified cells for other systems. It seems that most systems in a mature adult body contain stem and progenitor cells. These cells can regenerate specified cells in the event of cell loss due to injury and pathological disorders. Here, the epidermal and intestinal stem cells are used as examples for discussion.

There exist several types of stem and progenitor cells in the epidermal tissue. These include epidermal stem cells, follicle multipotent stem cells, melanocyte stem cells, dermal sheath stem cells, neural crest stem cells, and endothelial stem cells (Fig. 9.7). The epidermal stem cells are found in the basal layer of the epidermis and are also known as *basal cells*. These cells can primarily differentiate to epidermal cells. The follicle multipotent stem cells are found in the hair follicle bulge region and can differentiate to hair follicle epithelial cells, sebaceous gland cells, and epidermal cells. The melanocyte stem cells are also found in the hair follicle bulge and can transform into melanocytes. The dermal sheath stem cells are found in the hair follicle dermal sheath and can differentiate to dermal papilla cells and wound healing fibroblasts. The neural crest stem cells are present in the hair follicle dermal papillae and can transform into neural cells and mesenchymal cells. The endothelial stem cells are present in the dermal tissue and can differentiate to vascular endothelial cells. The dermis underneath the epidermal layer also contains stem cells. These cells can differentiate various cell types including smooth muscle-like cells and adipocytes under appropriate culture conditions (Fig. 9.8).

The intestinal system contains epidermal stem and progenitor cells. The epithelial stem cells are found mostly in the crypt region of the intestine (Fig. 9.9). These cells can differentiate to intestinal epithelial cells in response to epithelial injury. Compared to stem cell types from other organs, the epidermal and intestinal stem cells can be easily accessed and collected. These stem cells can be used as candidate cells for regenerative therapies.

REGENERATION OF ADULT TISSUES AND ORGANS

Cell regeneration occurs in selected adult tissues and organs of certain species. Typical examples include the limbs of salamander and crayfish as well as the liver of mammals. Salamander and crayfish can regrow their limbs after amputation. The regenerated limbs are identical to the original limbs in morphology and are fully functional. In mammals, the liver is the only organ that can regenerate completely after partial hepatectomy or liver

removal. The regenerated hepatic tissue possesses natural structure and physiological function. Based on the principles of developmental biology, these organs can reactivate their developmental processes that take place during the embryonic stage and thus regenerate missing or damaged tissues.

There are two general mechanisms for the regeneration of adult tissues and organs in salamander and mammals: epimorphogenesis and compensatory regeneration. *Epimorphogenesis* is a process by which undifferentiated cells (found in the embryonic stage) are reestablished from adult cells via dedifferentiation. The undifferentiated cells can be respecified into adult cells. This is a mechanism for the regeneration of the salamander and crayfish limbs. *Compensatory regeneration* is a process by which existing cells reproduce themselves via division. These cells are not able to transform themselves to undifferentiated cells. Liver regeneration is an example of compensatory regeneration. The investigation of these regenerative processes can provide essential information for understanding the underlining mechanisms of regeneration, for controlling the regenerative processes, and for enhancing the restoration of impaired tissues and organs. Here, the salamander limbs and mammalian liver are used as examples to demonstrate the principle of adult organ regeneration.

Regeneration of Salamander Limbs [9.5]

Salamander is a tailed amphibian of the order Caudata. It has long been observed that, when a limb of the salamander is severed, the remaining part of the limb can grow back to the original form. The limb system can precisely control the growth process. The growth is ceased when a complete limb is reconstructed. It has been a mystery how exactly a salamander reconstructs a severed limb and how it controls the morphology and distribution of different cells and tissues. During the past several decades, extensive investigations have been conducted on the regenerative mechanisms of the salamander limbs. These investigations have demonstrated a series of biological processes that are involved in the regeneration of the amputated limb.

Following limb amputation, the first step of repair is the formation of blood clots, which seal damaged blood vessels and prevent bleeding. The second noticeable step is the migration of epidermal cells, together with proliferation, from the remaining limb to the wound area. The newly generated epidermal cells form wound epidermis that covers the area of damage. A unique feature for the regenerative response of the salamander limb is the lack of scar formation. There are few fibroblasts and little fibrotic connective tissue in the layer of wound epidermis. Such a feature is critical to the reconstruction of the severed limb. The third step is the formation of the *regeneration blastema* underneath the wound epidermis. Several mesodermal cell types, including osteoblasts, chondrocytes, muscular cells, and fibroblasts, undergo dedifferentiation and regain the phenotypes of embryonic stem cells, capable of transforming to the cell types and producing extracellular matrix components necessary for limb regeneration. An example of muscular cell regeneration is shown in Fig. 9.13. The dedifferentiated mesodermal cells are clustered beneath the wound epidermis, forming a structure known as the regeneration blastema. This structure is the basis for the regeneration of a new limb.

Next step is the proliferation of cells in the regeneration blastema and the formation of a primary structure for the limb. Several growth factors, including fibroblast growth factor and glial growth factor, play an important role for cell proliferation. To form a limb, the regenerated cells must be organized into the right pattern with each cell type deployed to

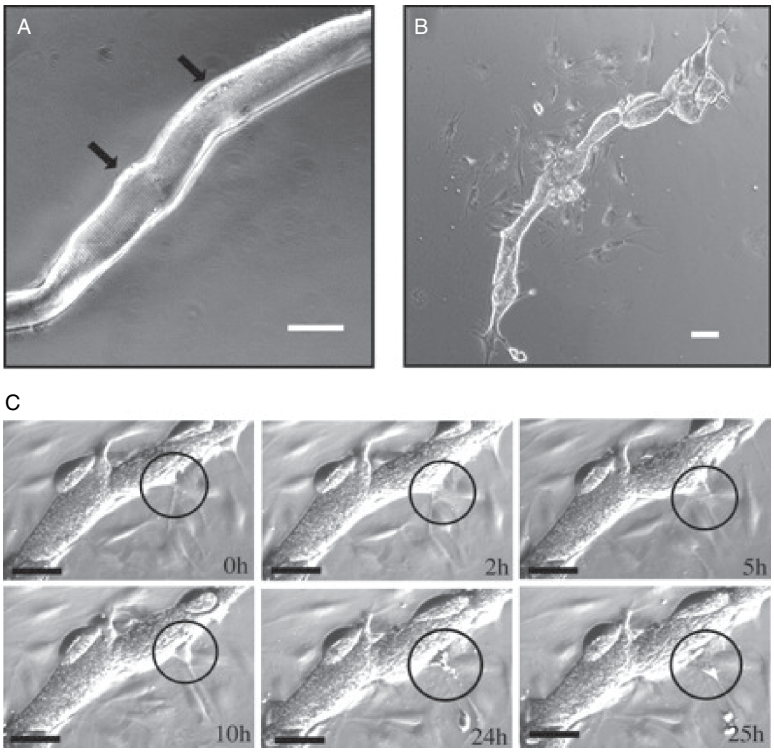


Figure 9.13. Budding of progeny cells from newt skeletal muscle cells. (A) Photomicrograph showing a freshly isolated single newt skeletal muscle cell. Arrows point to two visible nuclei. (B) Photomicrograph showing the same newt skeletal muscle cell at 15 days of culture. The myofiber morphology has changed and several lobular structures are seen while mononucleate progeny has been produced. (C) Time-lapse photomicrographs showing a sequence of a representative budding event, which leads to the derivation of a mononucleate cell. Note the protrusion of the myofiber in the circled area, which is concomitant with the appearance of a mononucleate progeny. Timepoints indicate the duration of the one specific budding event. Scale bars: 50 μm. (Reprinted from Morrison JI et al: *J Cell Biol* 172:433–40, 2006, copyright by permission of the Rockefeller University Press.)

the right location. Increasing evidence suggests that injury-induced limb regeneration resembles the process of embryonic limb formation. Molecules that regulate limb development during the embryonic stage, such as retinoic acid, sonic hedgehog, HoxA, and HoxD, can also be found in a regenerating limb in salamanders. These molecules participate in the regulation of the pattern formation of a regenerated limb. In particular, retinoic acid, produced by wound epidermal cells, is present with graded concentrations in the proximal–distal direction of the blastema. The concentration gradient of retinoic acid induces location-dependent activation of the HoxA gene. HoxA regulates the pattern formation of regenerated limb cells. Within about 2–3 months after limb amputation, a complete new limb can be regenerated with full function (Fig. 9.14).

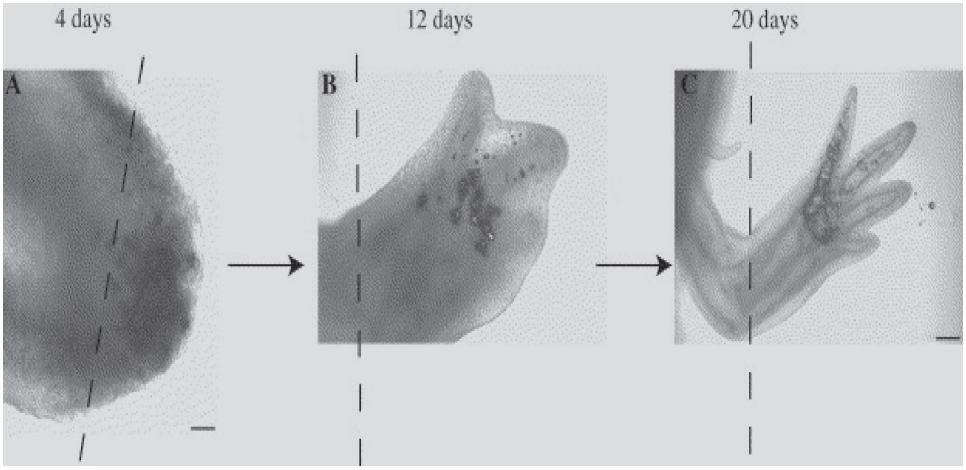


Figure 9.14. Salamander limb regeneration. (A) The blastema of a salamander limb at day 4 after amputation. The blastema was transfected with the CMV-DsRed fluorescent protein gene via the mediation of electroporation. The dark marker indicates the expression of the transfected gene. (B) By 12 days postamputation, the marked distal cells divided and remained in the most distal part. (C) At 20 days postamputation, the limb regenerated, and the marked cells contribute to the formation of the digits. The dashed line marks the plane of amputation. Scale bars: A,B = 100 μm; C = 500 μm. (Reprinted from Echeverri K, Tanaka EM: *Dev Biol* 279:391–401, copyright 2005 with permission from Elsevier.)

Regeneration of the Mammalian Liver [9.6]

The liver is an organ that can fully regenerate after partial hepatectomy or injury in mammals. Under physiological conditions, the liver is a quiescent organ with a turnover rate <0.01%. In response to injury, the liver can activate its mitotic mechanisms, initiating rapid cell proliferation and regenerating functional liver cells. A fundamental experiment that demonstrates liver regeneration is liver resection or partial hepatectomy. Such a procedure stimulates rapid liver regeneration (Fig. 9.15). Following partial hepatectomy (up to 60% of the liver size), the remaining liver can grow back to the original size within 10 days in the mouse and rat! Cell proliferation and liver regeneration cease once the liver returns to its original size. Few other cell types in mammals can grow or regenerate with such a rate.

Further experimental investigations have shown that liver regeneration is a precisely controlled process, depending on the ratio of the liver mass to the entire body mass. This phenomenon is demonstrated in several experimental models, including the parabiotic animal model and liver transplantation. In the parabiotic animal model, in which the circulatory systems of two animals are anastomosed, liver removal from one animal induces rapid liver regeneration of the liver of the other animal. The regenerative process continues until the liver mass doubles and reaches the original liver : body mass ratio. In contrast, the separation of the two animals induces rapid hepatocyte apoptosis and liver shrinkage in the animal survived (note that the animal without liver will die). Liver shrinkage continues until the liver mass returns to the original size of the individual animal. In the model of liver transplantation, a liver transplant derived from a smaller donor animal or

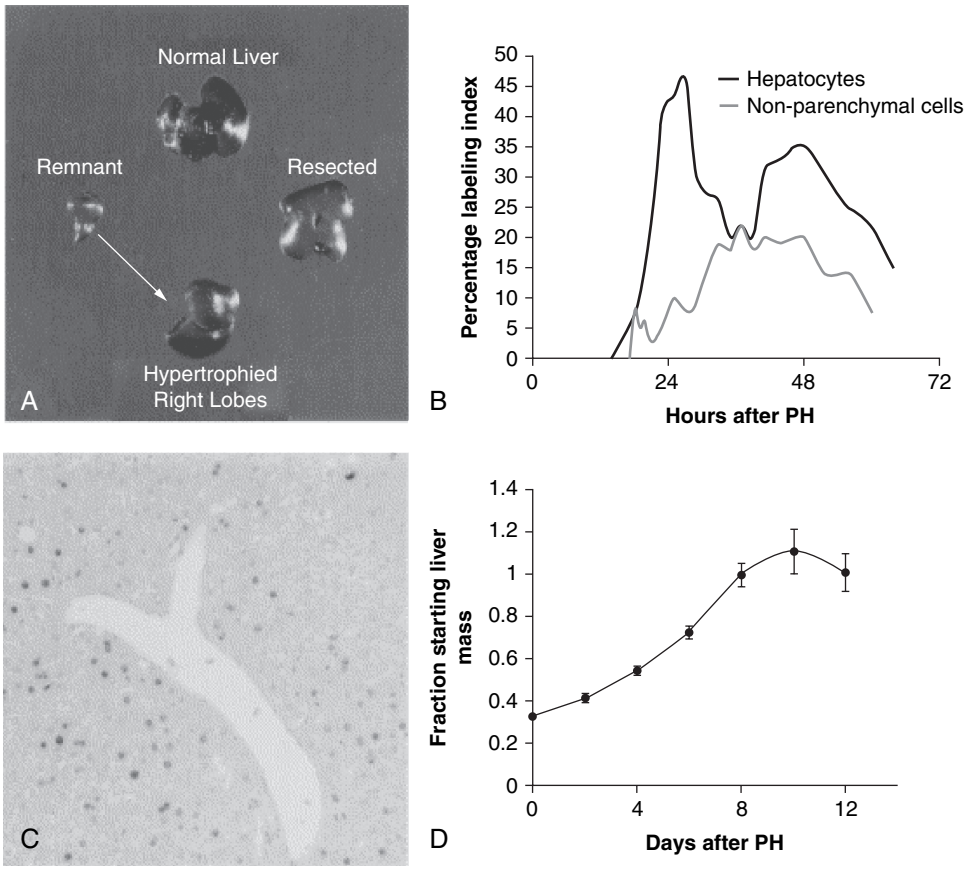


Figure 9.15. Liver regeneration after two-thirds partial hepatectomy (PH). (A) Mouse livers before and after partial hepatectomy and hypertrophied remnant 6 days after partial hepatectomy. (B) Percentage of ^{3}H-thymidine-labeled liver cells at timepoints after partial hepatectomy. (C) BrdU incorporation into proliferating rat hepatocytes after carbon tetrachloride treatment. (D) Time course of liver regeneration by mass after partial hepatectomy. (Reprinted from Koniaris LG et al: Liver regeneration, *J Am Coll Surg* 197:634–59, copyright 2003 by permission of the American College of Surgeons.)

human compared to the host will grow until reaching the size of the original liver of the larger host. In contrast, a liver transplant from a larger donor will shrink until reaching the size of the original liver of the smaller host. These investigations demonstrate that the mammalian liver can regenerate precisely in response to degree of liver loss and liver regeneration is a metabolism-controlled process. The investigations of liver regeneration have provided insights into the understanding of organ regeneration and the development of therapeutic approaches for degenerative diseases.

Biological Processes of Liver Regeneration [9.6]. There are a series of regenerative processes following partial hepatectomy. One of earliest known processes is the activation of mitogenic factors, such as hepatocyte growth factor (HGF) and interleukin (IL)6. Hepatocyte growth factor can be activated within the first hour following partial

hepatectomy and plays a critical role in the initiation and regulation of hepatocyte proliferation and liver regeneration. Under the stimulation of mitogenic factors, quiescent hepatocytes are activated to enter the cell division cycle, initiating DNA synthesis and cell division. DNA synthesis can be observed within 1–2 days following partial hepatectomy in small animals such as mice and rats. In larger animals, such as dogs and primates, DNA synthesis is often observed within 3–10 days. The DNA synthesis stage is followed with rapid cell proliferation, as detected by nucleotide incorporation assays. The rate of cell proliferation is usually proportional to the degree of liver resection. Cell proliferation slows down and ceases at about 10 days in mouse and rat hepatectomy models when the liver grows back to its original size.

Features of Liver Regeneration [9.6]. There are several features for liver regeneration:

1. Each liver cell type exhibits a distinct rate of regeneration. The hepatocytes can respond rapidly (within about 1 h) to stimulation induced by liver hepatectomy and has the highest rate of proliferation compared to other liver cell types. However, the duration of hepatocyte regeneration (about 2 days) is shorter than that of other cell types. The liver endothelial cells respond more slowly to stimulation (within about 2 h), but have a longer duration of proliferation (about 10 days) compared to the hepatocytes. The bile duct epithelial, Küpffer, and Ito cells fall in a range between the hepatocytes and endothelial cells in both rate and duration of proliferation (Fig. 9.16). Hepatocyte regeneration is dependent on the animal species. Large animals such as dogs, pigs, and primates usually exhibit delayed activation of cell prolifera-

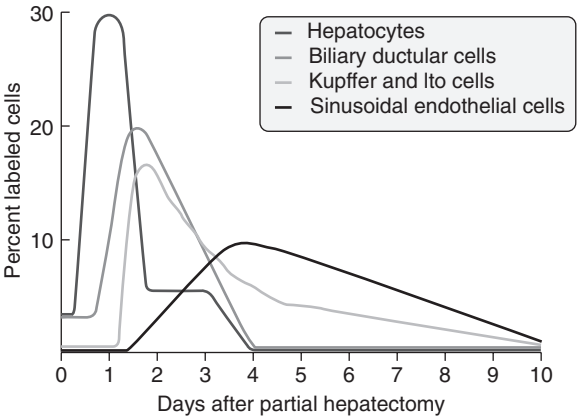


Figure 9.16. Time course of the proliferation of different liver cell types during liver regeneration after partial hepatectomy. The four major types of liver cells undergo DNA synthesis at different times. Hepatocyte proliferate peaks at 24 h, whereas the other cell types proliferate later. Regenerating hepatocytes produce growth factors that can function as mitogens for these cells. This has suggested that hepatocytes stimulate proliferation of the other cells by a paracrine mechanism. The figure was generated by graphic adaptation of the data presented in two publications (Grisham JW: *Cancer Res* 22:842, 1962; Widmann JJ, Fahimi HD: in *Liver Regeneration after Experimental Injury*, Lesch R, Reutter W, eds, Lesch R, Reutter W, eds, Stratton Intercontinental Medical Book Corp., New York, 1975, pp 89–98). (Reprinted with permission from Michalopoulos GK, DeFrances MC: Liver regeneration, *Science* 276:60–6, copyright 1997 AAAS.)

tion compared to small animals such as rats and mice. These observations suggest that liver cells contribute differently to liver regeneration following partial hepatectomy.

2. The rate of liver regeneration is proportional to the amount of liver removed. The larger is the removed portion, the faster is the rate of liver regeneration. Even a small resection (<10%) is followed by liver restoration to its full size.
3. Liver regeneration ceaseds when the injured liver grows back to its original size, suggesting that the functional demand for metabolism is a factor that initiates and controls the process of liver regeneration.
4. The liver possesses a large capacity of regeneration. A single rat hepatocyte can undergo at least 34 division cycles, resulting a clone of about 1.7×10^{10} cells (note that a normal rat liver has about 3×10^8 cells). A rat liver can regenerate to full size after 12 sequential operations of partial hepatectomy.
5. Liver cells undergo a clonogenic growth process. Each cell can proliferate to form a cell clone, according to which a liver nodule develops.
6. Mature hepatocytes are not terminally differentiated cells and can differentiate to different types of liver cells.

Liver regeneration is a compensatory process that is dependent not on the differentiation of stem cells, but on the proliferation and differentiation of liver cells, including hepatocytes, biliary epithelial cells, fenestrated endothelial cells, Küpffer cells, and Ito cells. All these cells can thus be considered liver progenitor cells. Among these cell types, the hepatocytes are the majority of liver cells and are responsible for basic liver functions, including metabolic processing of carbohydrates, fats, and proteins, degradation of toxic compounds, production of necessary proteins (e.g., albumin), and secretion of bile. The biliary epithelial cells line the internal surface of the bile ducts and participate in the transport process of biliary molecules. The fenestrated endothelial cells are found at the internal surface of the hepatic capillaries and the hepatic sinusoids and are responsible for molecular transport across blood vessels. The Küpffer cells are hepatic macrophages found in the hepatic sinusoids and are responsible for the destruction and clearance of bacteria and cell debris. The Ito cells can synthesize extracellular matrix components. In the case of liver injury or hepatectomy, all these cell types are stimulated to proliferate, differentiate, and participate in liver regeneration.

Experimental Models of Liver Regeneration [9.7]. Liver regeneration has been studied by using experimental models. Rats and mice are often used for such models. There are three common types of experimental models for liver regeneration: chemical ingestion, ischemia, and partial hepatectomy or liver resection. Chemical ingestion can induce cell injury and death. Common chemicals used for livery injury include alcohol, carbon tetrachloride, and D-galactosamine. These chemicals may target different regions of the liver. For instance, alcohol primarily induces hepatocyte injury near the portal veins, D-galactosamine induces overall hepatocyte injury, whereas carbon tetrachloride causes central hepatocyte injury. These models are often used to simulate toxin-induced human liver injury and failure. Liver ischemia can be induced by temporary occlusion of arteries. The ischemic area can be controlled by selectively occluding different generations of hepatic arteries. This model can be used to simulate human liver injury due to atherosclerosis and embolism.

Among the three types of liver injury models, partial hepatectomy is most commonly used in experimental investigation of liver regeneration. Selected liver lobes can be ligated and excised at the lobe base. Compared to the other types of liver injury, hepatectomy reduces the liver size, but does not induce significant hepatocyte injury or death. Thus, this model can be used to study liver regeneration with respect to mechanisms of physiological adaptation. The rate of liver regeneration is usually dependent on the relative resection size. The maximal size of resection without influencing the metabolism of the body is about 60% of the original liver size. Larger resection induces metabolic disorders and acute liver failure. In this chapter, partial hepatectomy is used as an example to demonstrate the processes and mechanisms of liver regeneration.

Regulation of Liver Regeneration [9.8]. Liver regeneration is initiated and regulated by growth regulatory factors produced in the liver as well as other organs. These factors include hepatocyte growth factor (HGF), interleukin (IL)6, epidermal growth factor (EGF), insulin, Wnts, transforming growth factor (TGF)β, and activin. Among these factors, hepatocyte growth factor, interleukin-6, epidermal growth factor, insulin, and Wnts exert mostly a stimulatory effect on liver regeneration, whereas transforming growth factor-β and activin serve as inhibitory factors for liver regeneration (Fig. 9.17). Both growth stimulatory and growth inhibitory factors are upregulated following partial hepatectomy with a time-dependent manner. The early dominant expression of growth stimulatory factors promotes hepatocyte proliferation and liver regeneration, whereas the following expression of growth inhibitory factors contributes to the cessation of liver regeneration when the liver mass returns to the original level. Thus, the control of the activities of these growth regulatory factors is critical to the regulation of liver regeneration. The redundancy of the growth regulatory factors is a mechanism that ensures effective, synergistic control of liver regeneration in response to injury. The role of these factors in liver regeneration is briefly discussed in this section.

Hepatocyte growth factor is one of the most important factors that regulate liver regeneration. This growth factor is expressed in the liver cells. A major function of hepatocyte growth factor is to stimulate hepatocyte growth and regulate hepatocyte survival. This function has been confirmed in experiments in vitro and in vivo. Hepatocyte growth factor can interact with and activate the c-met protein tyrosine kinase receptor, which induces activation of the Ras—mitogen-activated protein kinase signaling pathway. When the hepatocyte growth factor gene is deleted or modulated in the embryo, hepatic development is arrested or reduced, leading to death of the embryo. The administration of hepatocyte growth factor to animals with liver injury stimulates liver regeneration. After hepatectomy, the blood level of hepatocyte growth factor can be increased by 20 folds within 1 h. Hepatocyte growth factor serves as a signal that initiates early proliferation of liver cells. Other growth factors, such as *epidermal growth factor* and *fibroblast growth factor*, also activate protein tyrosine kinase receptor-mediated signaling pathways. These growth factors have been shown to serve as potent stimulators for hepatocyte proliferation following partial hepatectomy.

Interleukin (IL)6 is a cytokine that induces inflammatory reactions. Interleukin-6 exerts diverse effects on target cells, such as growth-stimulatory effect and growth-inhibitory effect, depending on cell types. For hepatocytes, IL6 serves as a growth stimulatory factor (Fig. 9.18). Hepatocytes express interleukin-6 following partial hepatectomy. Interleukin-6 can interact with the IL6 receptor α chain, also known as *gp80*, in the hepatocyte membrane and induce dimerization with another IL6 receptor chain known as

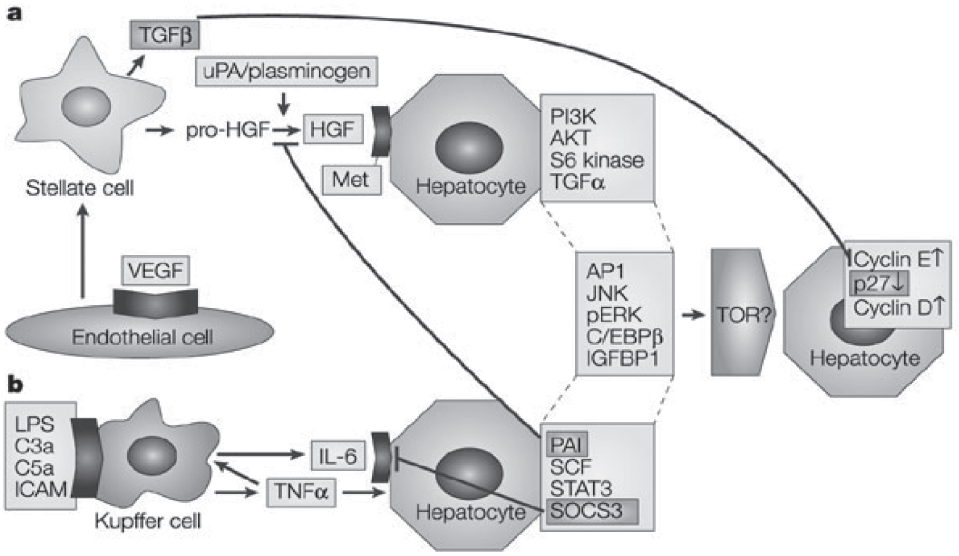


Figure 9.17. Growth factors and cytokines involved in liver regeneration. (a) Liver regeneration is regulated by several growth factors. Vascular endothelial growth factor (VEGF) binds to endothelial cells, which triggers the release of the hepatocyte growth factor (HGF) precursor, pro-HGF, from stellate cells. The urokinase-type plasminogen activator (uPA) and plasminogen proteases cleave pro-HGF, which releases HGF. HGF binds to the Met receptor on hepatocytes to activate the phosphatidylinositol 3-kinase (PI3K), AKT, and S6 kinase signal-transduction pathways. HGF signaling activates transforming growth factor (TGF)α and other downstream signals that are shared with the cytokine-mediated pathway, such as AP1, Jun *N*-terminal kinase (JNK), phosphorylated extracellular signal-regulated kinases (pERKs), CCAAT-enhancer-binding protein (C/EBP) β and insulin-like-growth-factor-binding protein (IGFBP)1. These factors are proposed to activate target of rapamycin (TOR), although this remains to be established, and this leads to cell cycle transition by increasing the expression of cyclins D and E and reducing p27. (b) Liver regeneration is also regulated by cytokines. Molecules factors, including lipopolysaccharide (LPS), complement factors C3a and C5a and intercellular adhesion molecules (ICAMs), can activate Kupffer cells, which produce tumor necrosis factor (TNF)α. TNFα in turn upregulates the expression of interleukin (IL)6 in Kupffer cells. Both TNFα and IL6 can activate the signal transducer and activator of transcription (STAT)3 and induce the expression of stem cell factor (SCF) and several proteins that are shared with the growth-factor-mediated pathway, resulting in hepatocyte activation and proliferation. During liver regeneration, various inhibitory proteins are also activated (shown in orange), including TGFβ (which is produced by stellate cells), plasminogen activator inhibitor (PAI), suppressor of cytokine signaling-3 (SOCS3) and p27 and other cyclin-dependent-kinase inhibitors. The effects of these inhibitors on liver regeneration are shown. (Reprinted by permission from Macmillan Publishers Ltd.: Taub R: *Nature Rev Mol Cell Biol* 5:836–47, copyright 2004.)

gp130. These activities further induce activation of the signaling cascade involving the Janus tyrosine kinase (JAK) and signal transducers and activators of transduction (STAT), resulting in hepatocyte proliferation. In mice with defect interleukin-6, liver regeneration is significantly impaired following partial hepatectomy. In transgenic mice with upregulated IL6, enhanced hepatocyte proliferation has been observed. These observations that IL6 serves as a potent stimulator for liver regeneration following liver injury.

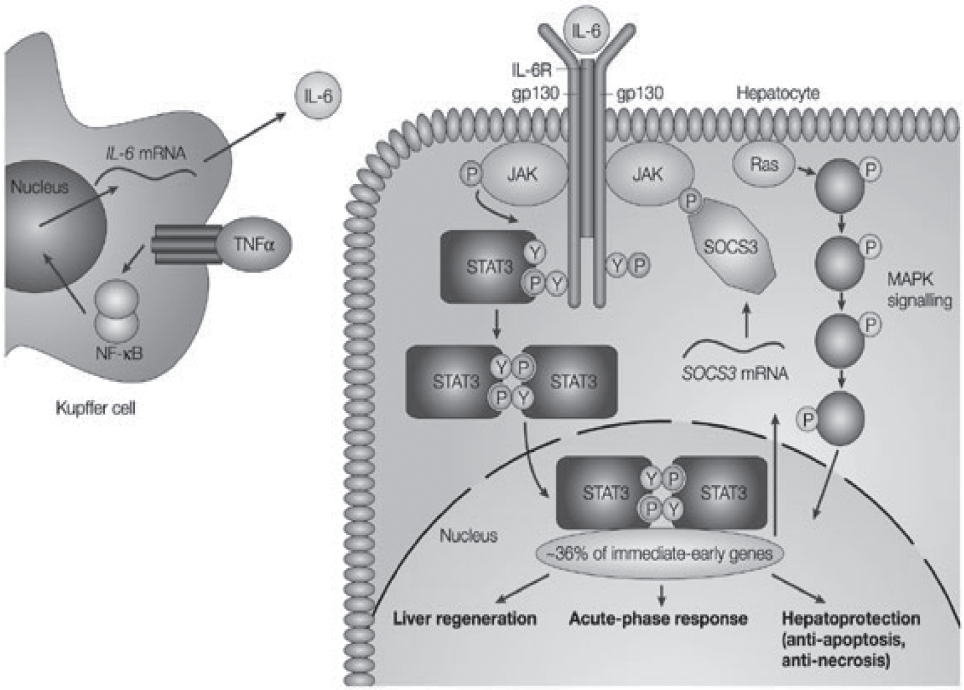


Figure 9.18. Role of the interleukin-6–STAT3-signaling pathway in regulating liver regeneration. Tumor necrosis factor (TNF)α binds to its receptor on Kupffer cells, resulting in the upregulation of interleukin-6 (IL6) transcription by the nuclear factor (NF)κB pathway. IL6 binds to the IL6 receptor (IL6R) on hepatocytes. The IL6 receptor interacts with two subunits of gp130, and activates Janus kinase (JAK). Activated JAK triggers the activation of two pathways: (1) the mitogen-activated protein kinase (MAPK) pathway, which is activated by SHP2–GRB2-SOS–Ras signal transduction (where SHP2 is SH2-domain-containing protein tyrosine phosphatase-2; GRB2 is growth-factor-receptor-bound protein-2; and SOS is "son of sevenless"); and (2) the signal transducer and activator of transcription (STAT)3 pathway, which is activated through JAK-mediated tyrosine (Y) phosphorylation. The STAT3 transcription factor dimerizes and translocates to the nucleus, where it activates transcription of ~36% of immediate–early target genes. In the liver, this process promotes liver regeneration, the acute-phase response, and hepatoprotection against Fas and toxic damage. Suppressor of cytokine signalling (SOCS)3 transcription is also regulated by IL6 signaling. SOCS3 interacts with JAK and blocks cytokine signaling. P, phosphate. (Reprinted by permission from Macmillan Publishers Ltd.: Taub R: *Nature Rev Mol Cell Biol* 5:836–47, copyright 2004.)

In addition, other biochemical factors, such as chemokines (transforming growth factor α, interferon-inducible protein 10, and macrophage inflammatory protein 1α), Wnts, norepinephrine, insulin, prostaglandins (prostaglandin E2, prostacyclin, and thromboxane), and steroid hormones (estradiol), are involved in the regulation of liver regeneration in response to liver injury. These factors, together with hepatocyte growth factor, epidermal growth factor, fibroblast growth factor, and IL6, control the initiation and progression of liver regeneration. Liver injury and hepatectomy can also activate matrix metalloproteinases. These enzymes can digest extracellular matrix, enhancing the proliferation and migration of hepatic cells.

Soluble biochemical factors may not only control the initiation, but also the cessation of liver regeneration. There are several factors, including *transforming growth factor* (*TGF*)β and *activin*, which serve as inhibitory factors for liver regeneration and may play a critical role in regulating the termination of liver regeneration. Transforming growth factor β is a member of the transforming growth factor β superfamily, which contains more than 30 members. This factor is upregulated in response to liver injury. Its expression is associated with suppression of hepatocyte proliferation in liver injury. Administration of exogenous transforming growth factor β to mice induces a significant reduction in liver regeneration following partial hepatectomy. In contrast, administration of inhibitors for transforming growth factor β elicits an opposite effect. The inhibitory effect of transforming growth factor β has been observed in experiments in vitro as well as in vivo. Activin is also a member of the TGFβ superfamily. This factor exerts a potent inhibitory effect on hepatocyte proliferation following liver injury. The effect of activin can be suppressed by the application of follistatin, a ligand that binds activin, inhibits the interaction of activin with its receptor, and thus suppresses the activity of activin. These observations suggest that TGFβ and activin serve as negative regulators for the regulation of liver regeneration in response to liver injury. This is a critical mechanism for the cessation of hepatocyte proliferation and control of liver regeneration.

The processes and regulatory mechanisms of liver regeneration discussed above are for normal livers or injured livers with regenerative capability. When a liver is composed of mostly damaged hepatocytes in disorders, such as liver failure, chronic hepatitis, and liver cirrhosis, the majority of hepatocytes are no longer capable of regenerating in response to additional liver injury due to exposure to toxic chemicals and partial hepatectomy. In these cases, hepatocyte or liver transplantation is necessary to restore the liver function.

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