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Concise Review: Recent Advances on the Significance of Stem Cells in Tissue Regeneration and Cancer Therapies

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ABSTRACT

In this study, we report on recent advances on the functions of embryonic, fetal, and adult stem cell progenitors for tissue regeneration and cancer therapies. We describe new procedures for derivation and maturation of these stem cells into the tissue-specific cell progenitors. The localization of the adult stem cells and their niches, as well as their implication in the tissue repair after injuries and during cancer progression, are also described. The emphasis is on the interactions among certain developmental signaling factors, such as hormones, epidermal growth factor, hedgehog, Wnt/β-catenin, and Notch. These factors and their pathways are involved in the stringent regulation of the self-renewal and/or differentiation of adult stem cells. Novel strategies for the treatment of both diverse degenerating disorders, by cell replacement, and some metastatic cancer types, by molecular targeting multiple tumorigenic signaling elements in cancer progenitor cells, are also illustrated. STEM CELLS *2006;24:2319–2345*

INTRODUCTION

There is great interest in the biology of adult stem cells because of their capacity to self-renew and their high plasticity. These traits enable adult stem cells to produce diverse mature cell progenitors that actively participate in the maintenance of homeostatic processes by replenishing the cells that repopulate the tissues/organs during a lifespan and regenerate damaged tissues during injury [1–13]. In general, embryonic, fetal, and adult stem cells show several common functional properties. Common properties include their high self-renewal capacity and potential to generate differentiated cell progenitors of different lineages under simplified culture conditions in vitro and after transplantation in the host in vivo [11, 13–22]. More particularly, the establishment of the functional properties of stem cells and their progenitors in vitro and in vivo has indicated that they may contribute to the regeneration of damaged tissues. Therefore, the use of stem cells and their progenitors is a promising strategy in cellular and genetic therapies for multiple degenerative disorders, as well as adjuvant immunotherapy for diverse aggressive cancer types [11, 13, 16, 17, 19, 23–27]. Parkinson and Alzheimer diseases, muscular degenerative disorders, chronic liver and heart failures, and type 1 and 2 diabetes, as well as skin, eye, kidney, and hematopoietic disorders, could be treated by the stem cell-based therapies (Table 1).

Genetic alterations and/or sustained activation of distinct developmental mitogenic cascades occurring in a minority of adult stem cells and their progenitors might also lead, in certain cases, to their oncogenic transformation [5, 9, 11, 28–34]. This implicates the activation of numerous tumorigenic cascades that are mediated through distinct growth factor signaling pathways and that assume a critical role for the growth and survival of cancer cells. More specifically, the upregulation of hormones, epidermal growth factor (EGF), hedgehog, Wnt/β-catenin, and/or Notch pathways seems to represent a critical event that might be implicated in the initiation and development of some aggressive cancer types, such as acute leukemia and lymphoma, brain, skin, lung, esophagus, stomach, pancreas, liver, breast, ovarian, prostate, and testis cancers [5, 11, 17, 18, 29, 33, 35–38]. Molecular targeting of these oncogenic signaling elements, therefore, constitutes a promising approach for the development of novel combination therapies against these metastatic and incurable forms of cancer. In this review, we focus on the growth factor signaling cascades that might be implicated in the control of the self-renewal and differentiation of embryonic, fetal, umbilical cord, and adult stem cells and their progenitors

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Table 1. Therapeutic applications of embryonic, umbilical cord blood, and adult stem cell progenitors

Tissue source/stem cell type	Cell progenitors	Cell-based therapy (treated diseases)
Embryo		
ESCs	Myeloid and lymphoid cells, platelets	Hematopoietic disorders, leukemias
	Neurons, motor neurons	Nervous system disorders
	Dopaminergic neurons	Parkinson disease
	Astrocytes, oligodendrocytes	Myelin diseases
	Skeletal muscle cells	Muscular disorders
	Cardiomyocytes	Heart failures
	Osteoblasts, chondrocytes	Osteoporosis, OI, osteoarthritis
	Insulin-producing β-like cells	Diabetes mellitus
	Dendritic cells	Immune disorders
	Endothelial cells	Vascular system disorders
	Retinal neurons	Retinal diseases
Umbilical cord blood		
HSCs	Myeloid and lymphoid cells, platelets	Hematopoietic disorders, leukemias
	Hepatocytes	Liver disorders
MSCs	Myoblasts	Muscular disorders
Adult tissues		
Bone marrow		
MPCs or MAPCs	Osteoblasts, chondrocytes	Osteoporosis, OI, osteoarthritis
	Myoblasts	Muscular disorders
	Hepatocyte-like cells	Liver disorders
	Endothelial cells	Vascular system disorders
MSCs or BMSCs	Osteoblasts, chondrocytes	Osteoporosis, OI, osteoarthritis
	Myoblasts	Muscular disorders
	Neurons	Nervous system disorders
	Astrocytes, oligodendrocytes	Myelin disorders
	Cardiomyocytes	Heart failures
HSCs	Myeloid and lymphoid cells, platelets	Hematopoietic disorders, leukemias
	Hepatocytes	Liver disorders
	Neural cells	Nervous system disorders
Hepatic oval cells	Hepatocytes	Liver disorders
	Insulin-producing β-like cells	Diabetes mellitus
	Cardiomyocytes	Heart failures
Muscle-derived stem cells	Muscle cells	Muscular disorders
	Neural cells	Nervous system disorders
	Myeloid and lymphoid cells, platelets	Hematopoietic disorders, leukemias
	Endothelial cells	Vascular system disorders
Adipose stem cells	Osteoblasts, chondrocytes	Osteoporosis, OI, osteoarthritis
Neural stem cells	Neurons	Nervous system disorders,
	Astrocytes, oligodendrocytes	Myelin disorders
	Myeloid and lymphoid cells, platelets	Hematopoietic disorders, leukemias
Pancreatic stem cells	Insulin-producing β-like cells	Diabetes mellitus
Limbal stem cells	Corneal epithelial cells	Corneal diseases
CE retinal stem cells	Retinal cell progenitors	Retinal diseases

Abbreviations: BMSC, bone marrow stem cell; CE, corneal epithelial; ESC, embryonic stem cell; HSCs, hematopoietic stem cells; MAPC, multipotent adult progenitor cell; MPCs, mesodermal progenitor cells; MSCs, mesenchymal stem cells; OI, osteogenesis imperfecta.

in vitro and in vivo. Moreover, we report a description of the in vivo localization and biological functions of adult stem cells. We also describe recent advances in potential therapeutic applications of stem cell progenitors in regenerative medicine and new combination therapies for cancer treatment.

Stem Cell Types

Distinct stem cell types have been established from embryos and identified in the fetal tissues and umbilical cord blood (UCB), as well as in specific niches in many adult mammalian tissues and organs, such as bone marrow (BM), brain, skin, eyes, heart, kidneys, lungs, gastrointestinal tract, pancreas, liver, breast, ovaries, prostate, and testis [1–7, 9–15, 18, 22, 39–41]. All stem cells are undifferentiated cells that exhibit unlimited self-renewal and can generate multiple cell lineages or more restricted progenitor populations that can contribute to tissue homeostasis by replenishment of cells or regeneration of tissue after injury.

Several investigations have been carried out with isolated embryonic, fetal, and adult stem cells in a well-defined culture microenvironment to define the sequential steps and intracellular pathways that are involved in their differentiation into specific cell lineages. More particularly, different methods for in vitro culture of stem cells have been developed, including the use of cell feeder layers; cell-free conditions; extracellular matrix (ECM) molecules, such as collagen, gelatin, and laminin;

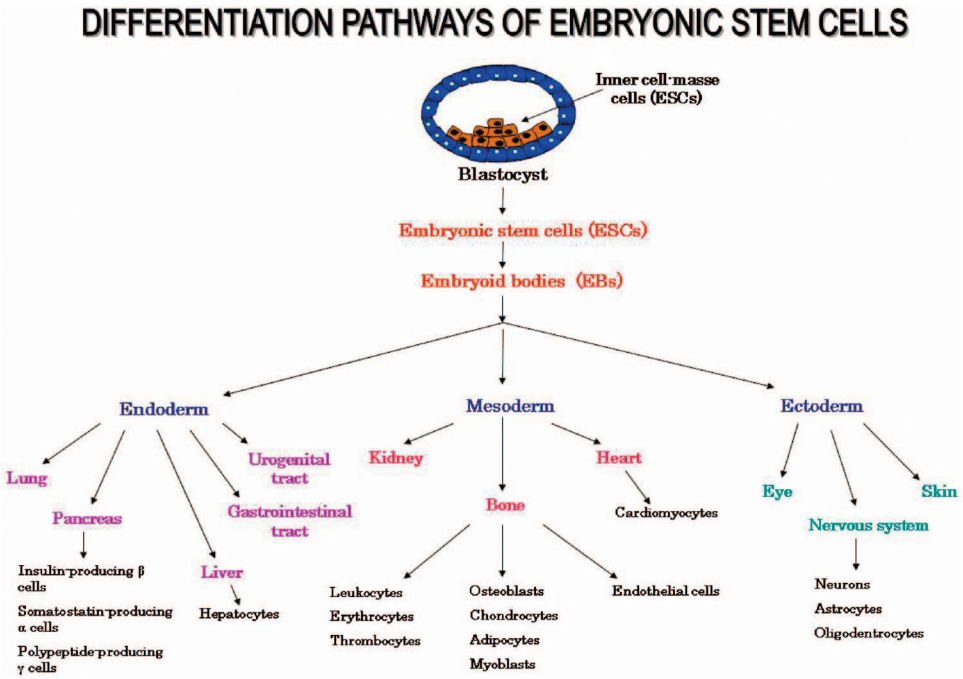

Figure 1. Schematic diagram showing the possible differentiation pathways of ESCs. The ESCs can give rise to three germ layers, endoderm, mesoderm, and ectoderm, during embryonic development. Similarly, ESC progenitors derived through the formation of EBs might also form teratomas corresponding to the complex structures containing the cell types from three germ layers in vivo. Moreover, the in vitro expansion and differentiation of ESC-derived progenitors in the presence of specific growth factors and cytokines in culture medium may also generate mature cell progenitors possessing the particular markers and biological features of cells constituting the tissues/organs of endodermal, mesodermal, or ectodermal origin. Abbreviations: EB, embryoid body; ESC, embryonic stem cell.

and diverse growth factors and cytokines [14, 21, 22, 42, 43]. We report here the structural and functional features of embryonic, umbilical cord, and adult stem cells and their niches, as well as the procedures that are used for their differentiation into particular cell lineages in vitro and in vivo.

Embryonic Stem Cells

Several mammalian pluripotent embryonic stem cell (ESC) lines derived from blastocyst-early-stage embryos have been established (Fig. 1) [22, 44]. More specifically, human embryonic stem cell (hESC) lines express many markers that are common to pluripotent and undifferentiated cells, such as CD9, CD24, octamer-binding protein (Oct-4), Nanog, alkaline phosphatase, LIN28, Rex-1, Cripto/TDGF1, DNMT3B, SOX2, EBAF, and Thy-1, as well as stage-specific embryonic antigen-3 and -4 (SSEA-3 and -4) and tumor-rejection antigen-1-60 and -1-81 (TRA-1-60 and -1-81) [22, 44, 45]. Moreover, all ESC lines generally exhibit high levels of telomerase expression and activity for prolonged periods in culture. In this matter, ESCs possess the dual ability to undergo unlimited self-renewal and to differentiate in all fetal and adult stem cells and their more differentiated progenitors. Therefore, they represent a useful source of stem cells for investigating the molecular events that are involved in normal embryogenesis and generating a large number of specific differentiated progenitors for cellular therapies.

In Vitro Derivation of ESC Progenitors. ESCs are generally isolated from the inner cell masses (ICMs) of blastocysts, which consist of pluripotent cell populations that are able to generate the primitive ectoderm during embryogenesis (Fig. 1). More specifically, in normal embryonic development, the primitive ectoderm gives rise during the gastrulating process to the primary germ layers, including ectoderm, mesoderm, and endoderm. These three germ layers might subsequently generate a variety of organized tissue structures involving complex epithelial-mesenchymal interactions. Similarly, the injection of ESC-derived progenitors into severe combined immunodeficient (SCID) mice might also result in the formation of teratomas corresponding to the complex structures containing the differentiated cell types from three germ layers [21, 22]. Moreover, ESCs can generate multiple cell progenitors that express the specific markers of three germ layers in vitro, including endoderm (α-fetoprotein and α1-antitrypsin), mesoderm (ζ-globin, enolase, kallikrein, cartilage matrix protein, myosin heavy chain, and muscle actin), and ectoderm (68-kDa neurofilament, class III β-tubulin, and keratin) [21, 22]. In this matter, the ESCs, when cultured in suspension in vitro, are able to spontaneously form embryoid bodies (EBs) that consist of spheres containing a variety of more differentiated progenitor cell types. The partial disaggregating and subculture of EBs might allow for the isolation and differentiation of a particular progenitor cell type that might be isolated on the basis of the expression of specific cellular markers. More particularly, the use of specific growth factors or cytokines during the outgrowth of EBs in culture in vitro might induce their differentiation into the specific cell lineages (Fig. 1) [21, 22, 42, 43]. For instance, it has been reported that the nerve growth factor (NGF) and hepatocyte growth factor (HGF) might induce the differentiation of ESC progenitors into cells from three embryonic germ layers,

whereas EGF, basic fibroblast growth factor (bFGF, also known as fibroblast growth factor 2 [FGF-2]), retinoic acid (RA), and bone morphogenic protein-4 (BMP-4) instead generate the progenitors expressing the ectodermal and mesodermal markers [42]. Furthermore, the differentiation of hESCs in the presence of the transforming growth factor-β (TGF-β) family-related protein, activin A, and a weak serum level might also generate a population containing up to 80% endodermal cells whose progenitors might be further enriched by using the cell-surface receptor CXCR4 [43]. More recently, the selective differentiation of hESCs into neural, definitive endoderm/pancreatic, and cardiac progenitors has also been performed on a Matrigel-coated surface under chemically defined conditions by using Noggin, activin A, and activin A plus BMP, respectively [21]. Among the ESC progenitors, there are the hematopoietic cell lineages, neuron-like cells, glial progenitors, dendritic cells, cardiomyocytes, skin cells, lung alveoli, hepatocytes, pancreatic islet-like cells, osteoblasts, chondrocytes, adipocytes, muscle cells, endothelial cells, and retinal cells (Table 1) [16, 19, 21, 22, 42, 43, 46–50]. More recently, in vitro derivation of hESCs into large amounts of functional osteogenic cells has also been performed without the intermediate step of the EB formation, by separation of the hESC population into single cells [49]. However, mouse and human ESCs express different marker profiles and might respond differently to certain growth factors, giving rise to distinct cell lineage progenitors. Therefore, these interspecies differences underline the importance of further establishing the particular differentiation pathways of hESC-derived progenitors for their clinical applications in humans.

One of the critical steps in the purification procedure appears to be the enrichment of EB-derived progenitor cells by the elimination of pluripotent and undifferentiated stem cells. Indeed, the elimination of undifferentiated stem cells that may form teratomas or teratocarcinomas in vivo appears to be essential for generating transplantable sources of differentiated stem cell progenitors for the treatment of diverse disorders [51–53]. As a matter of fact, it has been reported that the presence of less than 0.2% of specific markers of undifferentiated ESCs Oct-4 and SSEA-1 in the preparation of insulin-producing β-cells, which was detected in graft tissues by different techniques, including flow cytometry and immunohistochemistry, was sufficient to generate teratomas in vivo [53]. Therefore, the purification stages for the enrichment of differentiated ESC progenitors and the elimination of pluripotent and undifferentiated ESCs are essential for the development of safe ESC-based therapies. In this matter, it has been reported that the enrichment of nestin-positive neuroprogenitors in EB-derived cell populations and grafts in vivo may be performed by the elimination of pluripotent and proliferative Oct-4^+/prostate apoptosis response-4^+ (PAR-4^+) ESCs [54]. In fact, the selective apoptotic death of residual Oct-4^+/PAR-4^+ ESCs might be induced by the overexpression of PAR-4 or the treatment of these cells with ceramide analogs such as *N*-oleoyl serinol (S18), which also activate the PAR-4 pathway [54]. The activation of the PAR-4 pathway might lead to the downregulation of several survival cascades that are mediated through the atypical protein kinase Cζ, mitogen-activated protein kinase (MAPK), and nuclear factor-κB (NF-κB). Thus, the use of ceramide analogs might result in the selective elimination of the pluripotent and teratogenic Oct-4^+/PAR-4^+ ESCs during neural differentiation, and thereby prevent teratoma formation after the transplantation of EB-derived progenitors in brain. Moreover, the elimination of Oct-4^+ undifferentiated ESCs from the EBs, which has been performed by Percoll centrifugation and magnetic cell sorting, also produced a transplantable source of hepatocytes that formed no teratomas in vivo [55].

Hence, together these studies have contributed to the development of new procedures for the derivation and enrichment of hESCs. These new procedures can be used for studying the developmental pathways involved in hESCs maturation into their commitment lineages, as well as generating a variety of differentiated hESCs progenitor types for their in vitro and in vivo use. First, the development of novel purification methods to obtain a level of 100% purity of hESC-generated progenitors in the transplants seems to be necessary before they can be used in the clinic.

Embryonic and Primordial Germ Cell Carcinomas. Several works have revealed that pluripotent ESCs, as well as primordial embryonic germ cells (EGCs) that are derived from embryonic gonads, might also give rise to teratocarcinomas in vivo. As a matter of fact, the injection of adapted human ESC-derived progenitors that are obtained after prolonged culture in vitro into SCID mice might result in the formation of teratocarcinomas whose tumors are composed of cells derived from the three germ-like structures [45, 52]. The embryonic carcinoma cells, which are isolated from teratocarcinomas, may be used as models to study the factors that affect the stem cell self-renewal and embryonic development, as well as the relationship between the oncogenic events occurring in embryonic stem cells and cancer initiation [45, 56]. In fact, based on the hypothesis that certain adult cancer forms might also derive from the malignant transformation of tissue-specific adult stem cells into cancer progenitor cells, the genetic alterations observed in embryonic carcinoma cells might also provide information on oncogenic gene products that might be involved in initiation of certain adult cancer types.

Amniotic Epithelial Cells

Amniotic epithelial cells (AECs) derived from the amniotic membrane in human term placenta also express the markers that are present on pluripotent ESCs and EGCs, such as Oct-4, Nanog, and alkaline phosphatase [57, 58]. They can also differentiate as ESCs and EGCs in the cell lineages from three germ layers, including pancreatic endocrine cells and hepatocytes (endoderm), cardiomyocytes (mesoderm), and neural cells (ectoderm), in vitro [57, 58]. Of therapeutic interest, AECs do not express the telomerase and form no teratomas after transplantation in vivo. Therefore, AECs constitute a source of pluripotent stem cells that might be used in transplantation for tissue regeneration.

Fetal Stem Cells

Multipotent fetal stem cells (FSCs) are generally more tissue-specific than ESCs. Therefore, FSCs are able to generate a more limited number of progenitor types. One of the particular therapeutic advantages of FSCs as compared with ESCs is the fact that FSCs do not form teratomas in vivo. Moreover, the FSCs

obtained up to week 12 offer the possibility of transplanting these primitive stem cells without frequent rejection reactions, in contrast to UCB and BM stem cell transplants. As a matter of fact, recent work has revealed the possibility of using FSCs or their progenitors, isolated from particular tissues, for multiple therapeutic applications involving tissue regeneration [11, 15, 26, 59]. More particularly, the human fetal liver constitutes an alternative source of hematopoietic stem cells (HSCs) for the generation of hematopoietic cell lineages in vivo [15].

On the other hand, it is interesting to note that a reciprocal fetomaternal trafficking of cells and nucleic acids has also been shown through the placental barrier during pregnancy, which might contribute to tissue repair mechanisms in different maternal organs and the growing fetus [60, 61]. In fact, the cells from the growing fetus appear to be able to cross over the placenta and enter the mother's bloodstream and vice versa; the maternal cells can also pass into fetal circulation and persist into adult life, a phenomenon known as microchimerism. Hence, the fetal cells that are transferred to the mother during gestation can migrate to different damaged peripheral tissues, such as the liver and skin, or can cross the blood-brain barrier to enter damaged areas of the brain, where they actively contribute to the mother's tissue repair by generating mature cell progenitors [60–62]. More surprisingly, a small number of functional fetal cells with stem-cell-like properties, designated pregnancy-associated progenitor cells, appear also to persist in blood and tissues after pregnancy, and thereby continue to assume their protective role in tissue repair or contribute to certain pathophysiologies [60, 62]. This reflects the high plasticity and migratory potential of FSCs, which represent major advantages for their use in transplantation. More specifically, the establishment of the structural features of FSCs that enable them to cross both the placental and blood-brain barrier could allow for the improvement of therapeutic applications of neural stem cell progenitors in allowing their administration by i.v. infusion for repair of diverse brain disorders.

Umbilical Cord Stem Cells

Umbilical cord epithelium (UCE), which appears to derive from amniotic membrane epithelium, and UCB represent other sources of multipotent stem cells that might be used for generating diverse differentiated cell types [14, 17, 20, 27, 63]. More particularly, it has been reported that the multipotent UCE cells can differentiate into the surface of a fibroblast-populated collagen gel into functional keratinocyte-like cells [20]. These keratinocyte-like cells were also able to generate a stratified epithelial structure resembling that formed by keratinocytes in vivo, when grafted onto the back of nude mice. Moreover, the neonatal cord blood contains a primitive subpopulation of $CD34^+$ HSCs that shows a higher hematopoietic expansion capacity in the presence of FLT-3 ligand, KIT ligand and thrombopoietin (TPO) as compared with their counterpart in adult mobilized peripheral blood (MPB) [64]. In this matter, the elevated levels in plasma concentrations of testosterone, estriol, insulin-like growth factor-1 (IGF-1), and IGF binding protein-3, which were detected in human UCB from 40 women, have been associated with a high proliferative potential of $CD34^+/CD38^-$ cells and colony-forming unit granulocyte-macrophage [29]. Several works have also revealed the possibility of differentiating the UCB stem cells into diverse functional cell progenitors, including hematopoietic cell lineages, dendritic cells, neural cell progenitors, hepatocytes, pancreatic cells, and endothelium under specific culture conditions in vitro and in vivo (Table 1) [14, 63, 65]. For instance, the xenogeneic transplantation of $CD34^+$ or $CD45^+$ human cord blood cells in the neonatal nonobese diabetic (NOD)/SCID/β2-microglobulin^{null} animal mice models in vivo have revealed that these cells give rise to human hepatocytes showing morphological characteristics comparable to those of mouse hepatocytes [63]. Significantly, the results from reverse transcription-polymerase chain reaction analyses have also confirmed that the expression of human albumin mRNA occurred in these human engrafted cells, suggesting that they have effectively acquired the functional properties associated with mature hepatocytes. Furthermore, the trans-differentiation of human mononucleated cell population from UCB into insulin-producing β-like cells that express specific markers of developing pancreas, such as nestin, cytokeratin (K8), K18, and diverse transcription factors (Isl-1, Pdx-1, Pax-4, and Ngn-3), has also been performed in medium containing fetal calf serum [66]. Similarly, the differentiation and enrichment of $CD1^+$, $CD83^+$, $CD11c^+$, and $CDw123^+$ dendritic cells from UCB have also been carried out in vitro by the culture of cells in a medium containing diverse cytokines, such as granulocyte-macrophage-colony stimulating factor (GM-CSF), interleukin-3 (IL-3), recombinant human stem cell factor (SCF), and erythropoietin (EPO), for 2–4 weeks [65]. Hence, these dendritic cells may be used as an adjuvant in immunotherapy for diverse disorders and cancers.

In addition, human UCB also contains a more primitive subpopulation of mesenchymal stem cells than adult BM, the immature cells of which express adhesion molecules, such as CD13, CD29, CD44, CD90, CD95, CD105, CD166, and major histocompatibility complex class, but not the antigens of hematopoietic differentiation, such as CD34 [23, 67]. These mesenchymal stem cells can also differentiate into multiple lineages, including progenitors with bone, fat, and neural markers, under specific conditions in vitro. Interestingly, it has been reported that the systemic infusion of mesenchymal stem cell progenitors in immunodeficient mice resulted in their engraftment in BM, as well as in other diverse tissues, including the heart, teeth, and spleen [23]. Thus, it appears that the differentiation of UCB stem cells into tissue-specific adult stem cell progenitors might constitute an alternative strategy for cellular therapies of diverse disorders (Table 1).

Adult Stem Cells

Numerous studies have revealed that a population of adult stem cells and supporting cells reside within specific areas designated as niches in most of adult mammalian tissues/organs, including BM, heart, kidneys, brain, skin, eyes, gastrointestinal tract, liver, pancreas, lungs, breast, ovaries, prostate, and testis (Fig. 2) [1–7, 9–13, 18, 39–41, 68]. In fact, adult stem cells appear to originate during ontogeny and persist in specialized niches within organs where they may remain quiescent for short or long periods of time. Although adult stem cells, as observed for ESCs, FSCs, and UCB stem cells, might exhibit an uncontrolled growth in a specific microenvironment and enhanced telomerase activity, they generally show a more restricted differentiation potential and give rise to a more limited number of distinct cell progenitors. Adult stem cells can notably undergo proliferation

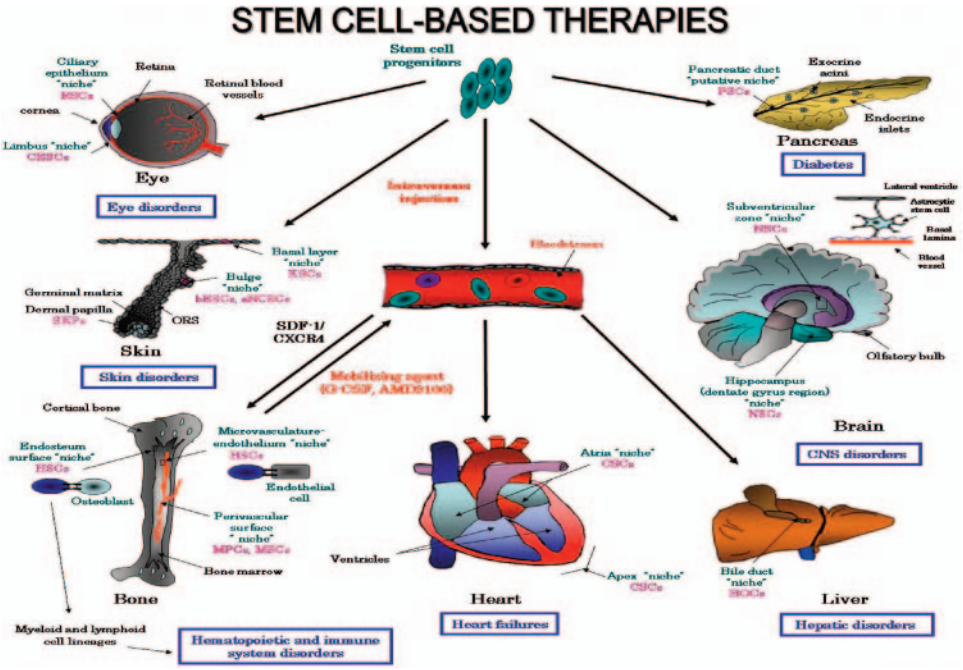

Figure 2. Scheme showing the tissues/organs constituting the potent targets for tissue regeneration by stem cell-based therapies. The localization of tissue-specific stem cells and their niches is shown. The tissue-specific degenerating disorders and diseases that might be treated by the transplantation of stem cell progenitors derived from embryonic stem cells, fetal stem cells, umbilical cord blood, and adult tissues/organs, including bone marrow (BM), are also indicated. The possibility of the mobilization of BM stem cells and their progenitors in the bloodstream by using the mobilizing agents is also shown. Abbreviations: bESC, bulge epithelial stem cell; CESC, corneal epithelial stem cell; CNS, central nervous system; CSC, cardiac stem cell; CXCR, CXC-chemokine receptor; eNCSC, epidermal neural crest stem cell; G-CSF, granulocyte colony-stimulating factor; HOC, hepatic oval cell; HSC, hematopoietic stem cell; KSC, keratinocyte stem cell; MPC, mesodermal progenitor cell; MSC, mechenchymal stem cell; NSC, neural stem cell; PSC, pancreatic stem cell; RSC, retinal stem cell; SDF, stromal-derived factor; SKP, skin-derived precursor.

and differentiation into more mature and specialized tissue-specific cell types following changes in their microenvironment within the niche [2, 4, 6, 69–71]. More specifically, stem cells and their supporting cells appear to interact reciprocally by forming diverse intercellular connections, such as gap and adherens junctions, for maintaining the niche integrity [69–71]. Hence, latent adult stem cells appear to be activated during cell replenishment to repopulate the tissue compartments under physiological and pathological conditions (Fig. 3) [1–13, 18, 30, 31, 33, 38, 72].

Certain concepts, based on the possibility of symmetric and asymmetric divisions or populational asymmetry, have also been proposed for explaining the balance between stem cell self-renewal and differentiation [1, 41, 70, 73]. During stem cell self-renewal, the expansion of each stem cell appears to involve its symmetric division into two identical daughter stem cells. In contrast, the asymmetric division of stem cells during differentiation involves each stem cell dividing to produce one daughter stem cell and one daughter transit-amplifying (TA)/intermediate cell (Fig. 3). The TA cells that are generated during asymmetric division are generally characterized by a high proliferative index and migratory properties. The TA cells may give rise to other intermediate cell progenitors and, ultimately, to more differentiated cells constituting tissues or organs where they originate or other, distant tissues. In fact, the migration of TA cells to distant sites, as well as the changes in their local microenvironment during amplification, might be the basis of a populational asymmetric division, which might result in the differentiation or trans-differentiation of TA cells into different cell lineage progenitors. In addition, many works have indicated that the adult stem cells, and more particularly BM stem cells, may be redistributed under certain physiological and pathological conditions to other distant tissues via circulation [41, 68, 74–76]. In fact, the new microenvironment of stem cells that is prevalent in another particular tissue might contribute to their new specification and trans-differentiation into tissue-specific cell progenitors. In this matter, we describe the localization of adult stem cells in diverse tissues/organs from endodermal (lungs, gastrointestinal tract, pancreas, liver, and urogenital tract), mesodermal (bone marrow and heart) or ectodermal (central nervous system [CNS], skin, and eyes) origin and the molecular events that might be implicated in their decision to undergo sustained proliferation or adopt a specific differentiation pattern under normal or pathological conditions. More particularly, the emphasis is on the adult stem cells that can give rise to a broad range of progenitors. These adult stem cells are also able to trans-differentiate in different mature cell lineages in vitro and in vivo.

Adult Stem Cells of Endodermal Origin

Pulmonary Epithelial Stem Cells. The multipotent pulmonary epithelial stem cells are able to differentiate into ciliated, secretory, intermediate, and basal cells and generate the submucosal glands. Although their specific markers and niche(s) have not yet been established precisely [9, 39, 41], it has been proposed

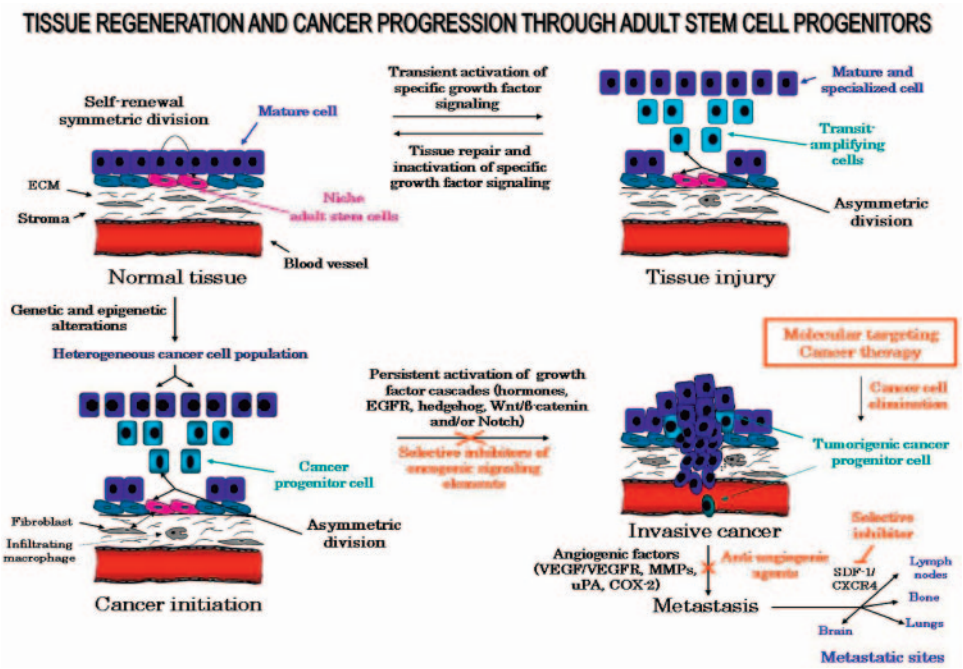

Figure 3. Proposed model of the cellular events associated with the tissue regeneration by adult stem cells and cancer progression originating in their malignant transformation into cancer progenitor cells. The cellular events that are implicated in the tissue regeneration after injury, including the asymmetric division of adult stem cells into transit-amplifying cells, which in turn may give rise to the mature cells that repopulate the tissue, are shown. Moreover, the malignant transformation of adult stem cells and/or their progenitors into cancer progenitor cells induced through genetic and epigenetic alterations (gene mutations, deletions, amplifications, and chromosomal rearrangements or change in DNA methylation) and whose genetic modifications might lead to the generation a heterogeneous cancer cell population is shown. The activation of numerous growth factor signaling cascades and tumorigenic signaling elements in cancer cells, which are implicated in cancer progression, as well as their molecular targeting by using the selective inhibitors for the development of novel combination therapies against the aggressive cancer forms, are also indicated. Abbreviations: COX, cyclooxygenase; CXCR, CXC-chemokine receptor; ECM, extracellular matrix; EGFR, epidermal growth factor receptor; MMP, matrix metalloproteinase; SDF, stromal-derived factor; uPA, urokinase-type plasminogen activator; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor.

that these stem cells reside within the surface airway epithelium. In this matter, a multipotent bronchioalveolar stem cell (BASC) population that is able to self-renew has been identified at the bronchioalveolar duct junction [9]. BASCs may contribute to the maintenance of the bronchiolar Clara cells and alveolar cells of the distal lung. The activation of EGF and hedgehog cascades in the airway epithelial stem cells appears to contribute to lung-tissue homeostasis by stimulating the proliferation and differentiation of stem cells, which repopulate the airway epithelium in the damaged tissues after airway injury [33, 77]. The extensive inflammatory lesions in the airway walls may, however, result in diverse lung disorders and hypersecretory lung diseases, such as chronic bronchitis, asthma, cystic fibrosis, and cancer [9, 31, 33, 39, 41, 77].

Gastrointestinal Tract Stem Cells. The replenishment of epithelial cell lineages within the gastrointestinal tract is a frequent process, occurring every 2–7 days under physiological conditions. This process may contribute to the generation of new cell progenitors, which repopulate the damaged tissues during diverse pathological disorders, such as inflammation and ulceration [1, 2, 5, 32]. This process is regulated by multipotent stem cells, which give rise to all gastrointestinal epithelial cell lineages and can regenerate whole intestinal crypts and gastric glands. Multipotent stem cells of the gastrointestinal tract are localized within the niches in the intestinal crypts and gastric glands [1, 2]. More specifically, it has been reported that the small intestinal epithelial stem cells expressing markers such as Musashi-1 (Msi-1), CD24, and the receptor for SCF, designated KIT, may reside in deep crypts [2, 5]. These stem cells can give rise to all cell types within the crypt, including absorptive cells, goblet cells, enteroendocrine cells and Paneth cells. Similarly, the stem cells in the large intestine or colon, which are localized at the bottom of crypts, may also give rise to the proliferative progenitors that differentiate toward all lineages during epithelium regeneration [5]. In addition, the gastric mucosa also contains multipotent stem cells and their progenitors, which may proliferate and undergo a bipolar migration associated with their differentiation into different stomach cell lineages, including parietal, zymogenic, and pit cells [2]. More specifically, the gastric stem cells have been proposed to reside in a niche localized in the isthmus region in each gastric gland. Each of these gastric glands is also composed of mesenchymal cells and ECM factors that regulate the functions of gastric stem cells through mesenchymal-epithelial interactions [2]. Multiple signaling pathways, including EGF, FGFs, IGF, hedgehog and Wnt/β-catenin, Notch, and/or TGF-β may contribute to the stringent regulation of the proliferation and differentiation of gastrointestinal stem cells into functional epithelial cells and gastric mucosa [1, 2, 5, 33]. In fact, these signaling cascades,

which assume an important role during embryonic gut development, are also involved in the replenishment of tissue-specific adult stem cells for maintaining normal homeostasis. These signaling cascades may also be altered in diverse disorders, such as gastrointestinal ulcers and gastric and colorectal cancers [1, 2, 32, 33].

Pancreatic Stem Cells. The mammalian adult pancreas has three tissue types: the ductal tree, the exocrine acini, which produces digestive enzymes, and the endocrine islets of Langerhans, composed of insulin-producing β-cells, glucagon-producing α-cells, somatostatin-producing δ-cells, and pancreatic polypeptide-producing γ-cells (Fig. 2). Much evidence indicates the presence of putative pancreatic stem cells (PSCs) in ductal and/or islet regions of the mammalian pancreas. The localization of the niche(s) of these putative PSCs is not yet precisely known. In this matter, the exocrine and endocrine cells appear to be produced from the differentiation of ductal cell progenitors during embryonal development [78]. Conversely, it has been reported that a ductal cell population might contribute to the regeneration of both the endocrine and exocrine pancreas after a 90% partial pancreatectomy in the young adult rat, suggesting that the ductal compartment could notably constitute a niche for the primitive multipotent PSCs [19]. In fact, it has been shown that the adult epithelial cells in ductal tissue from the human pancreas can be expanded ex vivo in culture and differentiated into islet cells, which are responsive to glucose-induced insulin secretion [19]. Moreover, the multipotent cell progenitors have also been identified within the ducts and islets in adult rodent and human pancreas [40, 78, 79]. More particularly, the nestin-positive cell progenitors from the islet region could be differentiated ex vivo into cells expressing the markers that are specific to the liver (α-fetoprotein), and pancreatic exocrine cells (amylase), ductal cells (K19), and endocrine cells (insulin, glucagon, and pancreatic and duodenal homeobox factor-1 [PDX-1], also known as IDX-1) under specific culture conditions [79]. Similarly, it has also been reported that the multipotent PSCs isolated from the human fetal pancreas and expressing stem cell markers, such as nestin, ATP-binding cassette transporter (ABCG2), and KIT, as well as epidermal growth factor receptor (EGFR), hepatocyte growth factor receptor (c-Met), and glucagon-like peptide receptor were able to form the islet-like cell clusters (ICCs) when cultured ex vivo [26]. These ICCs can give rise to diverse pancreatic cell lineages, including insulin-secreting cells.

In addition, the stem cells of other sources expressing the pancreas-developing markers, including nestin, K8, K18, neurogenin-3, and nuclear transcription factors such as IDX-1, Pax-4, Pax-6, and Isl-1, which are important for the β-cell differentiation, might be expanded and trans-differentiated under specific culture conditions in vitro. More specifically, the differentiation of ESCs, fetal and UCB stem cells, and adult stem cells from the liver, BM, spleen, and nervous system into pancreatic insulin-producing β-cell-like progenitors has been performed in vitro by using growth factors such as bFGF, SCF, nicotinamide, betacellulin, glucagon-like peptide, and activin A (Table 1) [19, 21, 22, 26]. Moreover, the differentiation of hESCs into ectodermal insulin-producing β-like cells expressing the specific markers related to pancreatic β-cells, including nuclear transcription factors, such as Foxa2, PDX-1, and Isl1, has recently been performed by cotransplantation of hESCs with the dorsal pancreas from mouse embryos [47].

Hepatic Oval Cells. Mature hepatocytes can undergo several cell division cycles and are responsible for continuous hepatic cell replacement during extensive liver injury. A small population of stem cells designated hepatic oval cells (HOCs) may also be involved in tissue regeneration [11, 25, 80]. These HOCs appear to reside in the smallest units of the bile duct epithelium within the periportal region, termed the canals of Hering and/or periductular region, from which they can migrate into the liver parenchyma (Fig. 2). HOCs, which express immature hepatocyte markers, such as α-fetoprotein, γ-glutamyl-transferase, albumin, K19, OV-6, and OC.2, as well as several hematopoietic cell markers, such as CD34, Thy1.1, Flt3-receptor, and KIT, can give rise to both hepatocytes and bile ductular cells in vitro and in vivo [11, 81]. In particular, the signaling networks involving HGF, EGF/TGF-α, vascular endothelial growth factor (VEGF), SCF, Wnt/β-catenin, TGF-β, and stromal-derived factor-1 (SDF-1) appear to assume a critical role in the regulation of the proliferation, survival, and differentiation of HOCs into hepatocytes during hepatic regeneration [11, 25, 33, 81]. In addition, several lines of evidence have also revealed that the hepatocyte-like cells can be derived from extrahepatic sources, including ESCs, BM, UCB, and pancreas under specific culture conditions in vitro and/or in vivo [11, 22, 63, 68, 75]. In regard to this, the transplantation of BM cells or enriched HSCs in the animal models in vivo has been observed to restore the HOCs and their differentiated progenitors, as well as the functions of the liver, after injury [82]. These observations suggest that stem cells from extrahepatic sources may also contribute to the repopulation of the liver compartment; however, results from more recent work have revealed that the trans-differentiation of HSCs from BM into hepatocytes may only occur at a low frequency under physiological and pathological conditions [11]. Thus, additional works are essential to establishing the factors that control the migration, incorporation, and homing of extrahepatic stem cells in the liver under specific in vivo conditions.

Urogenital Stem Cells

Mammary and Prostatic Gland Stem Cells. The regulation of the self-renewal, differentiation, and migration of urogenital stem cells and their progenitors that are localized in the mammary and prostatic glands appears to be assumed through distinct developmental signaling pathways such as hormones, EGF, hedgehog, Wnt/β-catenin, Notch, and/or BNPs [7, 8, 18, 83–85]. More specifically, the postnatal growth of the mammary gland during puberty, pregnancy, and lactation might notably be induced by estrogenic hormones that may regulate epithelial stem cell behavior in paracrine fashion. In this matter, the human adult mammary gland adopts a lubulo-alveolar structure that is composed of different epithelial cell types, including alveolar epithelial cells, contractile myoepithelial cells forming the basal layer of ducts and alveoli, and specialized epithelial cells constituting the luminal layer of ducts. Distinct undifferentiated and multipotent stem cell subpopulations have been identified in the mammalian mammary epithelium within the

niches localized near the basement membrane [7, 83–87]. These stem cell subtypes, which can express either estrogen receptor-α (ER-α-positive cells) or undetectable ER-α levels (ER-α negative cells), as well as specific stem cell markers, including Sca-1, K19, and Msi-1, are able to give rise to myoepithelial and luminal epithelial cells in vitro. Moreover, it has also been shown that the propagation of human undifferentiated mammary epithelial cells derived from the reduction mammoplasties under the form of nonadherent mammospheres in vitro, results in the generation of three mammary epithelial cell types [88]. These undifferentiated mammary epithelial cells may generate a functional ductal-alveolar structure resembling the mammary tree in reconstituted three-dimensional Matrigel culture system [88]. In addition, it has been observed that a suprabasal-derived mammary epithelial cell line, which was able to self-renew and differentiate into myoepithelial and luminal epithelial cells, could also form terminal duct lobular unit-like structures within a reconstituted basement membrane [86].

Similarly, a stem cell population found in the embryonic urogenital sinus epithelium from which prostatic epithelial buds develop also appears to persist in the adult prostatic epithelium and gives rise to basal and luminal epithelial cells. More specifically, a small pool of the adult prostate-specific stem cells, which show unlimited growth in a specific microenvironment and generate multiple more-differentiated epithelial cells due their striking plasticity, has recently been isolated from the proximal regions of prostatic ducts [18, 89, 90]. These prostate adult stem cells, which appear to be localized at the basement membrane, are also characterized by specific markers, such as $\alpha_2\beta_1^{(hi)}$-integrin, CD133, Sca-1, prostate stem cell antigen, telomerase, and K6a. Moreover, prostate stem cells also possess basal cell-like phenotypes, including their androgen independence due to a lack of androgen receptor (AR) and significant expression levels of K5, K14, p63, and anti-apoptotic Bcl-2 protein. Significantly, several recent works have also revealed that the aberrant activation of the developmental signaling cascades in mammary and prostatic gland stem cells and their progenitors occurring after tissue injury may lead to the initiation of precancerous lesions and progression into advanced and metastatic forms of breast and prostate cancers [7, 8, 18, 33, 83–85, 87, 90–92].

Ovarian and Testicular Stem Cells. Although several lines of evidence have indicated the presence of putative multipotent stem cells, which give rise to diverse differentiated epithelial and germ cells constituting the adult mammalian ovaries and testes, their precise localization needs to be identified. As a matter of fact, the multipotent stem cells have been established from neonatal gonads, testes, and ovaries in mouse and human [93–96]. These adult germ cells may differentiate into various types of somatic cells under specific conditions in vitro and form teratomas after inoculation into mice. In this matter, several morphogens from diverse developmental signaling, including BMP, Notch, and Wnt family members, might be involved in the control of gene expression in the gametes and their supporting somatic cells during gametogenesis [94]. Moreover, it has been proposed that the cytokeratin-positive mesenchymal cells in the ovarian tunica albuginea may undergo a mesenchymal-epithelial transition into ovarian surface epithelium (OSE) cells [95]. These bipotent OSE cells can subsequently differentiate into primitive granulosa and germ cells that assemble in the deeper ovarian cortex, thereby forming new primary follicles during the follicular cycle. Regardless, it has also been observed that the OSE cells from adult women may differentiate into oocytes and granulosa cells when cultured in vitro in the presence of estrogens [96]. These primary follicles that were formed by the oocytes and granulosa cells derived from the OSE cells could also give rise to the mature secondary oocytes in vitro. Therefore, these findings may constitute the basis for future stem cell-based research in the development of novel in vitro fertilization strategies; however, additional work is necessary to establish the functional properties of these in vitro generated human secondary oocytes before their putative use in the clinic.

Genetic modifications in testicular and ovarian somatic stem cells and germ cells in mice and humans may also lead to tumor formation. As a matter of fact, a tumor stem cell population showing stem cell-like characteristics has been established from human ovarian tumors, which proliferate and form tumors in animal models [34]. Moreover, the functional alterations in germ cells may also result in teratocarcinoma formation in vivo. The teratocarcinomas are also composed, like the primordial germ cell tumors, of a large variety of somatic cells and tissues from all three germ layers, which are disposed in a disorganized fashion. In this matter, the testicular tumor germ cells express a variety of pluripotent cell markers, such as Oct-4, Nanog, GDF3, CD9, EDR1 (PHC1), SCNN1A, Glut3, and Stella, that are also detected in the pluripotent ESCs [28]. It has also been reported that the RA treatment of these testicular tumor germ cells may suppress the expression of these marker molecules and tumorigenicity, suggesting that the high plasticity and undifferentiated state of tumor germ cells may be directly related to the formation of teratocarcinomas [28]. Additional investigations are necessary to establish the specific functions of these stem cell markers, as well as other genetic alterations that may occur in adult ovary and testicular stem cells and germ cells and that may be responsible for tumor formation in humans.

Adult Stem Cells of Mesodermal Origin

Bone Marrow. BM is a well-organized tissue composed of the basic elements from the stroma and hematopoietic system and located at the center of large bones (Fig. 2). BM contains HSCs and stromal stem cells that collaborate in a reciprocal manner at all stages, leading to the generation of different BM and bloodstream cell lineages [12, 69, 73, 97–99]. More specifically, the stromal osteoblasts express diverse soluble factors, such as granulocyte colony-stimulating factor (G-CSF), IL-6, and Notch ligand jagged 1, that can influence the proliferation and differentiation of HSCs. Reciprocally, HSCs regulate osteoblastic secretion. Hence, the differentiation of HSCs residing within BM, which express specific surface markers, such as Sca-1, KIT, CD34, and CD150, ensures the continuous replenishment of all types of circulating hematopoietic cell lineages by giving rise to leukocytes, erythrocytes, and thrombocytes, which show a limited lifespan (Table 1) [17, 73, 99]. In this matter, several recent works have indicated that the specific signals induced by numerous growth factors and cytokines such as EGF, Sonic hedgehog ligand (SHH), Wnts, Notch ligands, BMPs, HGF, ILs,

Myc, and chemokine SDF-1 and its receptor CXCR4 may be involved in the stringent control of the BM stem cell homing, migration, and differentiation, as well as in the regulation of the hematopoiesis and repair of damaged tissues [33, 73, 97–100]. Regardless, the SDF-1-CXCR4 axis appears to assume a major role in the maintenance of HSCs homing in BM, as well as in the specification of their differentiation into different myeloid and lymphoid cells [97]. In addition, the hemangioblast-like cells in BM and vascular walls, as well as the more differentiated endothelial progenitors designated monocytic cell-like progenitors in blood, may also represent other sources of circulating hematopoietic and endothelial cells involved in tissue repair [101, 102].

Hematopoietic Stem Cells. In the adult BM, the HSCs are localized near endosteal bone surface and sinusoidal endothelium, suggesting that these sites could constitute the principal niches for their homing in BM under physiological conditions and for engraftment of HSCs after systemic transplantation (Fig. 1) [12, 73, 99, 103]. More particularly, HSCs, which express a variety of cell surface adhesion molecules, including αL-, α5-, and β2-integrins and N-cadherin, are colocalized with the osteoblasts at the endosteal surface [12, 73, 99]. Thereby, these cells may be tightly connected by adherens junctions, such as homotypic N-cadherin interactions (Fig. 2). Moreover, HSCs express the receptor tyrosine kinase Tie2, which may interact with its ligand angiopoietin-1 provided by osteoblasts that are localized in close proximity in the niche [69, 73, 99]. Hence, these HSCs-osteoblast interactions may restrict HSCs homing in a quiescent state at the endosteal bone surface. Similarly, the association between HSCs and sinusoidal endothelium via the interactions of integrins VLA-4 and LFA-1 expressed by HSCs with their respective endothelial ligands, vascular cell adhesion molecule (VCAM)-1 and intercellular adhesion molecule-1, might also contribute to their BM homing [104]. In support with this, it has been observed that the blockade of the VLA4/VCAM-1 adhesion pathway by using anti-VLA-4 and/or anti-VCAM-1 antibodies inhibited stem cell homing and resulted in a mobilization of HSC progenitors into the circulation in vivo [104, 105]. In this matter, it has been reported that HSCs can migrate from endosteal surface into vascular niche under specific stimuli such as injury and after treatment with myeloablative agents [73]. Hence, the localization of HSCs and their progenitors within BM vascular niche may allow their rapid release into circulation. In addition, on the basis of the observation by immunohistochemical analyses that the mobilized $CD150^+$ HSCs were also localized at the sinusoidal endothelium on the spleen tissue sections, it has been proposed that this site could also constitute an extramedullary niche for HSC homing [103]. Further investigation is necessary to establish the specific signals that govern the rate of proliferation, differentiation, and migration of HSCs under physiological and pathological conditions. This should allow us to identify the specific functions that are associated with each type of HSC that is localized in BM and other distant sites, such as spleen and liver.

In addition, HSCs and their progenitors may also be obtained from different sources, including ESCs, FSCs, and UCB as well as adult BM and MPB, and differentiate in vitro and ex vitro into different hematopoietic cell lineages [17, 22, 50, 76, 100, 106–108]. In this matter, the results from a microarray and serial analysis have notably indicated that the $CD34^+/CD38^-/LIN^-$ HSCs from adult BM, cord blood, and MPB expressed several common genes [109]. Moreover, the ex vivo expansion and maturation of BM and MPB progenitors into the specific hematopoietic cell lineages have also been performed by using growth factors such as SCF, G-CSF, GM-CSF, ILs, Flk2/Flt3 ligand, and TPO [100, 106, 107]. More specifically, it has been reported that the downregulation of the expression of the endogenous myelomonocytic cytokine receptors for GM-CSF and M-CSF on the HSC progenitors may be related with their maturation into a common lymphoid precursor [100]. In contrast, the upregulated expression of these cytokine receptors, which might be induced by using IL-2, appears to lead to myeloid cell development [100]. It has also been reported that the ex vivo expansion of human or mouse HSCs in the presence of SCF, Flk2/Flt3 ligand, TPO, and IL-6/soluble IL-6 receptor may generate the transplantable sources of expended cells that are able to repopulate NOD/SCID mice in vivo [106, 107].

Stromal Stem Cells. The BM stroma is a highly vascularized, complex structure containing mesenchymal stem cells and extracellular matrix elements supporting for hematopoiesis [97]. Mesenchymal stem cell populations from BM stroma, which can give rise to stromal mature mesenchymal cells and regenerate the BM microenvironment, have been identified in a perivascular region in BM tissue (Fig. 2) [110]. Among the BM stromal stem cells, there are primitive mesodermal progenitor cells (MPCs) and multipotent adult progenitor cells (MAPCs), which have been copurified with other mesenchymal stem cells (MSCs), also as known as bone marrow stromal cells (BMSCs). MPCs might differentiate into different mesenchymal cell types that participate actively in the formation of calcified ECM, including the bone-forming cells, osteoblasts, and chondrocytes, which synthesize cartilage, and adipocytes or fat cells that can store energy in the form of fat (Table 1) [111]. In addition, MPCs may also differentiate in other cell lineage types, such as skeletal myoblasts, endothelial cells, hepatocytes, neuron-like cells, and hematopoietic cell lineages under specific culture conditions in vitro and in vivo [75, 111]. Similarly, MSCs may also give rise to the osteocytic, adipocytic, and chondrocytic lineages, as well as neuron-like cells and glial cells, as observed for MPCs in well-defined culture mediums in vitro [112, 113]. Furthermore, MAPCs and MSCs also show the ability to migrate extensively throughout the circulation. For instance, it has been reported that the transplantation of MAPCs leads to their engraftment and differentiation into the hematopoietic lineages and the epithelium of the liver, lungs, and gut in nonirradiated mice, which were further enhanced in an irradiated host [75]. The injection of stromal BM cells into neonatal mouse brain was also accompanied by their migration throughout the forebrain and cerebellum, where they differentiated into astrocytes [74].

It has been reported that the stromal stem cells from BM may be involved in continuous bone remodeling, as well as in the regeneration of injured bone, cartilage, and other distant tissues, such as the liver, lungs, gut, and heart, in adults [75]. In regard to this, it has been observed that MSCs were able to produce a bone-like mineralized tissue into a culture medium containing synthetic glucocorticoid dexamethasone ([Dex], Decadron;

Merck & Co., Inc., Whitehouse Station, NJ, http://www.merck.com), ascorbic acid, and β-glycerophosphate, as well as in diffusion chambers and porous calcium phosphate ceramics loaded with whole BM or cultured-adherent BM cells [17]. This was also accompanied by upregulating osteocalcin mRNA expression, bone-like nodule formation, and calcium deposition. Moreover, the addition of bFGF and BMP-2 to Dex-treated MSCs, whose factors act as mitogen and differentiation inducer, respectively, may markedly enhance their growth and osteoblastic differentiation [114]. In contrast, the activation of the heparin-binding EGF-like growth factor-EGFR axis may induce the proliferation of MSCs, whereas it reversibly prevents adipogenic, osteogenic, and chondrogenic differentiation induced with the differentiation inducers [115]. Since hESCs may be differentiated into diverse hematopoietic cell lineages when cocultured with BM stromal cells [108], it appears that the stromal cells can secrete diverse soluble factors that may promote hematopoietic cell differentiation. As a matter of fact, it has been reported that the mature osteoblasts, which are localized in close proximity to $CD34^+$ HSCs on the endosteal surface, may control their proliferation and differentiation into myeloid and lymphoid cells during hematopoiesis and engraftment after transplantation [97, 116]. More particularly, the stimulation by parathyroid hormone of its receptor on osteoblastic cells may lead to their proliferation and secretion of high levels of Notch ligand jagged 1, which in turn, may induce the activation Notch signaling in HSCs and stimulate their growth [116]. Moreover, osteoblasts secrete numerous growth factors and cytokines, including SDF-1, that can contribute to the retention of HSCs in the BM, as well as the specification of different mature hematopoietic cell lineages [97]. For instance, the secretion of SDF-1 by the stromal osteoblasts and endothelial cells from BM and expression of CXCR4 on the human hematopoietic progenitors may lead to their BM engraftment in vivo (Fig. 2) [97, 117, 118]. In contrast, the blockade of interaction of SDF-1 with its receptor CXCR4 by disrupting the SDF-1 gene or using a selective antibody or antagonist of CXCR4, such as AMD3100 may impair lymphopoiesis and myelopoiesis, as well as the BM engraftment of repopulating HSCs in NOD/SCID mice [97, 118]. More particularly, the chemokine SDF-1 expressed by human BM vascular endothelium assumes a critical role for the repopulation of BM by circulating $CD34^+$ progenitors, which express CXCR4 by promoting their adhesion to endothelial cells [117].

Cardiac Stem Cells. The clusters of multipotent cardiac stem cells (CSCs) expressing the cell surface antigen Sca-1, KIT, and/or multidrug resistance transporter gene 1 (MDR1) in variable combinations have recently been identified and localized throughout the myocardium, and more particularly, at the atria and lower region of the left ventricle of the heart, termed apex (Fig. 2) [13, 119]. CSCs possess the ability to self-renew and differentiate into three major cell types of the myocardium: myocytes, smooth muscle cells, and endothelial cells. In fact, CSCs appear to be involved in maintenance of heart homeostasis and repair of myocardial tissue after injury. It has been reported that the activation of c-Met and insulin-like growth factor-1 (IGFR-1) receptors by their respective ligands, HGF and IGF, in CSCs and the early TA cell progenitors may contribute to their proliferation, survival, and migration [120]. In addition, the undifferentiated and multipotent cardiac neural crest cells expressing specific neural cell markers, such as nestin, Msi-1, and MDR1, have also been identified in the heart [121]. These multipotent neural crest stem cells might be differentiated ex vivo in serum-free medium in cardiac cell progenitors expressing a different marker pattern including the neuron-, glia-, smooth muscle cell-, or cardiomyocyte-specific proteins. It has also been reported that the transplantation of cardiosphere-derived cells into chick embryos resulted in their migration to the truncus arteriosus and cardiac outflow tract, where they contribute to the cell population in dorsal root ganglia, spinal nerves, and aortic smooth muscle cells [121]. Thus, it appears that the multipotent quiescent CSCs and neural crest stem cells that reside in the adult mammalian heart may contribute to the regeneration of different cardiac cell lineages following injury.

Stem Cells of Ectodermal Origin

Neural Stem Cells. During prenatal development of the mammalian CNS, the neural stem cells (NSCs) and their progenitors may expand and give rise to the functional neurons and glial cells that constitute the growing brain [122, 123]. Similarly, in the adult CNS of mammals, a small number of NSCs, which possess astrocytic morphology and high telomerase levels, are also able to self-renew and generate different neural cell lineages including TuJ1-positive neurons, glial fibrillary acidic protein-positive astrocytes, and O4-positive oligodendrocytes under specific microenvironmental stimuli [6, 124–127]. NSCs appear to be principally localized in two regions in adult mammalian CNS (Fig. 2). More specifically, one population of NSCs resides in the subventricular zone (SVZ) around the lateral ventricle in the forebrain and can differentiate into neurons and glia. The other population, which is localized in the subgranular cell layer of hippocampus in a region termed dentate gyrus, may give rise to granule cell projection neurons. NSCs are also localized in close proximity to a perivascular basal lamina (BL) within the SVZ, which is composed of laminin, collagen-1, fibroblasts, and macrophages and appears to assume a critical function for the maintenance of NSC homing [6, 124, 126]. In fact, NSCs might expand within these niches and give rise to more differentiated progenitors that, in turn, may migrate to distinct distant sites. Although several reports revealed that neural crest stem-derived progenitors may migrate from SVZ into the olfactory bulb in the rodent brain, no available evidence suggests that this could also be prevalent in the human brain [127]. Hence, the generation of new differentiated neural cells from adult neural stem cells in the specific brain regions during a lifespan might assume the maintenance of CNS homeostasis and functions, particularly for learning and memory.

In Vivo Proliferation and Differentiation of NSCs. Several studies have been undertaken to establish the microenvironmental and intrinsic factors that might influence the behaviors of adult NSCs in vivo. Among the numerous growth factors and adhesion molecules that may be involved in the regulation of proliferation, maturation, and/or migration of adult NSCs, there are EGF, bFGF, SHH, Wnt/β-catenin, Notch 1 ligand jagged 1,

platelet-derived growth factors (PDGFs), ciliary neutrophic factor, VEGF, thyroid hormone T3, dopamine, NGF, neuregulins, BMPs, TGF-β, Ephrins/Ephs, leukemia inhibitory factor (LIF), and integrins [6, 122, 124, 126, 128–130]. More specifically, the EGF-EGFR system and β1-integrins appear to act in cooperation to promote the proliferation, survival, and migration of NSCs [129, 131, 132]. In contrast, ephrin-A2 and Eph-A7 may reduce the proliferation and/or migration of neural progenitor cells [128]. Furthermore, SHH is also expressed locally in both adult cortex and cerebellum, the regions that are associated with an elevated rate of cell proliferation and gliogenesis [133]. In vivo analyses of SHH expression and activity have indicated that the quiescent NSCs and their TA cell progenitors in the SVZ and dentate gyrus region in the adult mouse forebrain might respond to SHH by undergoing a marked expansion [133]. In support of the critical role of these mitogenic cascades in the regulation of the proliferation of NSC progenitors, it has been reported that the sustained activation of EGF-EGFR and SHH-patched receptor (PTCH) pathways may contribute to brain tumor formation [33, 38, 134]. A brain tumor stem cell population expressing the NSC marker CD133 and able to self-renew has been isolated from tumors of patients with medulloblastoma, suggesting that the malignant transformation of NSCs may lead to brain tumor development [135]. In addition, it has been observed that the adult mammalian NSCs also express Flk-1/VEGFR-2 and that the infusion of VEGF in the lateral ventricle may stimulate their proliferation [136]. This suggests that the endogenous VEGF from endothelial cells might also contribute of paracrine fashion to the NSC activation in vivo. Based on the knowledge of the factors involved in the regulation of embryonic and adult NSC growth, survival and differentiation in vivo, several new methods for in vitro expansion and differentiation of embryonic and adult NSCs have been conceived.

In Vitro Expansion and Differentiation of NSCs. Results from numerous studies have revealed that the human and rodent NSC progenitors derived from ESCs, UCB, fetal brain, MSCs, or skin-derived stem cells or isolated from adult brain tissues might be expanded in vitro or ex vivo in floating clusters called neurospheres in the presence of exogenous EGF, bFGF, SHH, and/or LIF. Moreover, the withdrawal of these mitogens and the addition of serum, RA, BNP, TGF-β type III, and/or ascorbic acid may promote their differentiation in the three major neuronal cell types, including neurons, astrocytes, and oligodendrocytes (Table 1) [14, 16, 21, 22, 59, 137]. In addition, it has been observed that the coculture of NSCs from mouse cerebral cortex at embryonic day E10–11 with endothelial cells leads to an extensive production of neuron-like cells in vitro, supporting the possibility that the endothelium within the niche might also contribute to the stimulation of NSC self-renewal [138]. Hence, together these works have identified certain growth factors and cytokines that might promote the expansion and differentiation of embryonic, fetal and adult NSCs into the specific neuronal cell lineages and possibly constitute the basis for the development of novel therapies for a variety of CNS disorders.

Skin Stem Cells. In adult mammalian skin, the epithelial compartment consists of the interfollicular epidermis (IFE) and its related appendages, such as the hair follicles and sebaceous glands. Numerous studies have revealed that the upper region of hair follicles, the bulge area, constitutes the principal niche of multipotent stem cells, which are responsible for the long-term growth of the hair follicles and epidermis regeneration after injury (Fig. 2) [4, 10, 139–143]. More specifically, multipotent epithelial stem cells (bESCs) within the bulge area, which express CD34, K5, and α6-integrin, are able to proliferate and give rise to the follicular epithelium, as well as to new cells constituting IFE and sebaceous glands after severe injury. In fact, bESC progenitors can emigrate along the outer root sheath (ORS), forming the outermost layer toward germinal matrix and dermal papilla during the hair cycle. It has been reported that the induction of telomerase in mouse skin epithelium caused a rapid transition from the resting phase of the hair follicle cycle (telogen) into the active phase (anagen) by promoting stem cell mobilization and proliferation [144, 145]. In this matter, several growth factors, including SHH, Wnt/β-catenin, and BMP appear also to contribute to the maintenance and/or regeneration of the hair follicles [142, 143, 146]. For instance, the upregulated expression of the HMG-box-containing gene Sox9 induced through the activation of SHH signaling cascade seems to assume an important role for hair growth induction by directing the differentiation of the hair stem cells that migrate from the bulge niche along ORS cells toward the hair bulb [146].

The bulge area in adult mammalian hair follicle also contains a pluripotent epidermal neural crest stem cell (eNCSC) population that shows several properties similar to embryonic neural crest stem cells (Fig. 2) [147]. In fact, it has been proposed that eNCSCs, like bESCs, might emigrate from the bulge region during the hair cycle and migrate along the entire length of hair follicle in the inner layers of ORS toward the base of hair follicles. More specifically, the dermal papilla is also enriched in eNCSC progenitors that might give rise to the progeny constituting the germinal zone matrix, whose cells, in turn, can proliferate and differentiate into mature cells and form a new hair follicle [147]. The pluripotent eNCSCs in the bulge area are also able to self-renew and give rise to multiple cell lineages in vivo, including melanocytes, neurons, Schwann cells, smooth muscle cells, and chondrocytes [147]. Moreover, eNCSCs in the bulge area may also differentiate to other neural crest derivatives, Merkel cells, which are characterized by specific marker K8 and whose cells remain localized around the bulge zone [147]. In this matter, the ex vivo culture of a tumorigenic cell subpopulation from metastatic melanomas corresponding to an enriched $CD20^+$ fraction of melanoma cells in the growth medium, which is used for human embryonic stem cells, may result in their propagation in the form of nonadherent spheres [30]. In fact, it has been observed that each individual multipotent cell from melanoma spheres was able to differentiate, like eNCSCs under well-defined conditions, into multiple cell types, including melanocytes, adipocytes, osteocytes, and chondrocytes [30]. These results suggest, then, that certain melanoma types may have derived from the malignant transformation of eNCSCs or their progenitors. In addition, the multipotent adult skin-derived precursors (SKPs), which reside within the dermal papillae of hair follicles, also appear to exhibit properties similar to those of eNCSCs [148, 149]. SKPs can be expanded in the presence of EGF, bFGF, and TGF-β and give rise to neurons, glia, smooth muscle cells, and adipocytes in culture in vitro. Thus, it will be important to establish whether SKPs correspond

to the progenitors that are generated from more primitive cells, such as eNCSCs, which can migrate from bulge area toward the bulb region. Altogether, these observations indicate that eNCSCs and their progenitors might represent another source of epidermal and neural cells for skin and brain engraftment. As a matter of fact, it has been reported that the multipotent human skin-derived $AC133^+$ cells expressing the CD34 and Thy-1 antigens were able to migrate throughout the forebrain and differentiate into astrocytes and endothelium after injection into the adult mouse brain [150].

In addition, the keratinocytes constituting the basal layer of epidermis also need to undergo a continuous expansion to replenish the terminally differentiated epithelial cells in the corneous layer of normal epidermis, which are shipped out of skin during the desquamation process [35]. The small clusters of multipotent stem cells, which express the specific markers, including K15, appear to reside near the basement membrane of the epidermis (Fig. 2) [151, 152]. These basal keratinocyte stem cells (KSCs), which possess a self-renewal capacity, express high levels of adhesion molecules such as β1-integrins, E-cadherin and β-catenin. In fact, the high expression levels of β1-integrins and Notch one ligand Delta one in putative KSCs may contribute to their intercellular adhesion and be responsible, in part, for their restricted mobility [151, 152]. Furthermore, the self-renewal versus differentiation of KSCs appears also to be regulated in part by the expression levels of Rho guanosine triphosphatase, Rac1, and Myc. As a matter of fact, it has been reported that Myc activation in undifferentiated keratinocytes or Rac1 epidermal deletion, which may result in the upregulation of c-Myc expression, might lead to exit of basal keratinocytes from stem cell niche into TA cell compartment [153]. In fact, the TA cells, which are characterized by specific markers, including CD98, are more mobile than KSCs and can give rise to more differentiated keratinocytes of all epithelial layers. In addition, it has been proposed that multiple growth factors, including EGF, might also be involved in regulating the high rate of proliferation of undifferentiated keratinocytes localized in the basal epidermal layer and preventing their differentiation [35, 154]. An opposed effect can be mediated by TGF-β. With regard to this, the sustained activation of EGF-EGFR and SHH-PTCH-GLI cascades or increased expression of Rac1 may also contribute to the maintenance of the proliferative basal and/or suprabasal cell populations in the benign hyperproliferative disorders such as wound healing as well as in basal cell carcinomas (BCCs) and/or squamous cell carcinomas (SCCs) [33, 35, 143, 153]. In this matter, since bESCs might also give rise to cells constituting IFE after injury, additional investigations appear necessary to determine the specific implication of bulge stem cells versus KSCs residing near the basement membrane during the different stages leading to the epidermis regeneration after trauma and carcinogenesis.

Ocular Stem Cells. The human ocular surface epithelium includes the corneal, limbal, and conjunctival stratified epithelia. Several recent lines of evidence have revealed that the corneal epithelial stem cells (CESCs) are localized at the basal cell layer of the peripheral cornea, and particularly at the limbus within the limbal epithelial crypts (Fig. 2) [3, 155–157]. Furthermore, the conjunctival epithelial stem cells appear to be enriched in the bulbar and forniceal conjunctiva. The limbal CESCs, which express several markers, including p63, ABCG2, α9- and β1-integrins, EGFR, K19, α-enolase, and CD71, possess the ability to reconstitute an intact and functional corneal epithelium in vivo [3, 157, 158]. More specifically, CESCs can give rise to the progenitors that are able to migrate at the corneal epithelium. These corneal cell progenitors show a rate of proliferation inferior to that of CESCs in vitro and correspond to a basal cell population that is involved in the replenishment of the corneal epithelium cells, which have a limited lifespan of less than 1 year [3]. On the other hand, the limbal CESCs in the adult cornea are also able to trans-differentiate into neurons and glia-like cells ex vitro in the presence of BMP and under heterotopic transplantation [159]. In addition, it has been reported that the putative stem cells, which are sequestered in a niche localized at the region between the corneal endothelium cells and the trabecular meshwork, may also give rise to both the corneal endothelium and trabeculae [160]. These stem cell-derived TA cells could also migrate at the endothelial periphery, suggesting their possible implication in eye endothelium regeneration.

A small population of mitotic quiescent neural stem cells has also been identified in the ciliary epithelium (CE) region adjacent to the retina in adult mammalian eyes, which may proliferate in response to retinal injury in vivo or after treatment with specific exogenous growth factors in vitro (Fig. 2) [161–163]. These multipotent CE stem cells also designated retinal stem cells (RSCs), which are able to self-renew, express several specific stem cell markers, including telomerase, neural markers such as nestin, and retinal progenitor markers such as Pax 6 [161, 162]. Moreover, RSCs in CE may differentiate in vitro into distinct adult retinal progenitor populations, including retinal ganglion cells, as well as rod photoreceptors, bipolar cells, and Müller glia, which are derived from early and late stages of retinal histogenesis, respectively [161, 162]. Analysis of the gene expression pattern indicated that RSCs show more resemblances to early than late born retinal neurons [162, 164]. More particularly, RSCs express the higher levels of FGF receptor 1 than EGFR and respond preferentially to bFGF than EGF in vitro. This then suggests that RSCs in CE may represent a primitive cell population that may respond preferentially to bFGF at early differentiating stage and, subsequently, to EGF at a later stage [137, 162]. Furthermore, the proliferation and/or differentiation of RSCs in CE may also be regulated through the activation of other mitogenic and differentiation signaling, such as hedgehog, KIT, and Notch signaling pathways, which are also known to be important regulators of neurogenesis [162, 164, 165]. The intraocular injection of SHH results in stimulation of the proliferation of RSCs in CE, whose proliferative effect was also inhibited in the presence of the Smoothened (SMO) signaling element inhibitor, cyclopamine [165]. Hence, the discovery in the adult eyes of limbal CESCs and RSCs in CE, which possess immature epithelial and neural cell properties, respectively, offers the possibility to use these stem cell types for the repair of corneal epithelium and retinal damages.

STEM CELL-BASED THERAPIES

The possibility of using stem cells and their more differentiated progenitors to treat numerous degenerative disorders has stim-

ulated great interest in developing safe transplantable sources of stem cells that are unable to form the teratomas but able to repopulate damaged tissues. Many recent investigations have provided interesting clinical findings about the possibility of using the progenitors derived from ESCs, AECs, FSCs, and UCB and BM stem cells, as well as other adult tissue-specific stem cells in genetic and cellular therapies for a wide variety of pathological processes, including degenerative, autoimmune, and genetic diseases. Clinical transplantation procedures for stem cells, which depend on patient state and diagnosis, generally involve the i.v. injection or subcutaneous administration of a specific number of stem cells directly into therapeutically targeted areas (Fig. 2). Among the disorders that might benefit from stem cell-based therapy are diabetes, acute liver and heart failures, muscular disorders, arthritis, brain damages and disorders, vision disorders, renal disorders, and hematopoietic and immune diseases, as well as acute leukemia and lymphoma and diverse solid tumor types [11, 13, 16, 17, 19, 23, 25–27, 78, 166]. In addition, the high plasticity and migratory potential of BM stem cells also offer the possibility of mobilizing them in vivo or injecting these stem cell types in circulation to regenerate the particular functional progenitors for the tissue regeneration (Table 1; Fig. 2) [68, 167, 168]. Moreover, gene-based strategies involving modifications or replacement of a particular gene product, such as MDR1, in stem cells and their more differentiated progenitors before their transplantation might now be conceived for the treatment of diverse genetic and incurable diseases [169, 170]. Nuclear transfer, in which the nucleus from donor somatic cells is transferred into an enucleated oocyte to obtain the pluripotent embryonic stem cells, offers another alternative source for the derivation of primitive stem cells for cell replacement therapy when no donor organ is available for transplantation [171]. For instance, it has been reported that the systemic infusion of human BMSCs transfected with a gene for human coagulation factor IX (hF1X) resulted in the secretion of the biologically active hF1X form into the circulation in SCID mice [170]. The transplantation of NSCs also constitutes a new stem cell-based delivery system for gene therapy in brain disorders and invasive tumors. As a matter of fact, it has been reported that NSCs might migrate throughout the brain and target the invading tumor cells, the effect of which appears to be mediated, at least in part, by upregulation of VEGF in the tumor that attracts the NSCs to tumoral sites [172]. In addition, molecular targeting of tumorigenic cascade elements in tissue-specific cancer progenitor cells, which are derived from the malignant transformation of adult stem cells, also represents a novel approach for the treatment of diverse metastatic and incurable cancer types by combination therapies. We report the recent advances on the more promising stem cell-based strategies that have been developed in past years for the treatment of numerous degenerative disorders and aggressive cancer types.

Regenerative Medicine

Pancreatic Diseases. Numerous studies have been undertaken to establish the insulin-producing β-cell-like progenitors for the development of new treatments of type 1 and 2 diabetes mellitus. In fact, the replacement of insulin-producing pancreatic islet β-cells that are destroyed or reduced in number results in the insulin deficiency and fasting hyperglycemia and constitutes a promising strategy. The β-cell-like progenitors derived from human and mouse ESCs, fetal and UCB stem cells, and adult stem cells from pancreas and BM, showing the capacity to secrete insulin, have been obtained in vitro [19, 22, 24, 40, 47, 78, 173–175]. Their in vivo characterization has also revealed their capacity to improve the symptoms of diabetic mice. For instance, it has been reported that the pancreatic ductal epithelial stem cells isolated from adult NOD mice might be differentiated ex vivo into the functional islet β-cell-like progenitors that are able to reverse type 1 insulin-dependent diabetes by implantation into diabetic mice [173]. It is of therapeutic interest that the subcapsular implantation of human fetal nestin-positive putative PSC-derived ICCs in NOD/SCID mice has also been observed to result in a marked reduction in blood glucose levels [174]. The results from a recent in vivo study have indicated the possibility of improving the functional properties of human fetal ICCs after transplantation in diabetic mice by post-treatment with a long-acting analog of glucagon-like peptide 1 and exendin-4 [175]. In fact, the transplanted diabetic mice treated with extendin-4 for 3 months were characterized by the presence of insulin-producing cells. The blood glucose levels and body weight were also improved as compared with the transplanted group not subjected to exendin-4 treatment. Similarly, it has also been reported that the combination of EGF and gastrin can stimulate the regeneration of β-cells in vivo and reverse hyperglycemia in NOD mice [176]. On the other hand, it has been observed, by using a murine sex-mismatched model, that the BM transplantation resulted in a small number of insulin-producing cells expressing the Y chromosome from donor mice in the pancreas of host mice [177]. However, the results from another study revealed that the trans-differentiation of BM cells in insulin-producing cells in vivo before their subcapsular renal transplantation into hyperglycemic mice resulted in a significant reduction of blood glucose levels that was maintained for up to 90 days post-transplantation [178]. Moreover, it has been observed that the implantation of HOCs in a diabetic NOD/SCID-mouse was accompanied by their trans-differentiation into insulin-secreting β-cells and that this event reversed hyperglycemia [179]. This observation then suggests that HOCs could also constitute another source of pancreatic cells for the treatment of diabetes. Thus, it now appears possible to conceive the development of cell replacement therapies for human diabetic patients by using human stem cell-derived β-cells. Nevertheless, additional work on the identification of specific factors that are involved in the migration and trans-differentiation of BM and liver stem cells into insulin-producing islet β-cell-like progenitors in vivo is needed. In addition, the functional properties of ex vivo and in vitro expanded β-cells in distinct animal models in vivo are essential. Optimization of these protocols is essential before their translation into clinical applications for humans. In this matter, the establishment of in vitro methods for obtaining a large amount of functional β-cells and the optimization of delivery site are also important factors that must be considered for improving their therapeutic efficacy in the long-time treatment of patients.

Cardiac Diseases. The generation of new myocytes from endogenous multipotent CSC pools offers the possibility for maintaining cardiac homeostasis and tissue regeneration after heart hypertrophies and failures. The stimulation of the proliferation, survival, and/or differentiation of the endogenous CSCs and their progenitors may be performed by using the specific growth factors that are involved in the control of cardiogenesis and neo-angiogenesis, such as VEGF, HGF, IGF-1, EPO, TGF-β, HIF-1α, and IL-8 [120, 168]. As a matter of fact, the injection of HGF and IGF-1 in the hearts of mice with infarcts that were up to 86% of the ventricle has notably been observed to promote myocardial regeneration and enhance their survival [120]. However, the senescence and death of primitive CSCs and their mature progenitors with myocardial aging, as well as in chronic ischemic cardiomyopathies, might lead to persistent heart disorders [180]. More specifically, the death of cardiomyocytes, which might be caused by an interruption of blood circulation in the heart, might result in heart failures that are associated with a gradual loss of pumping power of the heart and cardiac attack. In these pathological cases, the myocardial tissue regeneration by inducing the formation of new cardiomyocytes and coronary vessels might represent a putative therapeutic strategy. Certain works have indicated the possibility of delivering CSCs within the myocardium intravascularly to regenerate the cardiomyocytes after heart failure of ischemic and nonischemic origin [13, 119, 181]. More specifically, it has been reported that the injection of c-kit-positive CSCs into ischemic hearts leads to their engraftment and differentiation into new cardiomyocytes, smooth muscle cells, and vascular endothelial cells that reconstituted a well-differentiated myocardial wall [13, 119]. Similarly, the delivery of CSCs into the coronary arteries in the infarcted myocardium of rats after reperfusion has been reported to promote the myocardial repair and improve cardiac functions [181]. Despite this advance, the lack of knowledge of the molecular mechanisms that are responsible for human CSC (hCSC) senescence, as well as the difficulties associated with the procedures of the isolation and in vitro expansion of functional hCSCs, limit their clinical applications as effective therapies for myocardial regeneration.

In addition, another alternative may consist of the expansion and trans-differentiation of other stem cell types into cardiac cell progenitors in vitro or in vivo under specific conditions that may also enable them to engraft and regenerate the functional cardiomyocytes and endothelial cells for the reconstitution of damaged myocardium after injury. More particularly, several recent investigations indicated that the transplantation of ESC- and BM stem cell-derived cardiac cell progenitors might result in stable and functional intracardiac grafts in human and rodent, thereby improving cardiac functions by regenerating the infarcted myocardium [13, 182, 183]. Furthermore, the in vivo mobilization of HSCs and MSCs and their progenitors from BM into circulation by using specific cytokines, such as G-CSF, and increase of their rate of incorporation and homing in infarcted heart with growth factors such as VEGF might also represent another attractive strategy to improve the regeneration of the myocardium and coronary vessels (Fig. 2) [167, 168]. Hence, it appears that the ESC- and BM stem cell-derived cardiac cell progenitors could constitute a more accessible source of stem cells than CSCs for the treatment of diverse cardiac disorders. Additional long-term trials on large populations in human are necessary to establish the functional properties of these cardiac progenitors and their efficacy for the recovery of cardiac function after transplantation.

CNS Disorders and Diseases. The stimulation of adult neurogenesis in vivo by supplying specific growth factors and cytokines, such as G-CSF and SCF, that stimulate intrinsic NSC progenitors and BM-derived neuronal cells or delivery of exogenous NSC progenitors constitute the novel strategies that are very promising for restoring the impaired functions in numerous damaged brain areas after ischemia, seizures, and traumas or in diverse degenerative disorders (Table 1; Fig. 2) [14, 16, 46, 184–188]. As a matter of fact, it has been reported that the intraventricular infusion of recombinant FGF and EGF recruited the novel endogenous neuronal progenitors that contributed to a massive regeneration of hippocampal pyramidal neurons after ischemic brain injury in Wistar rats [184]. Moreover, several in vivo investigations on animal models have also revealed that the delivery of NSCs or their progenitors in brain regions could represent an effective strategy for the treatment of neurological diseases, such as stroke, and certain neurodegenerative disorders, including Huntington disease, Parkinson and Alzheimer diseases, and spinal cord injuries [185, 189]. For instance, it has been reported that the i.v. infusion of human UCB stem cells in the rat stroke model that had been previously subjected to a permanent middle cerebral artery occlusion rescued the behavioral deficits and reduced stroke infarct volume in a dose-dependent manner [185].

Parkinson and Alzheimer Diseases. The motor dysfunctions that are associated with Parkinson disease result from the progressive loss of dopaminergic neurons in the substantia nigra pars compacta, a region of brain that controls muscle movement. Therefore, cell replacement therapy by delivering new dopaminergic neurons represents a putative strategy for the treatment of this neurodegenerative disease [16, 46, 190]. A high yield of differentiation of the mouse and human ESCs into the midbrain TH^+-dopaminergic neurons has been obtained in vitro in a stromal cell-derived inducing activity system or by coculture with PA6 cells [22, 46]. Significantly, these neuron-like cells expressed the specific markers of dopaminergic neurons, including the dopamine transporter, aromatic amino acid decarboxylase, and the transcription factors associated with neuronal and dopaminergic differentiation (Sox1, Nurr1, Ptx3, and Lmx1b) [46]. These cells were also transplantable, and a small number survived in 6-hydroxydopamine-treated mouse or rat striatum. In addition, Alzheimer disease, which is characterized by regional neuronal degeneration and synaptic loss and associated with the presence of senile plaques, also represents a degenerative disorder that could benefit from neural cell delivery in the brain [191, 192].

Spinal Cord Injuries. Since the major cause of neurological disability in spinal cord injuries is oligodendrocyte death leading to demyelization and axonal degeneration, rescuing the oligodendrocytes and preserving myelin should result in a significant improvement in the functional outcome after such injuries. It has been reported that the addition of oligodendrogenic factors such as SHH, bFGF, and PDGF might result in the

differentiation of NSCs into oligodendrocyte progenitor cells (OPCs) in vitro. However, the rate of OPC differentiation of NSCs under these conditions was only approximately 10%. Interestingly, the transient expression of oligodendrogenic helix-loop-helix transcription factor Oligo 2 in NSCs, which is essential for oligodendrocyte lineage specification, may induce a high rate (approximately 55%) of OPC differentiation and generate the mature and functional oligodendrocytes expressing the transcription factor Nkx2.2 and all major myelin-specific proteins [193]. The transplantation of the oligodendrocyte precursors in combination with SHH also resulted in improved function and white matter sparing in the spinal cords of adult rats after contusion [186]. Furthermore, certain studies have also revealed the possibility of using the fetal spinal cord tissue from embryonic day 14 (E14/FSC) or the isolated neuronal and glia-restricted precursors from E14/FSC in transplantation for spinal cord injuries [194]. Thus, on the basis of these observations, it now appears possible to conceive novel stem cell-based therapies for a variety of brain disorders. At this time, there exist only palliative treatments for these diseases. In this matter, the development of new strategies for the delivery of NSCs and their progenitors in the specific brain damaged areas or selective activation of endogenous NSC-derived progenitor subset should also contribute to improving their potential therapeutic applications in humans.

Ocular Disorders and Diseases. CESCs and RSCs, isolated from the limbal epithelium and CE of adult eyes, respectively, are able to incorporate and reconstitute the damaged tissues in vivo. Therefore, they may be transplanted for the repair and regeneration of ocular surface damages. Several types of treatment for ocular disorders have been developed based on the use of limbal CESC transplants and bioengineered ocular surface tissue-equivalents for the replacement of ocular damaged tissues [156, 195]. More specifically, the choice of treatment depends on several factors, including the severity of corneal damage and the degree of limbal CESC loss. In a situation where the limbal area of the peripheral cornea is destroyed, such as in the Stevens-Johnson syndrome, ocular pemphigoid, and severe ocular burns, limbal CESC grafts combined with amniotic membrane transplantation might be used for reconstruction of the ocular surface [195, 196]. A new strategy for the treatment of limbal CESC deficiency includes a limbal biopsy in healthy donor eyes followed by the ex vivo expansion of limbal CESCs by using a substrate such as human amniotic membrane carrier, in which the cells might proliferate, migrate, and regenerate a new tissue [156, 196, 197]. Hence, this strategy provides the bioengineered corneal surface tissues with anti-angiogenic and anti-inflammatory properties due to the presence of amniotic membrane; the corneal surface tissues might be successfully transplanted onto diseased eyes to restore the structure and function of damaged ocular surfaces and the visual acuity. For instance, the results from a survival analysis of conjunctival limbal grafts concomitant with amniotic membrane transplantation in 33 eyes from 31 patients with total limbal CESC deficiency have indicated a graft cumulative survival of 33% after a mean follow-up time of 33 months [198]. There was an increase in postoperative visual acuity in 60.6% of patients during this period [198]. Moreover, the reconstruction of chemically burned rat corneal surfaces has also been performed by using human MSCs from healthy donors after expansion on amniotic membrane [199]. In fact, as suggested by the authors of this study, the therapeutic effect of the MSC transplantation may also be due, in part, to an inhibition of inflammatory and angiogenic processes rather than the epithelial differentiation of MSCs [199]. In addition, certain recent observations have also indicated that the transplantation of RSCs, NSCs, ESCs, and BM stem cells could also serve as the alternative sources of retinal cell progenitors for treating diverse degenerative diseases of the retina, including glaucoma, and certain disorders associated with retinal scarring by cell replacement [48, 165, 200]. Further investigation of the behaviors of endogenous CESCs and RSCs and their progenitors in eyes should allow us to conceive new methods for the functional recovery of partial ocular defects by their stimulation by diverse exogenous growth factors. Additional in vivo trials on the functional properties of ex vivo and in vivo expanded CESCs and RSCs, as well as the precise functions of amniotic membrane, are also necessary to establish the molecular mechanisms that are responsible for their therapeutic effects. These studies should contribute to improving the long-term survival rate of these adult stem cells after engraftment and thereby to their beneficial effects for the treatment of eye diseases.

Blood and Immune System Disorders. HSCs and their progenitors, which might be obtained from different sources including UCB, fetus, BM, and MPB, represent potent cells for autologous or allogeneic transplantation in the patients with hematological and autoimmune diseases [15, 17, 22, 23, 27, 76]. In this matter, the injection of HSCs or total bone marrow cells (BMCs) performed via the portal vein, intravenously, or directly into BM might generate the hematopoietic cell lineages in peripheral blood that, in turn, may contribute to immune system recovery and tissue repair in multiple organ systems. For instance, it has been reported that the infusion of large numbers of highly purified $CD34^+$ stem cells might promote immune recovery in children with refractory severe aplastic anemia and severe infections [201]. Moreover, it has been reported that the treatment of MRL/1pr mice with autoimmune diseases by fractionated irradiation followed by intra-BM injection of BMCs from allogeneic normal C57BL/6 mice resulted in an increase in donor-derived hematopoietic and stromal cells in BM concomitant with a rise of donor-derived hematopoietic progenitor cells [202]. Significantly, the MRL/1pr mice treated by this method survived more than 1 year and showed no sign of recurrence of autoimmune diseases. In addition, in vivo mobilization of HSCs and their progenitors from BM into the bloodstream might also be performed by using agents that are able to interfere with the SDF-1-CXCR4 system, such as G-CSF and AMD3100 (Table 2; Fig. 2) [97, 118, 203]. The use of a CXCR4 antagonist, bicyclam AMD3100, can mobilize $CD34^+$ stem cells and endothelial progenitor cells from BM into the bloodstream and thereby enhance their migration into damaged sites, where they may contribute to neovascularization and tissue regeneration [118]. Moreover, certain congenital diseases of a fetus may be treated by intracoelomic human HSC transplantation [204].

Table 2. Deregulated gene products in cancer cells and their molecular targeting as anticarcinogenic therapies

Type of therapy	Name of agent
Growth factor signaling inhibitor	
Anti-EGFR (erbB1) antibody	mAb-C225, IMC-C225, EKB-569
Anti-EGF antibody	ABX-EGF
Antisense oligonucleotide	As-EGFR, As-EGF, As-TGF-α
Anti-EGFR, EGF, or TGF-α toxin	425(scFv)-ETA′, DAB389EGF, TP40
EGFR-TKI	AG1478, gefitinib, erlotinib
Anti-erbB2 antibody	Trastuzumab
EGFR/ErbB-2	PKI-166, TAK165, GW572017 (lapatinib)
erbB1, erbB2, erbB3, erbB4 inhibitor	Cl-1033
FGF-1R signaling inhibitor	AdtrFGFR-1
EGFR, VEGFR	AEE788, ZD6474
PDGFR, VEGFR, FGFR	SU6668
IGFR	Adenovirus-IGF-Ir/dn, A12
Hedgehog	SMO inhibitor cyclopamine, anti-SHH antibody
Wnt signaling	Anti-Wnt antibody, WIF-1
Notch signaling	γ-Secretase inhibitor
AR	Bicalutamide, flutamide
ER	Tamoxifen, raloxifen
Element signaling inhibitors	
Telomerase	Telomerase template antagonist
Myc	As-Myc
PI3K inhibitor	LY294002, rapamycin, CCI-779
NF-κB inhibitor	IκBα inhibitor, sulfasalazine, or PS-341
Ceramide	S18 (teratomas), B13
VEGF	Anti-VEGF antibody
VEGFR	Anti-VEGFR antibody, SU5416
COX-2	NS-398, etodolax, celecoxib, rofecoxib
SDF-1	Anti-SDF-1 antibody
CXCR4	Anti-CXCR-4 antibody, CXCR4 antagonist (TC14012, TN14003, or AMD3100)
Chemotherapy	
Platinum derivative	Cisplatin, carboplatin, oxaliplatin, JM-216, AMD473
Taxanes	Paclitaxel, docetaxel
Topoisomerase II inhibitor	Etoposide
Mitoxantrone, Prednisone, Estramustine	
Gemcitabine, 5-fluoracil	
Immunotherapy	Dendritic cells
Combination therapy	
EGFR plus AR inhibitors	Gefitinib plus bicalutamide
EGFR plus ER inhibitors	Gefitinib plus tamoxifen
EGFR plus hedgehog inhibitors	Gefitinib plus cyclopamine
EGFR, VEGF, PDGF	AEE788 plus ST1571
EGFR inhibitor plus radiation, chemotherapy, or photodynamic treatment	
HDCT plus HSCs, G-CSF, or AMD3100	

Abbreviations: Adtv, adenoviral vector coding truncated FGFR-1; As, antisense; AR, androgen receptor; COX, cyclooxygenase; CXCR, CXC-chemokine receptor; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; ER, estrogen receptor; FGFR, fibroblast growth factor receptor; G-CSF, granulocyte colony-stimulating factor; HDCT, high-dose chemotherapy; HSC, hematopoietic stem cell; I$\kappa\beta\alpha$, inhibitor of nuclear factor-$\kappa\beta\alpha$; IGF, insulin-like growth factor; IGFR, insulin-like growth factor receptor; mAb, monoclonal antibody; NF-κB, nuclear factor-κ B; PDGFR, platelet-derived growth factor receptor; PI3K, phosphatidylinositol 3-kinase; SDF, stromal-derived factor; SHH, Sonic hedgehog; SMO, Smoothened; TGF, transforming growth factor; TKI, tyrosine kinase inhibitor; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor; WIF-1, Wnt-inhibitory factor-1.

Cancer Therapies

Numerous studies have indicated that several human cancer types, including those of the blood, brain, skin, lung, kidneys, gastrointestinal tract, pancreas, liver, ovarian, prostate, and testis, might arise from the malignant transformation of stem cells and their progenitors into cancer progenitor cells [2, 7–9, 18, 30, 31, 33, 37, 38, 72]. In fact, the formation of precancerous lesions during tissue injury such as chronic proliferative inflammatory atrophy might notably lead, in certain cases, to the development of certain cancer types. More specifically, somatic genomic alterations, such as mutations, deletions, amplifications, chromosomal rearrangements, and change in DNA methylation, might result in the aberrant activation of distinct developmental cascades in adult stem cells and/or TA cells. These activated cells may generate cancer progenitor cells (Fig. 3). These cancer progenitor cells, in turn, may subsequently give rise to a heter-

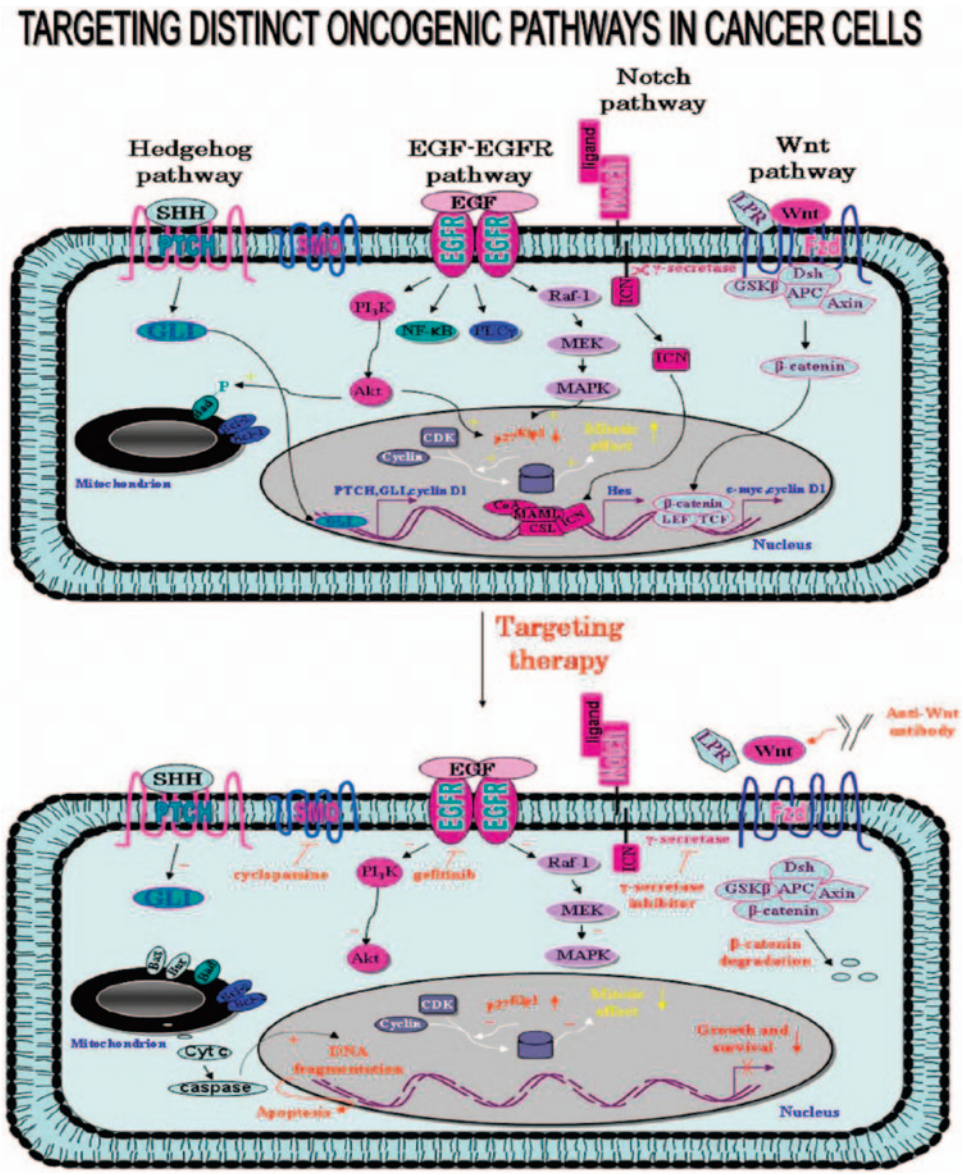

Figure 4. Proposed strategies for the development of novel combination therapies by targeting distinct oncogenic signaling pathways in cancer cells. The possible mitogenic and anti-apoptotic cascades induced through EGFR, hedgehog, Notch, and Wnt/β-catenin signaling pathways, which can be involved in the stimulation of sustained growth, survival, and/or migration of cancer cells, are shown. Moreover, the possible growth inhibitory and/or apoptotic effects induced by the selective inhibitors of EGF-EGFR system (gefitinib), Smoothened hedgehog signaling element (cyclopamine), Notch signaling (γ-secretase inhibitor), and Wnt cascade by using anti-Wnt antibody on the cancer cells are also indicated. Abbreviations: APC, adenomatous polyposis coli; CDK, cyclin-dependent kinase; CoA, coactivators; Cyt c, cytochrome c; Dsh, Dishevelled; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; Fzd, Frizzled receptor; GSK, glycogen synthase kinase; ICN, intracellular domain of Notch; LEF, lymphocyte enhancer factor; LPR, lipoprotein coreceptor; MAPK, mitogen-activated protein kinase; MEK, extracellular signal-related kinase kinase; NF-κB, nuclear factor-κB; PI3K, phosphatidylinositol 3-kinase; PLC, phospholipase C; PTCH, Sonic hedgehog-patched receptor; SHH, Sonic hedgehog; SMO, Smoothened; TCL, T-cell factor.

ogeneous population of cancer cells showing aberrant differentiation, unlimited division, and a decreased rate of apoptotic death. Cancer progenitor cells, which express stem cell markers and possess the capacity to self-renew, have been isolated from patients with acute myelogenous leukemia (AML), melanoma, brain, breast, prostate, and ovarian cancers [30, 34, 38, 72, 91, 135, 205, 206]. In fact, it has been observed that only these malignant cells were able to give rise to a heterogeneous population of more differentiated cancer cells in vitro and to reconstitute the original cancer-like tumors in NOD mice in vivo. Thus, on the basis of these observations, it appears that the elimination of this minority of cancer progenitor cells with stem cell-like properties, which are responsible for tumor formation, is essential for the development of more effective curative treatments against these aggressive cancer types.

Several in vitro and in vivo studies have been carried out with a variety of cancer cell line types and on different animal models to identify new therapeutic targets to block the growth

and/or survival of the cancer cells. Among them, the molecular targeting of distinct oncogenic signaling elements, which are activated in the cancer cells during the progression of numerous cancer, represents a promising strategy for the development of new chemopreventive treatments and combination therapies against some aggressive and metastatic cancers (Table 2; Figs. 3, 4). The aberrant expression and/or activity of diverse hormones, growth factors, cytokines and chemokines (androgens, estrogens, EGF and TGF-α/EGFR, IGF/IGFR, SHH/SMO, Wnt/β-catenin, Notch, TGF-β, and SDF-1/CXCR4), and tumorigenic signaling elements (telomerase, phosphatidylinositol 3-kinase [PI3K]/Akt, NF-κB, and Myc-1) may contribute to the sustained growth and survival of stem cells, as well as their malignant transformation during the initiation and cancer progression (Figs. 2, 4) [5, 7, 8, 18, 33, 35, 36, 99, 207, 208]. Therefore, their molecular targeting is of importance to the elimination of cancer progenitor cells, thereby inducing a complete tumor regression and cancer remission. We report here a brief description of new therapeutic drugs that are able to block the specific growth factor signaling cascades that are frequently deregulated in the stem cell-derived cancer progenitor cells, as well as the advantages that are associated with the use of high-dose chemotherapy (HDCT) with hematopoietic cell support.

Growth Factor Signaling Inhibitors

EGFR Family Member Inhibitors. Numerous preclinical and clinical trials have indicated that the selective blockade of the EGFR family member signaling, and particularly EGFR (ErbB1) or erbB2 (Her2), might represent a potent strategy, alone or in combination with other conventional treatments for numerous aggressive cancer forms [18, 35, 36, 77, 209–214]. The overexpression of EGFR, ErbB2, and/or their endogenous ligands has notably been associated with the development of skin, lung, colon, prostate, pancreas, breast, brain, and ovarian cancers. Among the selective agents targeting EGFR or ErbB2 signaling, there are antibodies or antisense oligonucleotides directed against EGFR or its ligands EGF and TGF-α, anti-ErbB2 antibody trastuzumab (Herceptin; Genentech, Inc., South San Francisco, CA, http://www.gene.com), and the selective EGFR tyrosine kinase inhibitors such as AG1478, gefitinib (Iressa; AstraZeneca Pharmaceuticals, Wilmington, DE, http://www.astrazeneca-us.com) and erlotinib (Tarceva; OSI Pharmaceuticals, Inc., Melville, NY, http://www.osip.com) (Genentech; Hoffman-La Roche, Basel, Switzerland, http://www.roche.com) (OSI-774) (Table 2) [18, 35, 36, 77, 210, 212–214]. Many in vitro and in vivo studies have revealed that these agents may induce the inhibition of the growth, invasiveness, and apoptotic death of diverse cancer cell types by counteracting distinct mitotic cascades, including MAPK, PI3K/Akt, NF-κB, phospholipase Cγ, and Shc (Fig. 4) [18, 35, 36, 210, 212, 215, 216]. Furthermore, it has been reported that the inhibition of EGFR activity by using AG1478, or with a neutralizing anti-EGFR antibody, may result in an inhibition of the c-Myc expression and suppression of the telomerase expression and activity in the human cutaneous squamous carcinoma cells HSC-1 [209]. The inhibition of EGFR was also accompanied by cancer cell growth inhibition.

Hedgehog, Wnt/β-Catenin, and Notch Signaling Inhibitors. The inactivating mutations in the hedgehog receptor patched (PTCH) and/or activating mutations in the hedgehog signaling element, SMO, might increase the incidence of BCC, medulloblastoma, and rhabdomyosarcoma [33]. Moreover, the aberrant activation of hedgehog cascade may result in the sustained growth and enhanced invasive properties of malignant cells in glioma and in the upper gastrointestinal tract, pancreas, small-cell lung, prostate, and breast cancers [7, 8, 18, 33, 33, 36, 38, 211]. Therefore, the blocking of this tumorigenic cascade represents a promising approach to counteract the initiation and progression of these aggressive cancer forms (Fig. 4). As a matter of fact, the inhibition of hedgehog cascade by using either SMO signaling element inhibitor, cyclopamine alkaloid, or the anti-SHH antibody has been observed to result in vitro and in vivo in a growth inhibition and the apoptotic death of the metastatic cancer cells, whereas normal cells were insensitive to the cytotoxic effects of these agents [18, 33, 36, 207, 208, 211].

In addition, molecular targeting of the canonical Wnt/β-catenin signaling elements (which may contribute to the malignant transformation of cancer progenitor cells and the progression of numerous cancers, including upper gastrointestinal tract, colorectal, pancreatic, mammary, prostatic, and epidermal cancers, as well as non-small-cell lung carcinoma, hepatoblastoma, medulloblastomas, and multiple myeloma) constitutes another anticarcinogenic strategy for the treatment of these cancer forms [5, 7, 18, 33, 36, 38]. Since the inactivating mutations in the Wnt signaling elements, such as adenomatous polyposis coli tumor suppressor gene product or activating mutations of β-catenin, which impairs its degradation, appear to frequently occur in colorectal tumors, it is important to block this cascade when treating this cancer type. In regard to this, the use of the selective anti-Wnt antibody, Wnt protein inhibitors (such as Wnt-inhibitory factor-1), or repressors disrupting nuclear lymphocyte enhancer factor/T-cell factor/β-catenin complexes might counteract the intracellular and nuclear accumulation of β-catenin, thereby inhibiting the proliferation of cancer cells that is induced through the Wnt/β-catenin pathway (Fig. 4) [5, 18, 217, 218]. Similarly, the blockade of the Notch signaling cascade, which also appears to participate in developing certain cancer types, including acute T-cell lymphoblastic leukemia and lymphoma, medulloblastoma, and mucoepidermoid, colorectal, pancreatic, mammary, ovarian, and lung carcinomas, may also represent another targeting approach in the therapeutic interventions against these hyperproliferative disorders [7, 212, 219–222]. For instance, the inhibition of the β- and γ-secretases, whose proteases can cleave the intracellular domain of the Notch transmembrane receptor, thereby permitting its translocation into the nucleus, where it participates in the transcriptional activation of genes, may notably represent a potent therapeutic target for these malignant disorders (Fig. 4) [212, 219–222].

Combination Therapies. The simultaneous inhibition of diverse hormone and growth factor signaling pathways, including ER, AR, IGFR, EGFR, hedgehog, Wnt/β-catenin, Notch, and/or G-protein-coupled receptors, as well as VEGFR and PDGFR cascades, which can act in cooperation by stimulating the growth, invasion, and metastatic spread of cancer cells at distant

sites during the different stages of cancer progression, may also constitute more effective therapies against the aggressive and highly metastatic cancer forms [18, 33, 36, 77, 211, 212, 215, 223]. As a matter of fact, some works have revealed that complex cross-talks may occur among the AR, ER-α/ER-β, EGFR/ErbB2 signaling cascades in prostate and mammary cancers [18, 224]. The combination of the agents that are able to block these tumorigenic pathways may be more effective to treat these epithelial malignancies as a single antihormonal therapy. Furthermore, our recent work has also indicated that the combined use of lower doses of gefinitib and cyclopamine resulted in vitro in the arrest of the growth and a more massive rate of apoptotic death of diverse metastatic and androgen-sensitive LNCaP-C33 and independent prostatic cancer cell lines LNCaP-C81, DU145, and PC3, as compared with the individual drugs [211]. This suggests that the simultaneous blockade of the EGFR and hedgehog pathways could represent a more effective and safe therapeutic treatment against certain metastatic cancer forms by decreasing the secondary effects that are associated with the use of high doses of these agents. Similarly, it has been reported that the activation of EGFR might lead to the cellular accumulation of β-catenin, and Notch and EGFR signaling may cooperate for the sustained growth and invasion of certain cancer cell types [18, 36, 212]. The simultaneous blockade of these cascades by using the aforementioned agents also merits further investigation. In support of this, it has notably been reported that the inhibition of Notch3 signaling in the lung cancer cell lines by using a dominant-negative Notch3 receptor enhanced their sensitivity to the anticarcinogenic effects induced by the EGFR tyrosine kinase inhibitor AG1478 [212]. The results from a recent study have also revealed that the combination of gemcitabine (Gemizar; Eli Lilly and Company, Indianapolis, http://www.lilly.com) with the dual EGFR/VEGFR tyrosine kinase inhibitor AEE788 and the PDGFR inhibitor ST1571 produced up to an 80% inhibition of tumor growth of highly metastatic L3.6pl pancreatic cells implanted orthotopically in the pancreas of nude mice and resulted in a significant increase in survival compared with the inefficacy of the treatment with gemcitabine alone [223].

Since the metastatic spread of diverse tumor cells, including those from glioblastomas, melanomas, and pancreas, breast, and prostate cancers to other specific tissues/organs, such as lymph nodes, bone, lungs, and/or liver, appears to be governed by the expression of diverse angiogenic factors, such as VEGF-VEGFR system, matrix metalloproteinases, urokinase-type plasminogen activator (uPA), cyclooxygenase-2 (COX-2), chemokines, and surface adhesion molecules, their molecular targeting may also constitute another adjuvant cancer therapy (Table 2; Fig. 3) [118, 225]. In this matter, the specific blockade of the SDF-1-CXCR4 axis by using a specific anti-SDF-1 antibody, anti-CXCR4 antibody, or CXCR4 antagonist (TC14012, TN14003, or AMD3100) has notably been observed to prevent the metastatic spread and interfere with the homing of breast and prostate cancer epithelial cells at their target metastatic sites, including lymph nodes, bone, and lungs [97, 118, 225]. The results from preclinical studies have also indicated that the use of the EGFR inhibitors in combination with COX-2 inhibitor or photodynamic treatment or as chemo- and radiosensitizers resulted in more effective chemopreventive and curative treatments for patients with advanced and metastatic cancer forms [214, 226, 227]. It is noteworthy that the sequence of treatment with the EGFR inhibitor and chemotherapy appears to be a critical factor that should be considered for clinical application. Indeed, it has been observed that the treatment of KYSE30 human esophageal squamous epithelial cancer cells by using a platinum derivative (cisplatin [Platinol; Bristol-Myers Squibb, Princeton, NJ, http://www.bms.com], carboplatin [Paraplatin; Bristol-Myers Squibb], or oxaliplatin [Eloxatin; Sanofi-Synthelabo Inc., New York, http://www.sanofi-synthelabo.us]) or a taxane (docetaxel [Taxotere; Aventis Pharmaceuticals Inc., Bridgewater, NJ, http://www.aventispharma-us.com] or paclitaxel [Taxol; Bristol-Myers Squibb]) followed by adding an EGFR signaling inhibitory agent (gefitinib, cetuximab [Erbitux; ImClone Systems, Inc., New York, http://www.imclone.com], or ZD6474) resulted in the synergistic antiproliferative and cytotoxic effects of the drugs [228]. In contrast, the treatment of cells with the EGFR inhibitor before chemotherapy induced an antagonistic effect instead. In addition, since the resistance of several metastatic cancer cells to radiotherapy and chemotherapy has been associated with the aberrant response elements in ceramide and caspase cascades, targeting these apoptotic pathways also may represent another antitumoral strategy (Table 2) [229]. As a matter of fact, our studies have notably indicated that the combination of an EGFR inhibitor with an activator of cellular ceramide production might induce a massive rate of apoptotic/necrotic death of metastatic prostate cancer cells [215, 216]. Altogether, these recent studies have indicated that molecular targeting of EGFR signaling, alone or in combination with other cytotoxic agents, may constitute a putative strategy for conceiving more effective clinical treatments against a variety of aggressive cancers.

High-Dose Cancer Therapy Plus HSCs. Stem cell transplantation may also constitute an option as adjuvant therapy for cancer, particularly in the patients receiving high doses of chemotherapeutic agents and/or radiation that, along with killing cancer cells, cause the severe damage to normal tissues and/or destroy the hematopoietic cells. Thus, the stem cell transplants might replace the endogenous stem cells destroyed by high-dose cancer treatment, thereby producing healthy hematopoietic cell lineages and improving the immune system defense. The autologous or allogeneic transplantation of UCB, BM, or MPB stem cells and their progenitors might be effectuated in combination with HDCT for numerous aggressive cancer forms to replace BM and blood-forming cells that have been destroyed by chemotherapy. AML and high-grade lymphoma are among the principal types of cancer that are usually treated with hematopoietic cell support as adjuvant therapy [230, 231]. The different subtypes of AML appear to result from distinct mutations at the level of HSCs, the appearance of which may give rise to primitive leukemic stem cells (LSCs) possessing a specific phenotype, such as $CD90^-$, $CD117^-$, and $CD123^+$ [232]. These malignant LSCs, which are able to self-renew, might generate a heterogeneous AML cell population, thereby maintaining leukemic blasts [37, 72]. Interestingly, it has been proposed that the maintenance of LSCs in quiescent status might contribute to their survival after chemotherapeutic treatment and leukemia relapse. Hence, the selective apoptosis of LSCs by

using agents such as proteasome inhibitor MG-132 may constitute an adjuvant treatment for AML [232].

In addition, transplantation or mobilization of HSCs and their progenitors in systemic circulation is often used as immune support in combination with HDCT for the treatment of patients with certain highly aggressive solid tumors, and more particularly in advanced and metastatic stages of germinal cell tumors, retinoblastoma, myeloma, brain, lung, kidney, breast, and ovarian cancers [17, 233–235]. However, the timing of the injection of HSCs during the disease and the number of grafted cells are among the major factors influencing the success of the engraftment and/or survival of patients. In this matter, the in vivo elimination of circulating tumor cells by purging prior to treatment may decrease the cancer progression in high-risk patients [76, 235]. Moreover, the ex vivo expansion of HSCs or the mobilization of HSCs from BM into the peripheral blood by using mobilizing agents such as G-CSF and AMD3100 might also lead to a great number of stem cells and their progenitors in bloodstream, thereby decreasing the recovery time after HDCT (Table 2; Fig. 2) [118]. The differentiated HSC-derived progenitors, such as dendritic cells, which are among the most efficient cells of the immune system in presenting an antigen to helper/cytotoxic T lymphocytes, might also to be used as an adjuvant treatment in cancer immunotherapy to eliminate the neoplastic cells that express immunogenic antigens at their surface (Table 2) [17, 65, 236]. Furthermore, UCB also contains a substantial amount of $CD16^{-}/CD56^{+}$ natural killer cells that might be expanded in the presence of IL-12 or IL-15 and that show a high rate of proliferation and cytotoxic effects against some cancers, particularly leukemia [27]. In addition, the chemoprotection against myelotoxicity induced by HDCT may also be counteracted by genetic manipulations in HSCs conferring to their progenitors resistance to certain cytotoxic effects of drugs, such as the expression of MDR1 [169].

CONCLUSIONS

These recent works in the field of stem cell biology have identified intrinsic mitogenic signaling cascades that are activated in mammalian embryonic, fetal, and adult stem cells during the normal process of self-renewal and differentiation. These cellular events may also be implicated in the regenerating process after tissue injuries. Hence, this offers the possibility of differentiating these stem cell types into the specific mature cell lineages in vitro, ex vivo, and in vivo by using appropriate growth factors and cytokines for their use in basic research, as well as in transplantation for diverse degenerative disorders. Several molecular targeting therapies may also be conceived by blocking distinct developmental signaling cascade elements, such as EGFR, hedgehog, Wnt/β-catenin, and/or Notch pathways, which are frequently upregulated in cancer progenitor cells during the initiation and development of a variety of aggressive and metastatic cancers.

PERSPECTIVES AND FUTURE DIRECTIONS

Further in vitro and in vivo investigations with the human embryonic, fetal, UCB, and adult stem cells and their more differentiated progenitors in different systems, animal models, and humans appear to be essential to determine their functional properties during long time periods. Detailed analyses of structural and biological properties of differentiated progenitors established from human stem cells in vitro in comparison with those of endogenous adult stem cells are highly needed before they are used in the clinic. In this direction, additional studies on human adult stem cell biology appear to be particularly essential because of the interspecies differences that may influence their capacity to give rise to the specific cell lineages under well-defined conditions in vitro and regenerate a particular tissue in vivo. In addition, the optimization of purification and delivery methods of stem cells and their progenitors appear generally to be important before their possible use under safe conditions in cellular therapies. More specifically, the establishment of the procedures of derivation from EB-derived progenitor cells in culture for the elimination of residual pluripotent and undifferentiated hESCs, which are able to form the teratomas or teratocarcinomas in vivo, is necessary before they are used in transplantation for diverse degenerative disorders in humans. In addition, further work is important to identify the specific biomarkers of adult stem cells and their progenitors, as well as the microenvironmental factors that might induce their self-renewal, maturation, and migration during each stage, leading to tissue regeneration, and during their relocation to other distant damaged tissues. These future studies should elucidate the sequential cellular events that are implicated in the maintenance of tissue homeostasis, thereby establishing the deregulated signaling that may contribute to diverse disorders and cancer types. Furthermore, since certain lines of evidence have also indicated that certain adult stem cells might trans-differentiate into mature and specialized cells from other tissue types under specific culture conditions in vitro and after transplantation in vivo, it will be important to establish more precisely the factors influencing their incorporation in healthy and damaged tissues/organs in vivo.

Several similarities appear to exist between the behavior of adult stem cells and the cancer progenitor cells, including their high capacity to self-renew and give rise to a heterogeneous population of more differentiated cells and their high capacity to migrate at distant sites, where they may establish their novel homing through interactions with their new microenvironment. Therefore, the knowledge that will be acquired on the adult stem cell biology under physiological and pathological conditions should aid particularly in the design of new cancer therapies targeting cancer progenitor cells. In this matter, it will be important to establish the specific oncogenic alterations occurring in cancer progenitor cells versus their more differentiated progenitors within the global tumor cell population during localized tumor development and dissemination to other distant tissues. Additional analyses of possible interactions between diverse developmental signaling (such as hormones, EGFR, hedgehog, Wnt/β-catenin, and Notch pathways that are involved in the regeneration of numerous tissues and frequently activated during the malignant transformation of adult stem cells into cancer progenitor cells) are notably of particular interest. The possibility that the pharmacological agents acting on the cancer progenitor cells may also alter the behaviors of normal stem cells also emphasizes the importance of using these types of agents at lower doses in combination with conventional cancer treatments.

Altogether, these future works should establish molecular changes occurring in adult stem cells and their progenitors

during tissue repair and etiopathogenesis. Hence, these further studies could lead to the development of more effective treatments for numerous genetic and degenerative disorders by cell replacement. Moreover, the identification of specific markers and targeting distinct tumorigenic cascades in cancer progenitor cells should also contribute to developing novel early detection methods and combination therapies for diverse aggressive and lethal cancers derived from the malignant transformation of adult stem cells.

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DISCLOSURES

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