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Drug Discovery

Regenerative Medicine and Stem Cell Based Drug Discovery

Kazuhiro Sakurada, Fiona M. McDonald,* and Fumiki Shimada**

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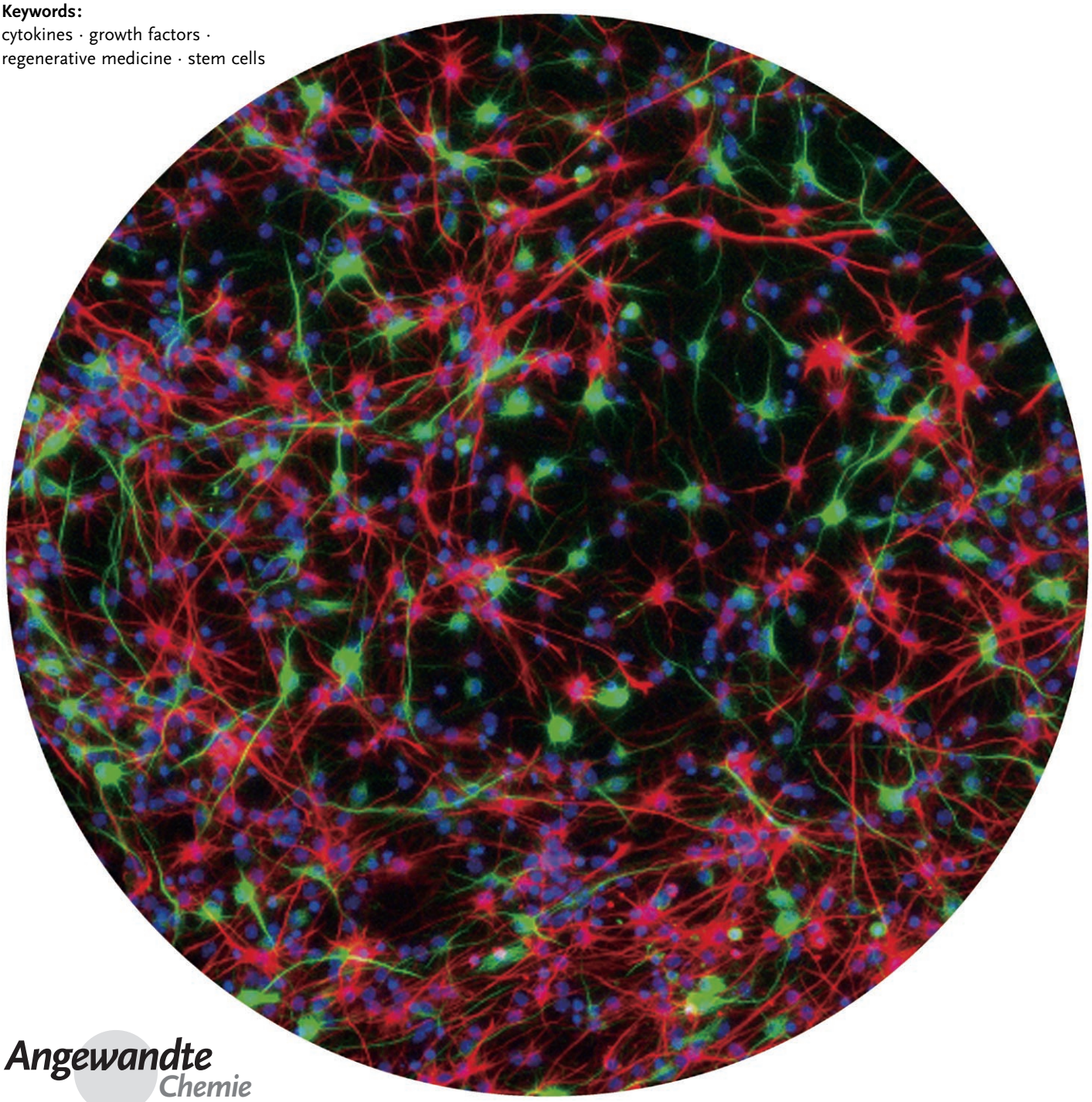


"All the world's a stage,
And all the men and women merely players:
They have their exits and their entrances;
...
Last scene of all,
That ends this strange eventful history,
Is second childishness and mere oblivion,
Sans teeth, sans eyes, sans taste, sans everything."
(As You Like It, Act 2, Scene 7)

As William Shakespeare beautifully described, increasing age often causes loss of tissue and organ function. The increase in average life expectancy in many countries is generating an aging society and an increase in age-related health problems. Regenerative medicine is expected to be a powerful actor in this drama, and stem cell technology may hold the key to the development of innovative treatments for acute and chronic degenerative conditions. This Review surveys the present situation and some future prospects for regenerative medicine and stem cell based drug discovery.

From the Contents

1. Introduction

Over the last century, progress in healthcare and medicines has brought large benefits to many patients. With some exceptions, such as antibiotics, many of these drugs can be divided into two categories: those which provide symptomatic relief and those that treat asymptomatic conditions, such as hypertension and hyperlipidemia, which are risk factors for other diseases. However, there are still many chronic intractable degenerative diseases for which no adequate treatment is available. In addition to the tissue degeneration and organ dysfunction associated with these diseases, many other conditions whose incidence increases with age (including myocardial infarction, stroke, diabetes, and arthritis) are associated with tissue degeneration and can result in sustained organ dysfunction. Regenerative medicine is a new branch of medicine which addresses the use of regenerative treatment to restore organ function in such conditions.

2. The Therapeutic Concept of Regenerative Medicine—Then and Now

Transplantation of donor-derived tissues and organs, for example, kidneys, is widely used and can be a very effective treatment of tissue injury and severe sustained organ dysfunction. Bone marrow transplantation is widely used to replace bone marrow hematopoietic stem cells (HSCs) in a number of hematopoietic diseases and cancers. A major limiting factor for all these transplantation therapies is the shortage of transplantable organs, tissues, and cells. For this reason, the initial concept of regenerative medicine was to produce transplantable material in vitro by using cultured human stem cells. This area of regenerative medicine can be referred to as "cell-replacement therapy" or "stem cell therapy". Multiple clinical trials have been initiated in this field, and a few of these are shown in Table 1. The NIH database of clinical trials (www.clinicaltrial.gov) lists more than 700 active stem cell related clinical trials.

In addition, there are two other important streams in regenerative medicine. The first of these is "induction of regeneration". For a long time it was supposed that organisms such as the salamander might hold the answer to the induction of regeneration in humans. These animals are able to regenerate complex structures, such as an entire limb, by

[*] Dr. K. Sakurada, Dr. F. M. McDonald, Dr. F. Shimada
Bayer Yakuhin Research Center
BMA 3F, 1-5-5 Minatojima-minamimachi
Chuo-ku, Kobe 650-0047 (Japan)
Fax: (+81) 78-304-7242
E-mail: kazuhiro.sakurada@gmail.com
fiona.mcdonald@bayerhealthcare.com
fumiki.shimada@bayerhealthcare.com

Dr. K. Sakurada
Present address:
iZumi Bio, Inc., 4th Floor
1650 Owens Street, San Francisco, CA 94158 (USA)

Dr. F. M. McDonald
Present address:
Bayer Schering Pharma AG
D-13342 Berlin (Germany)

Dr. F. Shimada
Present address:
Bayer Yakuhin Ltd., 6-64 Nishimiyahara 2-chome
Yodogawa-ku,Osaka 532-0004 (Japan)

Table 1: Summary of some clinical trials in the field of stem cell based regenerative medicine.[a]

Therapeutic area/indication	Company/institution	Cell source	Cell type	Development stage
hematology/immunology				
hematological cancer	Gamida-Cell Ltd.	umbilical cord blood	HSC	phase II
GVHD	Osiris Therapeutics	bone marrow	MSC	phase III
Crohn's disease	Osiris Therapeutics	bone marrow	MSC	phase III
cardiovascular				
AMI	Amorcyte Inc.	bone marrow	CD34+ cells	phase I
AMI	Osiris Therapeutics	bone marrow	MSC	phase I
CHF/AMI	Angioblast Systems	bone marrow	MPC	phase I
chronic myocardial ischemia	Cytori Therapeutics	adipose tissue	MSC	phase I
chronic myocardial ischemia	Arteriocyte	bone marrow	CD133+ cells	phase I
chronic myocardial infarction/CHF	Mytogen Inc.	skeletal muscle	myoblasts	phase I
chronic myocardial ischemia	Kobe IBRI	mobilized peripheral blood	CD34+ cells	phase II
myocardial infarction	University of Rostock	bone marrow	CD133+ cells	phase I/II
angina pectoris	Baxter Healthcare Corp.	mobilized peripheral blood	CD34+ cells	phase II
CHF	BioHeart Inc.	skeletal muscle	myoblasts	phase II/III
AMI	Frankfurt University	bone marrow	mixed progenitor cells	phase III
PAOD/CLI	Frankfurt University	bone marrow	mixed progenitor cells	phase I/II
CLI	Kobe IBRI	mobilized peripheral blood	CD34+ cells	phase I/II
PAOD/CLI	Aastrom Bioscience Inc.	bone marrow	MSC	phase IIb
neurological				
Batten disease	Stem Cells Inc.	fetus	NSC	phase I
orthopedic				
bone repair	Aastrom Bioscience.	bone marrow	MSC	phase III
cartilage repair	Osiris Therapeutics	bone marrow	MSC	phase II

[a] The abbreviations are defined at the end of the Review in tabular form.

recreating an embryonic environment at the site of injury. Thus, it was proposed that "the general approach of regenerative biology should be to identify the cellular and molecular differences that distinguish tissue embryogenesis from wound repair (scarring) and then to recreate an embryonic (regenerative) environment in the injured adult tissue".[1] However, over the past decade, no significant progress has been made in the induction of a full regenerative response for complex structures in mammals.

The third concept, "potentiation of self-repair processes", arose from the study of mammalian adult tissue stem cells. Although it varies among the tissues, the lifespan of an individual cell in the human body is shorter than the lifespan of the human organism. Therefore, it is necessary to replace old cells with new ones by a process known as proliferative homeostasis. In addition to this, cells may become damaged by injury or inflammation, and these damaged cells should also be replaced by new ones—in this case by a process called insult-induced cell genesis. Adult tissue stem cells take a central role in both processes. The role of stem cells during development is to generate tissues and organs according to strict temporal and spatial patterns. Adult tissue stem cells, however, should generate an appropriate number of new cells only when required. In concordance with this concept, it has been shown that the function of tissue stem cells differs between the adult system and the embryo. This observation also indicates that mammals have a limited capacity for full regeneration of complex structures—such as is seen in the salamander and some other amphibians and invertebrates. Nevertheless, medical interventions which can activate proliferative homeostasis and insult-induced cell genesis could be beneficial in many human diseases.

Kazuhiro Sakurada, born in Okayama/Japan, studied molecular genetics and received his PhD from Osaka University in 1993. He joined Kyowa Hakko Kogyo Co. Ltd. Research Laboratories in 1988, and was a visiting scientist at Kyoto University School of Medicine (1991–1992) and the Salk Institute (1997–1998). In 2004 he joined Nihon Schering KK (part of Schering AG, now Bayer Schering Pharma AG) and is head of the Bayer Yakuhin Research Center in Kobe. His research focuses on the development of regenerative medicine for intractable diseases.

Fiona McDonald studied pharmacy and received her PhD in Pharmacology from the University of Strathclyde, Glasgow in 1980. This was followed by postdoctoral and research scientist positions in the Department of Applied Physiology of the University of Cologne, before moving to Berlin to join Schering AG (now Bayer Schering Pharma AG) in 1986. She is currently head of Regenerative Pharmacology at the Bayer Yakuhin Research Center in Kobe, Japan.

3. Biology of Stem Cells

In the context of the cell source, human stem cell systems can be categorized into three groups: 1) stem cells derived from fetal tissue and embryos, 2) stem cells derived from umbilical cord and placenta, and 3) stem cells from adult gonadal and somatic tissues. All these stem cell systems can be considered as cell sources for cell replacement therapy. Furthermore, stem cells in adult somatic tissues are the target of interventions designed to potentiate self-repair processes. In this section, we will summarize the biology of these stem cell systems.

3.1. Stem Cells in Fetal Tissue and Embryos

Human pluripotent stem cells can be harvested from human blastocysts five days after fertilization in vitro or from primordial germ cells from human embryos five to nine weeks after fertilization; the former are embryonic stem cells (ES cells) and the latter embryonic germ cells (EG cells).[2] Although mouse ES cells were developed in 1981, human ES cells and EG cells were not reported until 1998. Most of the studies performed in the past eight to ten years, with the aim of developing cells for future clinical application, have focused on human ES cells.

The generic criteria for pluripotent ES cells and EG cells are: 1) they originate from a blastocyst or from primodial germ cells; 2) they maintain normal karyotype during propagation, are immortal, and can be propagated in a self-renewing manner; 3) they are capable of spontaneous differentiation into extraembryonic tissues, and give rise to all three embryonic germ layers in vitro and to teratomas in vivo; and 4) they can give rise to any cell type in the body, including germ cells, when the clone is transplanted into a blastocyst.[2] Human ES cells and EG cells meet the first three criteria. For ethical reasons, the fourth test can not be applied to human ES cells. Therefore, at present, human cells are referred to as being ES cells if they meet all the other criteria. Recently, the International Stem Cell Initiative characterized 59 human ES cell lines from 17 laboratories worldwide.[3] Despite differences in genetic origin and in technical procedures for their establishment and maintenance, all the lines showed similar expression patterns for several ES cell markers. For the application of human ES cells in the clinic, the development of common standards and quality control procedures is essential. In this context, the prospective identification and definition of the origin of ES cells is another important challenge, to ensure consistency of the starting material. Human ES cells and mouse ES cells use different signaling pathways to maintain their pluripotency.[4] Mouse ES cells depend on leukemia inhibitory factor (LIF) and bone morphogenetic protein (BMP), whereas their human counterparts rely on, for example, activin/nodal and fibroblast growth factor (FGF). Recently, pluripotent stem cells were isolated from mouse epiblasts which show several differences to mouse ES cells and similarities to human ES cells.[5] Pluripotent stem cells derived from mouse epiblast cells could provide a useful tool for studying the differences between mouse and human ES cells, to determine whether these have their basis in a species difference or in differences in the developmental (namely, temporal) origin of the cells.

Fumiki Shimada studied pharmacy at Teikyo University and obtained his PhD in molecular pharmacology from Kobe University Medical School. In 1992 he joined Mitsui Pharmaceuticals, where he worked on CNS drug discovery. In 2001 he moved to Nihon Schering K.K., and also worked in Neurology Research at Schering AG, Berlin from 2002–2003. He joined the Nihon Schering Research Center in Kobe in 2004, and became head of Stem Cell Based Drug Discovery for Bayer Schering Pharma in 2007. His current research interest is drug discovery focused on regenerative medicine.

3.2. Stem Cells from Umbilical Cord Blood and Placenta

Umbilical cord blood, umbilical cord, placenta, and amniotic membranes and fluid are normally discarded after birth, but might represent a valuable source of stem and progenitor cells. Umbilical cord blood has long been recognized as a source of hematopoietic stem cells, and is routinely used as an alternative to bone marrow as a source of cells for transplantation in the treatment of hematopoietic disorders.[6] The use of umbilical cord blood is associated with a lower incidence of severe graft versus host disease; however, its use is limited by the relatively small number of cells that can be obtained from the umbilical vein.

Both amniotic fluid and the placenta contain a heterogeneous population of progenitor cells, and the placenta has an important role as a hematopoietic organ during embryogenesis. The cell profile of amniotic fluid changes as it receives cells from the placenta and embryo during gestation. Several studies have reported that the human placenta and amniotic fluid contain fibroblast-like cells with multilineage differentiation capability.[7a] These cells share some properties of mesenchymal stem cells (MSCs) derived from bone marrow, and can be induced to differentiate into mesenchymal lineages, neurons, and hepatocytes.[7b]

3.3. Adult Stem Cells from Gonadal Tissue

It is well known that germline stem cells (GSCs) are present in adult testes and give rise to spermatozoa.[8] However, it was supposed that female mammals are born with a fixed number of oocytes and that the adult female does not have GSCs. This dogma was challenged by the report that new oocytes can be generated from putative GSCs from bone marrow and ovary.[9] The contribution of cells derived from bone marrow is called into question by data from Eggan et al.,[10] who found no evidence for mature oocytes derived from bone marrow. However, although controversial, the concept of oogenesis in the adult ovary has not been completely refuted.[11]

GSCs isolated from adult mouse testes have been reported to acquire ES cell properties when cultured under specific conditions.[12] If similar stem cells can also be generated from human GSCs, and this has already been claimed,[13] they might have potential as a source of human ES-like cells.

3.4. Stem Cells from Adult Somatic Tissues

3.4.1. Tissue Stem Cells

Adult tissue stem cells and progenitor cells are required for both proliferative homeostasis and insult-induced cell genesis, and are present in all tissues in the human body. These tissue stem cells are usually lineage-restricted to the generation of cells inherent to that tissue. Adult tissue stem cells can be grouped into three categories.[14] The first group contains cells with high turnover and high regenerative ability, such as HSCs, gut epithelial stem cells, and epidermal stem cells. The second group represents cells with low turnover but high regenerative potential, such as oval cells of the liver, pancreatic progenitor cells, and skeletal muscle satellite cells. The third group contains cells with low turnover and low regenerative potential, such as neural stem cells (NSCs)[15] and cardiac progenitor cells.[16] Although the physiological role of adult NSCs and cardiac progenitor cells is still controversial, multiple studies have demonstrated proliferative homeostasis and insult-induced cell genesis in the brain and heart. The cells on which most work has been done are listed in Table 2.

Table 2: Tissue stem cells/progenitor cells in the adult human body.

Tissue	Stem cell	Niche
blood	hematopoietic stem cell	bone marrow
gut	gut epithelial stem cell	gut crypt
skin/hair	epidermal stem cell	hair follicle bulge
liver	oval cell	biliary ductule
pancreas	pancreatic progenitor cell	pancreatic duct
skeletal muscle	satellite cell	between sarcolemma and basal lamina
brain	neural stem cell	subventricular zone and dentate gyrus
heart	cardiac progenitor cell	not determined

3.4.2. Mesenchymal Stem Cells

Besides tissue stem cells, mesenchymal stem cells are also present throughout the body.[17] When bone marrow cells are cultured on plastic in medium containing 10% serum, MSCs can be identified as fibroblastic, plastic-adherent cells.[18] MSCs can differentiate not only into mesenchymal tissue lineages (such as adipocytes, bone, muscle, cartilage, and tendon), but also into non-mesenchymal lineages such as neurons.[19] MSCs have also been propagated from brain, spleen, liver, kidney, lung, heart, muscle, thymus, pancreas, adipose, and vascular tissue, and it has been hypothesized that the original stem cells giving rise to MSCs are located in a perivascular niche.[17] Mesenchymal stem cells have the ability to home in on sites of injury, in some studies even when injected intravenously,[20] and to promote tissue regeneration and vascularization. Although the mechanisms remain obscure, many studies have demonstrated multiple functions for mesenchymal stem cells in vivo, including differentiation into somatic cells, paracrine activation of endogenous repair processes, and immunosuppression.

Although much work has been done with MSCs, questions remain concerning their developmental derivation and in vivo behavior. It was widely believed that MSCs are derived from mesoderm. However, a recent study demonstrated clearly that early MSCs are derived from neuroepithelium and neural crest cells.[21] In addition, this study indicated that MSCs recruited from the neuroepithelial pathway are transient and are replaced by MSCs from unknown sources in the postnatal stage. The concept of MSCs originating in multiple waves derived from distinct sources is in agreement with the finding that MSCs can be isolated throughout the body. Perhaps some adult MSCs could be derived from adult neural crest stem cells that persist in mesenchymal tissues.[22] The identification of the origin of adult MSCs may help answer the question of their physiological role.

The use of different cell culture conditions for MSCs resulted in the identification of a multipotent stem cell which is able to differentiate into cells of all three germ layers. Multipotent adult progenitor cells (MAPCs) were the first population of these stem cells with the potential to give rise to cells of all three germ layers.[23] They were purified initially from adult bone marrow and subsequently also from brain and muscle. Another class of these multipotent stem cells with the ability to differentiate into cells of the three germ layers is the marrow-isolated adult multilineage inducible (MIAMI) cell, which resembles MAPCs.[24] The identification of the origin of adult MSCs will clarify whether multipotent cells with three germ layer potentiality exist within the organism or whether the multipotent phenotype is acquired in vitro during the culture process.

3.4.3. Circulating Cells Derived from Bone Marrow which are Recruited to Sites of Injury

In addition to tissue stem cells, many different circulating cells derived from bone marrow are involved in tissue repair. Cytokines generated in insult-induced inflammation can directly or indirectly mobilize bone marrow cells.[25] Activation of sympathetic neurons also contributes to mobilization.[26] The largest population of cells recruited in response to cytokine cues appear to be monocytes/macrophages.[27] A specific population of blood-borne CD34+/CD11b+ cells, fibrocytes, may contribute to tissue repair,[28] either directly by differentiation into the appropriate cell lineage[29] or by cell fusion.[30]

Under normal conditions, bone marrow stem cells/progenitor cells, such as HSCs, endothelial progenitor cells (see Section 4.2), and MSCs, are present only in very low numbers in peripheral blood. These cells are mobilized by injury or inflammation, and although their total number is much lower

than the number of macrophages, they might play an important role in tissue regeneration. For example, HSCs can induce neovascularization at sites of injury.[31]

3.5. Dysfunction of Adult Stem Cell Systems as a Cause of Tissue Degeneration and Cancer

Monogenic mendelian disorders generally arise early in childhood, whereas the incidence of complex disease starts to rise in middle age and increases age-dependently. These complex diseases show dysfunctions in cell genesis such as atrophy, dysplasia, and hyperplasia, which result from disturbances in proliferative homeostasis and insult-induced cell genesis.[32] Cancer (neoplasia) is also a problem of proliferative homeostasis and the incidence of many cancers increases with age. According to the cancer stem cell hypothesis, cancer arises from the transformation of adult tissue stem cells (see Section 5.4). Aged tissue stem cells can show not only tumorigenic alterations but also suppression of self-renewal, which then leads to tissue atrophy (see Figure 1).

Proliferation and differentiation of adult tissue stem cells are regulated by both paracrine factors produced by the stem cell niche[33] and systemic endocrine factors. Tissue repair processes may be impaired by a lack of these factors or by the presence of an inhibitory environment. Stem cell intrinsic dysfunction also inhibits repair processes, as has been demonstrated for hematopoietic stem cells.[34] The different variations are summarized schematically in Figure 2. Given that adult tissue stem cells are probable candidates for the cellular culprits of these age-related problems of cell proliferation, it seems likely that understanding the mechanisms of adult stem cell dysfunction will hold promise for preventing and treating many common human diseases.

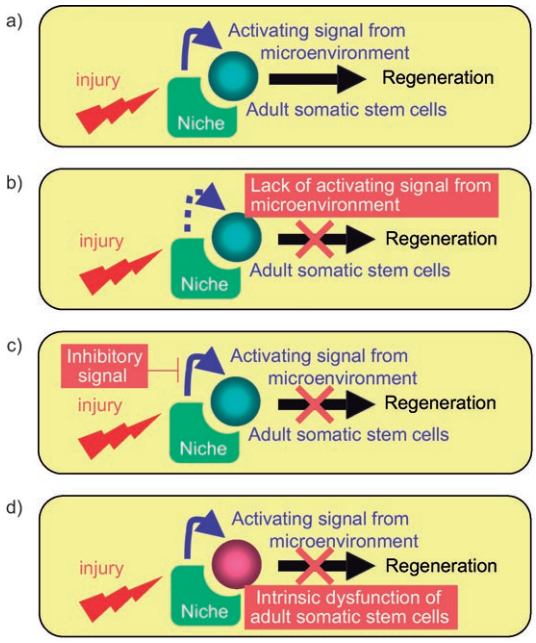


Figure 2. Factors contributing to dysfunction of tissue repair: a) normal repair process; b) absence of paracrine/endocrine repair signals; c) presence of repair inhibitory signals; and d) failure of repair because of stem cell intrinsic dysfunction.

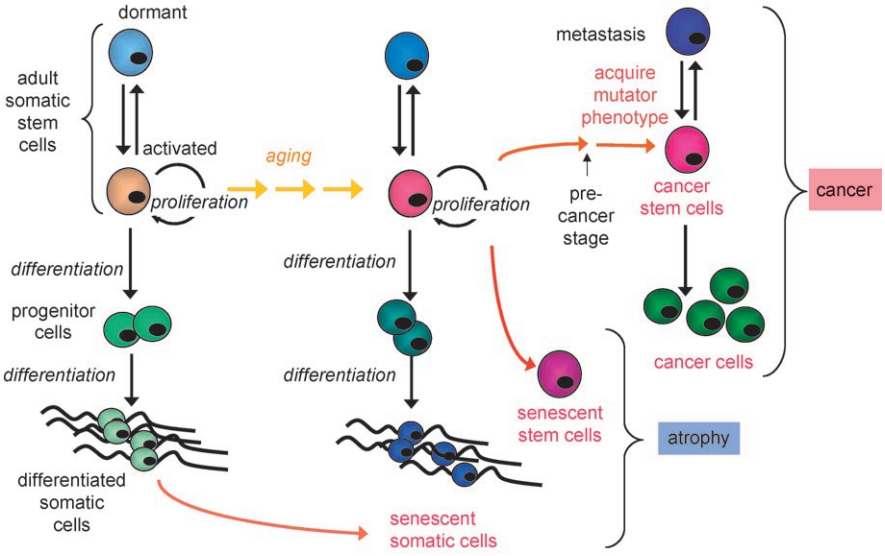


Figure 1. Age-dependent stem cell intrinsic dysfunction.

3.5.1. Changes in Stem Cell Function Associated with Stem Cell Intrinsic Changes

Although multiple studies demonstrated an age-related decline in HSC function in mice, much less is known about the processes driving these changes. Two of the most clinically significant age-associated hematological conditions are reduced function of the adaptive immune system and elevated incidence of myeloproliferative diseases.[35] Since these age-dependent hematological conditions in mice are transferable by HSC transplantation, it has been suggested that autonomous changes in the HSCs are the origin of the dysfunction.[35] Telomere shortening, DNA mutation, and epigenetic alteration are major triggers in aging-associated stem cell intrinsic dysfunction.[36] Several studies have demonstrated that in the mouse, deficiencies in DNA-damage repair, such as the repair of a double-strand break, repair of nucleotide excision, and telomere maintenance, impair the function of HSCs with age.[37,38] Choudhury et al.[39] demonstrated clearly that deletion of p21 protects against the aging effects of telomere dysfunction in stem cells without accelerating the formation of cancer. Reactive oxygen species, which induce DNA damage, also limit the function of HSCs age-dependently in

Atm-deficient mice in a p38-MAPK pathway-dependent manner.[40,41]

The age-dependent dysfunction of HSCs in *Atm*-deficient mice also indicates a role for epigenetics in the senescence pathway, since overexpression of *Bmi-1*, an epigenetic control factor, can repair the HSC dysfunction in an *INK4a*-dependent manner.[40] It has also been reported that loss of *Bmi-1* reduces self-renewal in NSCs[42] and HSCs[43] in mice, and that the Werner syndrome gene, a DNA repair gene,[44] is epigenetically suppressed in cancer tissue. It is well known that global hypomethylation and promoter hypermethylation are generally observed in tumor cells.[45] Recently, Suzuki et al.[46] reported that epigenetic alterations in colon and gastric cancer patients correlated with age, but accumulation of DNA mutations did not, which suggests that epigenetic changes may precede DNA mutation. However, DNA mutations did correlate with epigenetic changes, particularly hypomethylation. This finding suggests that the epigenetic changes precede and may predispose to the development of DNA mutations and cancer development/progression.

3.5.2. Changes in Stem Cell Function Associated with Changes in the Stem Cell Niche

Functional alteration in the stem cell niche is another trigger of age-dependent dysfunction of stem cells, and one example of this is seen in spermatogenesis. Spermatogenesis is maintained by spermatogonial stem cells and exhibits age-related deficits that can result in infertility. Ryu et al. showed that when mouse spermatogonial stem cells, which usually decline between 12 and 24 months of age, were consecutively passed at 3 month intervals to the testes of young males, they continued to give rise to sperm for more than 3 years.[47]

As mentioned above, age-dependent dysfunction of HSCs in mice is generally thought to be driven by cell autonomous change. However, a recent study also demonstrated the importance of the HSC niche.[48] In this study, B-cell lymphogenesis was found to be impaired and myeloid proliferation increased when bone marrow cells from wild-type donors were transplanted into aged mice with dysfunctional telomeres. The age-dependent trigger of human HSC dysfunction is still unidentified, but this finding indicates that the stem cell niche might also be one of the targets involved in age-dependent HSC dysfunction in humans.

3.5.3. Changes in Stem Cell Function Associated with Aging of Endocrine Systems

Regeneration of injured skeletal muscle or liver is inhibited in old mice. However, when old mice were physically connected to young mice of the same strain by parabiosis (heterochronic parabiosis), in which the animals share their circulatory system, the regenerative capacity of skeletal muscle and liver in the old mice was restored.[49] This finding suggests that the age-related decline in stem cell function can be modulated by systemic factors in the stem cell's environment and that such factors normally present in the blood of young animals are being lost as the animals age.

The function of hormone-secreting endocrine organs also decreases with age, and results in reductions in the concentration of several hormones produced by these tissues.[50] One of these is growth hormone (GH), which is secreted from the pituitary gland. GH secretion is highest at puberty, and then falls with increasing age.[51] This decrease in GH is thought to be caused by increased production of somatostatin by the hypothalamus, leading to reduced production of GH-releasing hormone.[50,51] As a result, the production of GH-dependent insulin-like growth factor-1 (IGF-1) by the liver is reduced. IGF-1 plays an important role in stem cell proliferation and survival, so the reduction in GH might be involved in aging-associated dysfunction of stem cell systems.

4. Therapeutic Areas for Regenerative Medicine and Stem Cell Based Therapeutics

Therapeutic areas in which regenerative medicine and stem cell based therapeutics can play a role are potentially huge. In this section, we summarize the potential in hematological, cardiovascular, neurological, and orthopedic disorders as well as type I diabetes.

4.1. Hematological Disorders

Multiple hematological disorders, such as leukemia, Hodgkin's disease, multiple myeloma, myeloproliferative disorders, myelodysplastic syndromes, phagocyte disorders, primary immunodeficiency diseases, and metabolic disorders are treated with HSC transplantation. Bone marrow has represented the main source of HSCs in pediatric and adult individuals in the past. However, difficulties in finding suitable donors limit the clinical application. Recently, mobilized peripheral blood (that is, blood from patients treated with, for example, granulocyte colony-stimulating factor (G-CSF) to mobilize cells from the bone marrow into peripheral blood to give increased numbers of circulating bone marrow derived stem cells) and umbilical cord blood were identified as new sources for HSC transplantation.[52] Although mobilized stem cells from peripheral blood are widely used in the clinic, poor mobilization in some individuals and the risk of splenic rupture in the donor limit the application. HSCs derived from umbilical cord blood have the advantage of a lower risk of inducing graft versus host disease by the less-mature lymphocytes, but the small numbers of stem cells obtained limit the recipients to pediatric patients. These limitations could be resolved if the technology to expand HSCs can be developed; this will be reviewed in Section 5.3.1.

4.2. Cardiovascular Disease

Cardiovascular diseases are a group of disorders of the heart and blood vessels, and include coronary heart disease, cerebrovascular disease, peripheral arterial disease, rheumatic heart disease, congenital heart disease, deep vein

thrombosis, and pulmonary embolism. Stem cell based treatments are expected to have an impact on some of these conditions by promoting cardiac myogenesis and vascularization. It is widely believed that after birth, new endothelial cells are derived from resident endothelial cells during the process of angiogenesis. However, multiple studies have indicated that endothelial progenitor cells (EPCs) derived from bone marrow contribute to postnatal vascularization.[53] It has also been shown that hematopoietic lineage cells can become entrapped at the site of injury and induce neovascularization by production of growth factors.[31] In agreement with these findings, transplantation of mobilized peripheral blood in patients with critical limb ischemia as a secondary disease of diabetes improved the ischemia.[54]

In some studies on animal models of myocardial infarction, cultured MSCs derived from bone marrow have been shown to home in on the site of injury and to promote cardiomyocyte differentiation and vascularization,[55] thereby resulting in a near normalization of ventricular function. However, the mechanisms remain obscure.

The observation that bone marrow elements such as EPCs, HSCs, and MSCs can contribute to cardiac repair in the infarcted heart generated a therapeutic strategy for the use of adult bone marrow cells after myocardial infarction. Although the degree of the effect varies between reports, independent studies have shown a beneficial effect of bone marrow derived cell transplantation in animal models of myocardial infarction.[56–58] Based on these findings, mononuclear cells derived from bone marrow have been administered by intracoronary injection in patients. Two clinical trials indicated that the transplantation of mononuclear cells from bone marrow is safe and may improve cardiac function after myocardial infarction.[59,60a] However, follow-up data from one of these trials showed that after 18 months there was no longer a significant difference between the cell-treated group and the control group.[60b] A further trial did show a positive effect of bone marrow derived progenitor cells on clinical outcome one year after treatment.[61]

In addition to cell therapy approaches, the identification of cardiac progenitor cells[16] in the adult heart opens up new therapeutic possibilities for pharmacological stimulation of the endogenous repair process in cardiac disorders.

4.3. Neurological Disorders

The goal of regenerative medicine in the domain of neurological disorders is to reverse the damage to the nervous system caused by disease or injury, by utilizing ex vivo expanded stem cells or endogenous adult neural stem cells.[15,62] Stroke, Parkinson's disease, spinal cord injury, Alzheimer's disease, amyotrophic lateral sclerosis, Huntington's disease, and multiple sclerosis are some of the possible targets for neural regenerative therapy. Compared to hematological and cardiovascular indications, the major difficulty in neurological disorders is that, in addition to neurogenesis, reconstruction of the correct neuronal circuitry is required. Parkinson's disease is a possible exception to this, as the generation of dopamine-producing cells in the striatum, while not regenerating the lost neurons in the Substantia nigra, may be sufficient for successful treatment. A surgical approach to reconstruct neuronal circuitry is not feasible, so most activities in the field of neural regeneration are in the context of stimulation of the endogenous repair system. This will be discussed in more detail in Section 5.3.2. Several companies are developing cell-based treatments for neurological indications using fetal brain derived neural stem cells, adult bone marrow stem cells, and embryonic stem cells.

4.4. Type 1 Diabetes Mellitus

Type I diabetes results from autoimmune degeneration of pancreatic β cells. Insulin replacement is the major treatment for this disease; however, stable control of blood glucose is a major challenge. Lack of adequate metabolic control can result in severe secondary consequences of diabetes, such as nephropathy, neuropathy, retinopathy, critical limb ischemia, and other cardiovascular problems. The restoration of physiologically regulated insulin secretion should overcome these problems and, in recent years, islet transplantation has been increasingly employed in an attempt to provide this.[63] However, like other transplantation therapies, this is limited by the lack of suitable donor material. Thus, the major goal for regenerative medicine in type I diabetes is the in vitro generation of insulin-producing islet β cells suitable for transplantation.[63] The use of an appropriate encapsulation technology to shield the cells from immune attack (while still allowing free passage of glucose and insulin, thereby giving physiologically regulated insulin production)[64] would mean that autologous cells are not necessary, and an allogenic approach could be adopted using embryonic stem cells as the source of cells for differentiation. The present limitation of this strategy is the efficient differentiation of fully functional islet cells from ES cells.

Although the existence of progenitor cells, which can give rise to new β cells in adult individuals is controversial,[65] such progenitor cells could open up new therapeutic possibilities for pharmacological stimulation of the endogenous repair process in diabetes.

4.5. Orthopedic Disorders

Degenerative disorders of bone and joints, such as bone fracture, osteoporosis, and arthritis, represent another field with significant potential for regenerative medicine approaches. Osteocel, developed by Osiris Therapeutics, contains MSCs from bone marrow and has been used in the USA since its introduction in 2005 for promoting the repair of bone fractures and bone lost by malignancy. Bone marrow MSCs can differentiate into bone-forming osteoblast cells[66] and contribute to bone regeneration[67] when transplanted into the site of injury using biocompatible scaffolds.

Inflammatory diseases of the joints, such as osteoarthritis, represent further indications in this domain, with cartilage regeneration as the treatment goal.[68] MSCs are being used as

a stem cell source for the regeneration of cushioning and surface cartilage.

5. Pharmacological Approaches to Regenerative Medicine and Stem Cell Based Therapeutics

Synthetic small molecules and natural products have provided useful pharmacological interventions to modulate cellular processes in stem cells in the past. Indeed, several studies have shown that many drugs in current clinical use have the potential to modulate the function of stem cells.[69] In addition, new chemical entities have been identified which have modulatory activity on stem cells.[70–72] These chemicals might have potential for ex vivo expansion and differentiation of stem cells as well as for pharmacological intervention to stimulate self-repair processes. In this section we summarize the potential of chemical approaches to regenerative medicine and stem cell based drug discovery. Some of the compounds referred to in this section are summarized in Table 3.

5.1. Expansion and Differentiation of ES Cells

The long-term efficient self-renewal of ES cells is the first key step in ES cell based regenerative medicine. Mouse ES cells are typically expanded (increased in number) in the presence of LIF[73] and BMP.[74] The combination of these two factors allows the ES cells to proliferate in the absence of serum and feeder cells. LIF activates STAT signaling, which promotes self-renewal and inhibites mesodermal and endodermal differentiation.[73] BMP inhibits MAPK signaling and neuroectodermal differentiation.[74,75] Recently, by using high-throughput chemical screening, the 3,4-dihydropyrimido[4,5-*d*]pyrimidine analogue SC1 was identified as having the ability to expand mouse ES cells in the absence of LIF.[76] SC1 has been demonstrated to act through two targets: RasGAP and ERK 1/2. BIO (6-bromoindirubin-3′-oxime), a synthetic derivative of the natural product 6-bromoindirubin (which is one of the constituents of the mollusk-derived dye Tyrian purple), has also been shown to promote the self-renewal of human and mouse ES cells without feeder cells by inhibiting glycogen synthase kinase 3 (GSK3) and activating Wnt signaling.[77]

Compared to mouse ES cells, human ES cells are more vulnerable to apoptosis upon cellular detachment and dissociation (anoikis), which causes major problems for manipulations, such as subcloning, which require dissociated cultures. A recent study showed that the selective Rho-associated kinase (ROCK) inhibitor Y-27632 markedly diminished dissociation-induced apoptosis.[78] Although the mechanism of Y-27632′s action in blocking apoptosis is not known, it was shown that another ROCK inhibitor (Fasudil) had a similar effect, but inhibitors of several unrelated kinases did not.[78]

Several methods for the differentiation of ES cells to specific lineages are currently being pursued. Neural differentiation can be induced, for example, by treatment of embryoid bodies (EBs) with retinoic acid,[79] by multistep induction/selection culture[80] or by using specific growth factors in serum-free and feeder-cell-free culture.[81,82] Robust cardiac myocyte differentiation is observed in spontaneously differentiating ES cells when cultured under appropriate conditions and stimulated through transient inhibition of BMP signaling by Noggin.[83] Compared to the generation of these two lineages, the generation of insulin-producing islet cells from ES cells appears more complex. It has been shown that cells must go through multiple steps for the induction of functional islet-like clusters: definitive endoderm induction, pancreatic endoderm formation, endocrine induction, and islet-like cluster maturation. Recently, Jiang et al. described a protocol for the generation of pancreatic islet-like cell clusters containing insulin-producing cells from human ES cells.[84]

Although great progress in the differentiation of ES cells has been made in the past decade, it is clear that better methods are still required. In this context, Ding et al. studied a large number of synthetic small molecules in cell-based phenotype screening assays, which resulted in the identification of the neurogenesis stimulator TWS119[85] and four diaminopyrimidines—cardiogenol A–D,[86] the most potent of which was cardiogenol C—which stimulate cardiac myogenesis.

5.2. Bone Marrow Stem Cell Mobilization

The mobilization of bone marrow cells is one of the key factors in getting stem cells, progenitor cells, macrophages, and monocytes to sites of injury, where they can induce vascularization and support regeneration. The injection of G-CSF is one of the most common methods to mobilize HSCs for transplantation in the clinical setting.[52] In addition, several other pharmacological agents, such as granulocyte-macrophage colony-stimulating factor (GM-CSF),[87] erythropoietin,[88] statins,[89] rosiglitazone,[90] estrogens,[91] angiotensin II receptor antagonists,[92] and ACE inhibitors[93] have been shown to have an effect on bone marrow cells. HMG-CoA reductase inhibitors and recombinant human erythropoietin have been shown to augment the number of circulating endothelial progenitor cells through the PI3 kinase/Akt pathways.[88,89] The role of angiotensin II in the mobilization of endothelial progenitor cells was identified by the observation that the ACE inhibitor enalapril modulates endothelial progenitor cell function through the CD26/dipeptidyl peptidase IV pathway.[93]

It has long been believed that G-CSF-induced HSC mobilization is controlled by proteases, including matrix metalloproteinase-9 (MMP-9), neutrophil elastase, and cathepsin G, which alter the bone marrow microenvironment by degrading vascular cell adhesion molecule-1 and SDF-1, and by remodeling the extracellular matrix.[94] However, this hypothesis was challenged by the observation that G-CSF still induced HSC mobilization in mice deficient in neutrophil serine proteases and/or after treatment with MMP-9 inhibitors.[95] A recent study by Katayama et al. demonstrated clearly that the sympathetic nervous system also contributes to mobilization of HSCs through down-regulation of SDF-1

Table 3: Summary of some compounds that have effects on stem cell mobilization, expansion, or differentiation, or which have been shown to have regenerative effects in vivo.

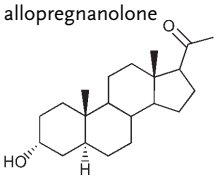
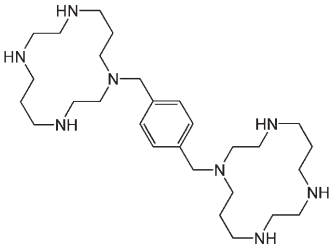
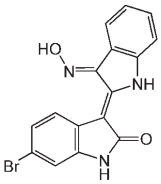
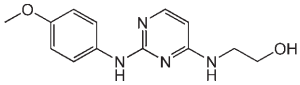
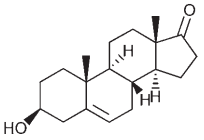
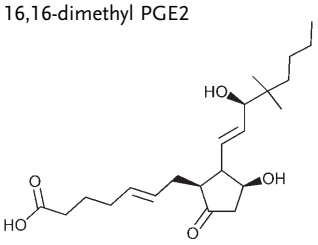
Compound	Effects; mechanism	Ref.
allopregnanolone 	increased proliferation of rat hippocampal neural progenitor cells and human neural stem cells in vitro	[135]
AMD3100 (plerixafor) 	increased HSC mobilization (AMD3100 + G-CSF compared to G-CSF alone); CXCR4 antagonist	[96]
antidepressants, e.g. fluoxetine (SSRI), tranylcypromine (MAOI), desipramine (tricyclic), venlafaxine (SNRI), rolipram (PDE4 inhibitor)	increased hippocampal cell proliferation and neurogenesis	[126]
BMP	mouse ES cell expansion (in combination with LIF); Smad activation and inhibition of p38 and ERK	[74, 75]
6-bromoindirubin-3′-oxime (BIO) 	mouse and human ES cell expansion; GSK3 inhibition increased proliferation of cardiac precursor cells derived from human heart	[77] [154]
cardiogenol C 	cardiac differentiation of ES cells	[86]
decHOX peptide	enhanced ex vivo expansion of HSCs derived from umbilical cord blood; HOX mimetic	[116]
dehydroepiandrosterone (DHEA) 	increased hippocampal neurogenesis	[133]
16,16-dimethyl PGE2 	increased HSC numbers and HSC repopulation	[118]

Table 3: (Continued)

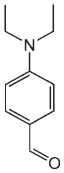
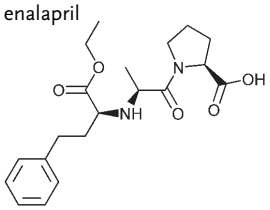
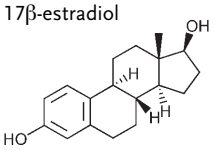
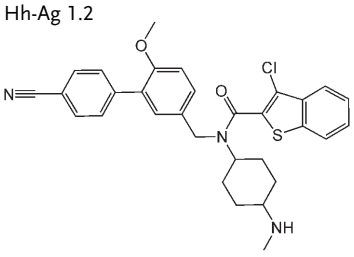
Compound	Effects; mechanism	Ref.
diethylaminobenzaldehyde 	HSC expansion in vitro; aldehyde dehydrogenase inhibitor	[117]
enalapril 	increase in endothelial progenitor cell mobilization following ischemia stress; ACE inhibitor	[93]
erythropoietin	increase in circulating hematopoietic progenitor cells and in endothelial progenitor cells in EPO-treated patients	[88]
17β-estradiol 	increase in circulating endothelial progenitor cells increase in osteogenesis	[91] [161]
G-CSF	mobilization of bone marrow stem cells into peripheral blood improved functional recovery after experimental myocardial infarction reduction in infarct size and improved functional recovery after experimental stroke [103]: in combination with stem cell factors increased neurogenesis from neural stem cells in vitro	[52, 98, 99] [99] [103, 104] [104]
GM-CSF	mobilization of bone marrow stem cells into peripheral blood	[87]
GROβ	HSC mobilization; CXCR2 ligand	[97]
HGF + IGF-1	migration, proliferation, and differentiation of cardiac progenitor cells and improved functional recovery after experimental myocardial infarction	[157]
Hh-Ag 1.2 	increased proliferation of neuronal precursor cells; Smoothened agonist	[122]
LIF	expansion of mouse ES cells (in combination with BMP); STAT3 activation	[73]
lithium	increased proliferation of neural progenitor cells in high density culture; induced neuronal differentiation of hippocampal neural progenitor cells; ERK/CREB activation increased bone formation; activation of Wnt signaling	[128] [166]

Table 3: (Continued)

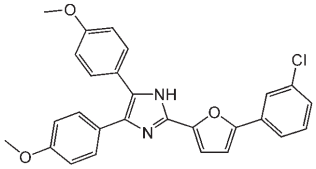
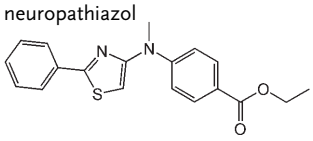
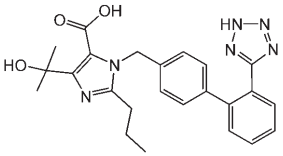
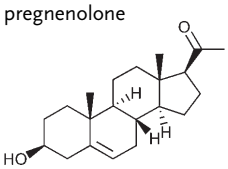
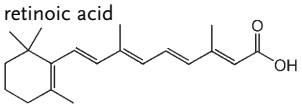
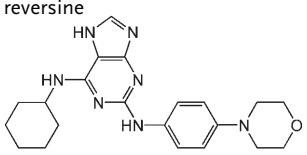
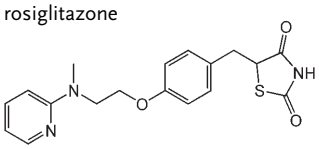
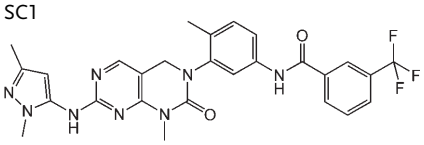
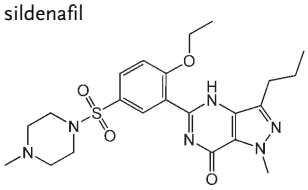
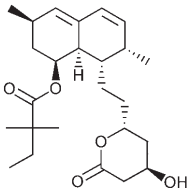
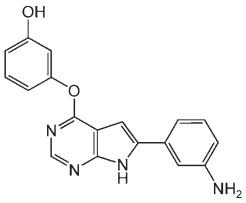
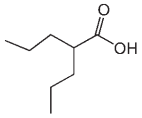
Compound	Effects; mechanism	Ref.
neurodazine 	generation of neuronal cells from skeletal muscle cells	[179]
neuropathiazol 	induced neuronal differentiation of adult hippocampal neural progenitor cells	[124]
neuropeptide Y	increased proliferation of hippocampal neural progenitor cells; ERK1/2 activation	[136]
olmesartan 	increase in endothelial progenitor cells in patients with type 2 diabetes mellitus; AT1-receptor antagonist	[92]
oxytocin	differentiation of adult murine heart-derived Sca-1$^+$ cells to cardiomyocytes	[149]
PACAP	increased proliferation of neural stem cells in vitro and in vivo; PKC-dependent effect	[137]
parathyroid hormone	increased bone formation	[164]
pregnenolone 	increased hippocampal neurogenesis	[134]
retinoic acid 	neuronal differentiation of embryoid bodies	[79]
reversine 	dedifferentiation of myogenic lineage-committed cells to multipotent mesenchymal progenitor cells	[178]
rosiglitazone 	increase in endothelial progenitor cells and promotion of differentiation towards endothelial lineage	[90]
SC1 	expansion of mouse ES cells; inhibition of RasGAP and ERK 1/2	[76]

Table 3: (Continued)

Compound	Effects; mechanism	Ref.
sildenafil 	increased neurogenesis and improved functional recovery after experimental stroke; PDE5 inhibitor	[131]
	increased neurosphere generation from subventricular zone-derived cells; increased neuronal differentiation of neurospheres; activation of PI3 K/Akt pathway and GSK3 phosphorylation/inhibition	[132]
statins (e.g. simvastatin) 	increase in endothelial progenitor cell differentiation from peripheral blood mononuclear cells; stimulation of PI3 K/Akt	[89]
TWS119 	neuronal differentiation of ES cells; GSK3β inhibition and activation of β-catenin signaling	[85]
valproic acid 	increased HSC proliferation and self-renewal; histone deacetylase inhibitor; GSK3β inhibition and HoxB4 up-regulation	[115]
	induced neuronal differentiation of adult hippocampal neural progenitor cells; histone deacetylase inhibitor; up-regulation of NeuroD	[130]

gene expression in osteoblastic cells of the bone marrow niche.[26]

The critical role of the SDF-1/CXCR4 axis in the mobilization of bone marrow cells was also demonstrated by the use of the specific CXCR4 antagonist AMD3100.[96] AMD3100 (plerixafor) is under development as an HSC-mobilizing agent for patients with non-Hodgkin's lymphoma or multiple myeloma.[96] Other chemokines may also be involved in the mobilization of HSCs, and the CXCR2 ligand GROβ has been shown to induce effective mobilization of HSCs in mice and monkeys.[97]

5.2.1. *Application of Stem Cell Mobilizing Agents in Tissue Repair*

G-CSF has received attention as a possible treatment for heart failure after myocardial infarction, and it has been shown to have beneficial effects on cardiac function and cardiogenesis in animal models of myocardial infarction.[98,99] On the basis of these findings, G-CSF has been tested in patients with acute myocardial infarction (AMI) or chronic myocardial ischemia. G-CSF treatment seems to be safe, and open trials in patients with AMI were encouraging. However, three double blind placebo-controlled trials did not show any effects of G-CSF treatment.[100–102]

In a mouse stroke model, the combination of G-CSF and the stem cell factor (SCF) induced neurogenesis from NSCs and MSCs in the infarct area and induced functional recovery.[103] In this model, G-CSF + SCF showed better efficacy in the subacute phase than in the acute phase. It is thought that MSCs can be mobilized into peripheral blood and migrate to the injury site, although the function of MSCs in CNS regeneration is still unclear. In addition to its effects on bone marrow, G-CSF plays multiple roles in CNS regeneration. These include direct proliferative activity on NSCs and a protective effect on neurons.[104] G-CSF can also inhibit the production of inflammatory cytokines such as TNF-α, IL-1β, and IL-12,[105] and this anti-inflammatory effect may also play a role in CNS regeneration. A small clinical study of G-CSF, administered to 10 patients for 5 days starting between 1 and 5 days after stroke, showed a remarkable recovery of neurological function at 12 months in the G-CSF-treated group compared to the control group, with no major side effects.[106] However, as G-CSF may cause a transient hypercoagulable state,[107] a thorough investigation of safety and efficacy aspects of G-CSF treatment in a larger collective of stoke patients is needed.

5.3. Self-Renewal and Differentiation of Tissue Stem Cells

The enhancement of self-renewal and differentiation of tissue stem cells is another important target for pharmacological modulation. Tissue stem cells usually exist in somatic tissues in a dormant state, but can be transiently activated by insult-induced signals to divide either symmetrically to regenerate the stem cell pool or asymmetrically to generate one daughter stem cell and one daughter progenitor cell, which proceeds along the differentiation pathway.[108] This is a major difference to embryonic stem cells, which undergo symmetric division. Understanding the molecular mechanisms of asymmetric cell division and insult-induced cell genesis might lead to a breakthrough in the development of regenerative medical interventions. In this section, we discuss the potential of chemical approaches to the ex vivo expansion of HSCs and the modulation of insult-induced neurogenesis, cardiogenesis, osteogenesis, and the genesis of islet β cells.

5.3.1. In Vitro Expansion of HSCs

Combinations of hematopoietic cytokines, including SCF, Flt3 ligand, thrombopoietin, and IL-6/soluble IL-6 receptor, were found to expand HSCs significantly compared to a control in a SCID repopulating cell assay.[109] However, cytokine expanded HSCs derived from umbilical cord blood did not improve the efficacy of transplantation in the clinic,[110] thus indicating that further improvement in HSC expansion is necessary. Recent studies demonstrated that Notch,[111] HOXB4,[112] Wnt,[113] and BMP[114] signaling pathways regulate the self-renewal of HSCs. The goal for drug discovery here is to discover compounds which mimic the effects of these signaling pathways.

Valproic acid stimulates the self-renewal of HSCs by inhibiting GSK3β, accompanied by activation of Wnt signaling and of the *HOXB4* gene.[115] A decoy peptide containing the YPWN motif from the HOX protein (decoy HOX) enhances the ex vivo expansion of HSCs derived from human umbilical cord blood by mimicking the function of the HOX protein.[116] Inhibition of aldehyde dehydrogenase with diethylaminobenzaldehyde also induces HSC expansion, by inhibiting the production of endogenous retinoids and thereby reducing differentiation signals.[117] This study showed that inhibition of aldehyde dehydrogenase also up-regulates *HOXB4* expression in human HSCs, which may contribute to the increased expansion.

A panel of compounds was screened in a zebrafish assay system to identify new modulators of HSC expansion.[118] Linoleic acid increased the number of HSCs whereas celecoxib, a cyclooxygenase inhibitor, decreased the number of HSCs in this system. Since PGE_2 is the main effector prostanoid in zebrafish, PGE_2 was picked up as a candidate modulator of HSCs. By using a stable PGE_2 derivative, 16,16-dimethyl-PGE_2, it was demonstrated that PGE_2 induced the proliferation of mouse HSC.[118] This study also indicated that administration of COX inhibitors to patients who receive HSC transplants might impair recovery of the hematopoietic function.

5.3.2. Insult-Induced Neurogenesis

Although some spontaneous insult-induced neurogenesis is observed after stroke,[119] the number of new neurons derived from NSCs is less than the number of neurons lost. A drug that can induce neurogenesis could be a major therapeutic advance for degenerative CNS disorders. However, it is essential that neurogenesis inducers regenerate only what has been lost, in terms of number, type, and location of neurons, as a nonspecific, constitutive neurogenesis could result in severe side effects, such as epilepsy and increased intracranial pressure. Clarification of the mechanisms of physiological neurogenesis and selection of key biological activities controlling proliferation, differentiation, migration, and survival decisions along the pathway from NSCs to fully differentiated neurons is essential for the development of "context-specific" neurogenesis inducers.

The development of drugs to promote proliferation/differentiation of NSCs and progenitor cells is a current focus of attention. Growth factors and neurotrophic factors have the ability to induce proliferation and differentiation of NSCs. However, the systemic administration of growth factors and neurotrophic factors has been unsuccessful, because, for example, of insufficient penetration into the brain, and they may also induce significant side effects, such as pain (BDNF and NGF)[120] or anemia (FGF-2).[121] An alternative approach is the search for low-molecular-weight compounds that can substitute for growth factors or neurotrophic factors. Examples of these are Smoothened agonists, which enhance Hh signaling,[122] and the Wnt signaling enhancer 2-amino-4-[3,4-(methylenedioxy)benzylamino]-6-(3-methoxyphenyl)pyrimidine.[123] However, since Hh and Wnt have multiple actions both within and outside the CNS, it is uncertain whether these compounds will have the required specificity for the injury site. To solve these issues, high-throughput screening with cultured adult NSCs has been performed to identify NSC-specific neurogenesis inducers. As a result, 4-aminothiazole derivatives with neuronal differentiation activity were identified from a screen of about 50000 compounds.[124] Comprehensive target gene profiling has also been used to identify novel drug targets related to neurogenesis.[125]

As well as these efforts to identify novel compounds and targets, neuroregenerative effects have been reported for some known drugs. Interestingly, it has been observed that antidepressants with different molecular targets have the ability to induce neurogenesis in the hippocampus.[126] Although the molecular mechanisms underlying neurogenesis for these drugs have not been elucidated, it has been reported that antidepressants increase the production of BDNF.[127] The mood stabilizer lithium also induces proliferation and differentiation of NSCs in the hippocampus both in vitro and in vivo,[128] and lithium also enhanced the production of BDNF.[129] The mood stabilizer and antiepileptic drug valproic acid has been shown to promote neurogenesis by inhibition of histone deacetylase.[130] Sildenafil, a PDE V inhibitor, increased the cGMP concentration in the brain and promoted neurogenesis in a stroke model,[131] and an increasing cGMP

concentration was found to promote neuronal regeneration through activation of Akt/PI3 kinase in NSCs.[132]

Hormones and neurotransmitters can also induce neurogenesis. In addition to IGF-1, estrogen, prolactin, and the thyroid hormones, the induction of proliferation/differentiation of NSCs has been reported for other hormones, such as dehydroepiandrosterone,[133] pregnenolone sulfate,[134] and allopregnanolone,[135] as well as for neuropeptide Y,[136] PACAP,[137] and neurotransmitters such as dopamine, serotonin, norepinephrine, and acetylcholine.[138–141]

Most studies on insult-induced neurogenesis have focused on stroke. How much of the knowledge gained there can be applied to other indications such as chronic neurodegenerative diseases such as Parkinson's disease and Alzheimer's disease is an open question. There are some indications of increased neurogenesis in Parkinson's disease, but this remains to be confirmed.[142] Insult-induced neurogenesis has not been shown in the brain of patients with Alzheimer's disease. However, if transgenic animals which show amyloid deposition are kept in an enriched environment, which is known to increase hippocampal neurogenesis, amyloid deposition is reduced.[143] This finding suggests that an environment which can promote neurogenesis might suppress the production and deposition of amyloid. Clarification of the molecular mechanisms underlying this might reveal new targets for the treatment of Alzheimer's disease.

5.3.3. Insult-Induced Cardiogenesis

Similar to neurons of the CNS, it was believed that the postnatal heart is a postmitotic organ, and that the only response to loss of cardiomyocytes is hypertrophy of the remaining myocytes.[144] However, in 1998, proliferation of myocytes in healthy human hearts and an increase in proliferation after AMI were reported.[145] These results indicate that the heart is capable of proliferative homeostasis and that myocyte proliferation might compensate, at least in part, for insult-induced myocyte death. One of the most important breakthroughs in this field in recent years was the isolation of putative cardiac progenitor cells from postnatal heart.[146–155] However, the very limited turnover capacity under normal conditions has meant that studies on insult-induced cardiogenesis and the role of cardiac progenitor cells are the subject of controversy.

It has been reported that cardiomyocyte turnover increases in acute myocardial infarction, and the net output is positive (more new cells are generated per day than are lost per day).[156] However, the net output becomes negative in chronic myocardial infarction. Despite the net increase in myocyte generation in the acute phase of myocardial infarction, spontaneous cardiogenesis is often not sufficient to give full functional recovery.

Most cardiac progenitor cells express functional receptors for hepatocyte growth factor (HGF) and IGF-1. Based on the hypothesis that cardiac progenitor cells are lost in the infarcted area, the effects of HGF and IGF were studied in the context of increasing recruitment of cardiac progenitor cells from the nondamaged area to the site of injury (by HGF) and the stimulation of cardiac progenitor cell proliferation and differentiation (by IGF-1).[157] Administration of HGF and IGF-1 in a mouse myocardial infarction model stimulated myocyte regeneration and neovascularization.[157] The proliferation of cardiac progenitor cells can also be activated by, for example, GSK3β inhibitors,[154] and differentiation to cardiomyocytes by FGF-2[158] or oxytocin.[149] Detailed study of insult-induced cardiogenesis should result in the discovery of new drug targets for cardiac regeneration.

5.3.4. Osteogenesis

Two types of cells are involved in bone remodeling: the bone-resorbing osteoclasts and bone-forming osteoblasts.[159] The overall balance between these two activities maintains bone mass throughout life. However, in osteoporosis patients, an imbalance in this system leads to bone loss. Estrogen receptors are expressed in osteoblasts, and estrogen has a beneficial effect on bone formation.[160,161] Since the use of estrogens is associated with an increased risk of uterine and breast cancer, selective estrogen receptor modulators (SERMs) such as raloxifen are used for the prevention and treatment of osteoporosis.[162] Ideally, a SERM for osteoporosis treatment would have antagonistic effects on the breast and uterus, and an agonistic effect on bone.[163] Human parathyroid hormone is another agent which has been approved for the treatment of postmenopausal osteoporosis.[164]

It is well established that the Hedgehog family and bone morphogenetic proteins regulate bone formation during embryogenesis. Recently, it has been shown that the Wnt signaling pathway contributes to bone formation in adults.[165] One of the drug targets in the Wnt signaling pathway is GSK3β; GSK3β inhibitors have been shown to induce osteoblast differentiation and bone formation.[166]

5.3.5. Generation of Islet β cells

The identification of regenerative pathways for pancreatic islet β cells is also an active area of research. Three major routes for the generation of new β cells in the adult pancreas have been proposed: 1) neogenesis from ductal progenitor cells, 2) proliferation of existing β cells, and 3) transdifferentiation of pancreatic exocrine progenitor cells.[65] However, similar to the situation in the heart, the very limited turnover capacity of these cells under normal conditions means that these studies are the subject of controversial discussion. Glucagon-like peptide-1 (GLP-1) and its related peptide exendin-4 have been shown to prevent diabetes by enhancing β cell mass by inhibition of β-cell apoptosis.[167] Therefore, in addition to cell replacement therapy using islet β cells derived from ES cells, pharmacological intervention could be an another important approach to the regenerative treatment of diabetes.

5.4. Cancer Stem Cells

Despite the long history of cancer research, the origin of human tumors has not been identified. It has long been

believed that tumors arise from somatic cells which accumulate mutations in DNA associated with six characteristics: 1) self-sufficiency in growth signals, 2) insensitivity to anti-growth signals, 3) evading apoptosis, 4) tissue invasion and metastasis, 5) limitless replicative potential, and 6) sustained angiogenesis.[168] Recently, many studies demonstrated the presence of small numbers of cancer-initiating cells (called cancer stem cells) in many but not all tumors. This finding raised a new hypothesis that tumors result from the disruption of the function of tissue stem cells. Indeed, many genes identified as carcinogenic factors, such as Wnt, Sonic Hedgehog (Shh), Notch, Bmi-1, are related to stem cell function.[169,170] Furthermore, tissue stem cells are likely to accumulate DNA mutations and epigenetic change, since they exist in somatic tissues throughout life. The role of cancer stem cells in human tumor pathophysiology is not yet well characterized. Where they exist, however, the elimination of cancer stem cells would be necessary to cure the cancer. At present most of the pharmacological approaches to the elimination of cancer stem cells are focused on signaling pathways such as Wnt, Shh, and Notch, which are also involved in the growth of normal tumor cells. Cyclopamine, a steroidal alkaloid, was demonstrated to inhibit Shh signaling specifically and to inhibit tumor growth in the brain.[171] Gleevec (ST1571/imatinib mesylate) is a tyrosine kinase inhibitor which was shown to inhibit β-catenin activity in human colon cancer.[172] γ-Secretase inhibitors interfere with Notch signaling, and several inhibitors such as *N*-[*N*-(3,5-difluorophenacetyl)-L-alanyl]-S-phenylglycine *tert*-butyl ester (DAPT), dibenzazepine, IL-X (cbz-IL-CHO), and others have been shown to suppress cancer growth and induce apoptosis.[173] Another Notch inhibitor, MK0752, is in phase I of clinical development for the treatment of T-cell acute lymphoblastic leukemia and advanced breast cancer.[173] Long-term data from studies with compounds which target cancer stem cells as well as "normal" tumor cells should help us to understand the role of cancer stem cells in disease progression and therapy outcome.

5.5. Reprogramming of Tissue Stem Cells

As discussed in Section 3.5.1, mutations in DNA and epigenetic changes are considered as major causes of stem cell intrinsic dysfunction. Telomere shortening limits the proliferative life span of human stem cells by activating the DNA damage pathway. As mentioned above, inhibition of p21 (Cdkn1a, Clip1, and Waf1) is a potential target to improve age-dependent stem cell dysfunction caused by telomere shortening without accelerating the formation of cancer, and could improve proliferative homeostasis and insult-induced cell genesis in patients with telomere dysfunction.[39]

Recent studies clearly demonstrate that epigenetic changes are involved in human disease as well as during normal development.[45,174,175] A general principle of disease epigenetics is defects in phenotypic plasticity, that is, the ability of tissue stem cells to change their function in response to internal or external environmental cues. The goal of therapeutic research in this area is to identify the mechanisms of age-dependent epigenetic modification of tissue stem cells and to develop pharmacological interventions to reprogram the inappropriate epigenetic status of aged tissue stem cells. To achieve this, we need to identify biomarkers specific for normal stem cells, aging stem cells, and pre-oncogenic stem cells. *INK4a/ARF*, a cancer suppressor gene, might be a biomarker to distinguish between normal stem cells, senescent stem cells, and cancer stem cells.[176] It has been shown that $p16^{INK4a}$ accumulates in aged stem cells. On the other hand, the disruption of $p16^{INK4a}$ is viewed as a hallmark of cancer. Thus, the expression of $p16^{INK4a}$ might be used as a surrogate biomarker in diagnostics and drug-discovery research.

Recent studies demonstrated that gene transfer of *Oct4*, *Sox2*, *c-Myc*, and *Klf4* into adult mouse somatic cells converts these cells into ES cell like pluripotent stem cells.[177] This surprising report indicates that it might be possible to recover normal tissue stem cells from senescent/pre-oncogenic tissue stem cells, although this might require different factors to those used for reprogramming somatic cells. The goal of drug discovery in this case is to identify small molecules or biologics that can induce reprogramming of differentiated tissue cells and/or aged tissue stem cells. In this context, Chen et al. screened a library of small heterocyclic compounds, including 2,6-disubstituted purines, for their ability to induce dedifferentiation in the lineage-committed myoblast cell line C2C12, and identified 2-(4-morpholinoanilino)-6-cyclohexylamino-purine (reversine) as being able to convert C2C12 cells into multipotent progenitor cells which can subsequently differentiate into adipocytes and osteoblasts.[178] Williams et al.[179] recently showed that treatment of C2C12 cells or mononucleate cells and satellite cells derived from human skeletal muscle with neurodazine resulted in them developing a neuronal phenotype. Neurodazine was identified from a neurogenesis screen of approximately 300 imidazole derivatives. These data show that small-molecule approaches to cell reprogramming are feasible.

6. Translational Medicine Using Human Pluripotent Stem Cells

Differences in pharmacological effects between animals and humans can be an obstacle to the selection of appropriate drug candidates for development. Techniques which allow the translation of the knowledge obtained from animal experiments to clinical application are vital for the improvement of drug development productivity, and include in vitro and in vivo assay systems that mimic effects in human subjects.

In addition to human ES cells, it might be possible to isolate ES cell like stem cells from testis[13] or to generate them from adult somatic cells by somatic cell nuclear transfer.[180] Such pluripotent stem cells can to some extent be instructively differentiated in vitro to adult somatic cells and somatic stem cells. Cells and tissues differentiated from pluripotent stem cells should better mimic the in vivo state than do conventional human cell lines. Furthermore, adult somatic stem cells can form complex human tissue when transplanted into immune-deficient mice. Thus, human stem

cells could be used to establish in vitro and in vivo assay systems that mimic the true biological state of human subjects.

Although reprogramming-induced human pluripotent stem cells are not yet available, this technology would be a most efficient tool for translational medicine as well as for the generation of human cells and tissues with specific genetic characteristics. They would provide useful tools for predicting the effects and side effects of drug candidates where these are related to genetic polymorphisms.

The application of human pluripotent stem cells to a wide variety of drug-development needs will require the development of technologies to finely regulate their growth and differentiation.

7. Conclusion

The knowledge and technological methods associated with stem cells are enabling a paradigm shift in drug discovery and disease treatment. Originally, regenerative medicine focused on using stem cell technologies to contribute to cell therapy. However, progress in developmental biology and in the biology of adult stem cells allows us to adopt new approaches to the treatment of degenerative diseases. We believe that technologies based on adult stem cells and ES cells have the potential to provide innovative medicines which will dramatically improve the quality of life in an aging society.[181]

Abbreviations

ACE	angiotensin converting enzyme
AMI	acute myocardial infarction
AT1	angiotensin II type 1 (receptor)
BDNF	brain-derived neurotrophic factor
BMP	bone morphogenetic protein
cGMP	cyclic guanosine monophosphate
CHF	congestive heart failure
CLI	critical limb ischemia
CNS	central nervous system
COX	cyclooxygenase
CREB	cyclic AMP response element binding protein
DNA	deoxyribonucleic acid
EB	embryoid body
EG	embryonic germ (cell)
EPC	endothelial progenitor cell
EPO	erythropoietin
ERK	extracellular signal-regulated kinase
ES	embryonic stem (cell)
FGF	fibroblast growth factor
G-CSF	granulocyte colony-stimulating factor
GH	growth hormone
GLP	glucagon-like peptide
GM-CSF	granulocyte-macrophage colony-stimulating factor
GSC	germline stem cell
GSK	glycogen synthase kinase
GVHD	graft versus host disease
HGF	hepatocyte growth factor
Hh	Hedgehog
HMG-CoA	3-hydroxy-3-methyl-glutaryl-CoA
HSC	hematopoietic stem cell
IBRI	Institute for Biomedical Research and Innovation
IGF	insulin-like growth factor
IL	interleukin
LIF	leukemia inhibitory factor
MAPK	mitogen-activated protein kinase
MAPC	multipotent adult progenitor cell(s)
MIAMI	marrow-isolated adult multilineage inducible
MMP	matrix metalloproteinase
MPC	mesenchymal precursor cell
MSC	mesenchymal stem cell
NGF	nerve growth factor
NIH	National Institutes of Health
NSC	neural stem cell
PACAP	pituitary adenylate cyclase activating polypeptide
PAOD	peripheral arterial occlusive disease
PDE	phosphodiesterase
PGE	prostaglandin E
PI3	phosphatidylinositol triphosphate
PKC	protein kinase C
ROCK	Rho-associated kinase
SCF	stem cell factor
SCID	severe combined immunodeficiency
SDF	stromal cell derived factor
SERM	selective estrogen receptor modulator
STAT	signal transducer and activator of transcription
TNF	tumor necrosis factor

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