Part I
Stem Cell Biology

Stem Cell Transplantation. Biology, Processing, and Therapy.
Edited by Anthony D. Ho, Ronald Hoffman, and Esmail D. Zanjani

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Clinical Potentials of Stem Cells: Hype or Hope?

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1.1
Introduction

The present enthusiasm for and controversy around stem cell research began with two breakthroughs: (i) the successful cloning of "Dolly" by Ian Wilmut, Keith Campbell and coworkers in 1997 [1]; and (ii) the establishment of human embryonic stem cell (ESC) lines by the laboratory of James Thomson in 1998 [2]. Without any doubt, these technologies have opened up novel avenues for tissue engineering and organ transplantation [3]. Never in the history of biomedical research have scientific discoveries spawned such tremendous repercussions on a global scale. The ability to rejuvenate or even replace defective organs and the tissues of the human body has been a centuries-old dream. Stem cells have demonstrated their potential to develop into practically all types of specialized cells and tissues in the body, and have therefore been compared to the "fountains of youth" that mankind have searched for since time immemorial. Recent discoveries using both adult and embryonic stem cells as starting cell populations have led to speculations that out of such "raw material" we might be able to produce all sorts of replacement parts for regenerative medicine. Hopes are high that many age-related degenerative disorders such as heart disease, Parkinson's disease, diabetes, and stroke could some day be cured by stem cell therapy.

1.2
What are Stem Cells?

All life forms begin with a stem cell, which is defined as a cell that has the dual ability to self-renew and to produce progenitors and different types of specialized cells in the organism. For example, in the beginning of human life, one fertilized egg cell – the zygote – becomes two, and two becomes four [4]. In these early stages, each cell might still be totipotent – that is, a whole organism can be derived out of each of these cells. Within 5 to 7 days, some 40 cells are formed which

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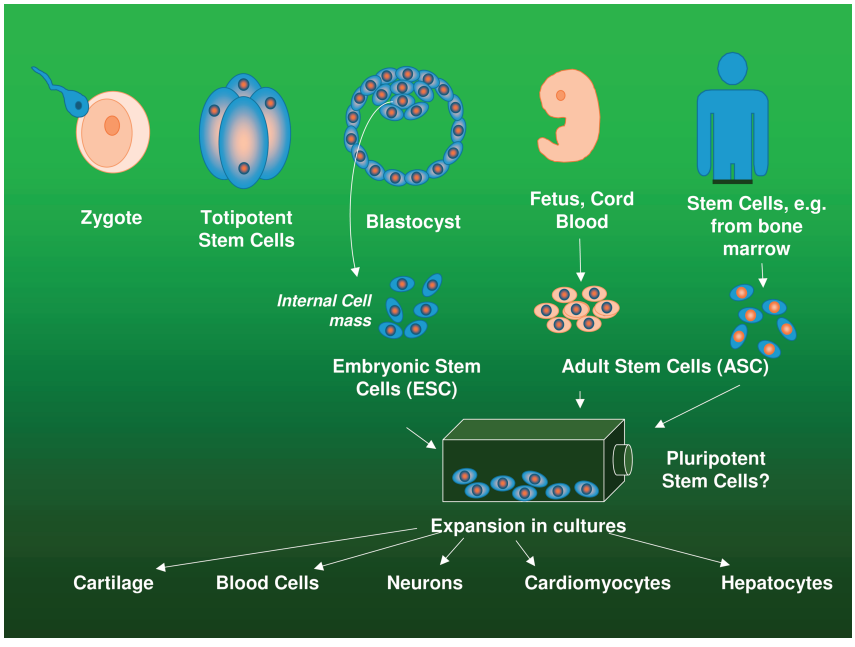

Figure 1.1 Sources for embryonic and adult stem cells.

build up the inner cell mass, surrounded by an outer cell layer forming subsequently the placenta. At this stage, each of these cells in the inner cell mass has the potential to give rise to all tissue types and organs including germ cells – that is, these cells are pluripotent (Fig. 1.1). Ultimately, the cells forming the inner cell mass will give rise to the some 10^{13} cells that constitute a human body, organized in 200 differentiated cell types [5]. Many somatic, tissue-specific or adult stem cells are produced during fetal development. Such stem cells have more restricted ability than the pluripotent ESC and they are multipotent – that is, they have the ability to give rise to multiple lineages of cells. These adult stem cells persist in the corresponding organs to varying degrees during a person's whole lifetime.

1.3 Stem Cells and Regeneration

Lower life forms have amazing prowess of regeneration which mammals and especially humans woefully lack [6]. Upon decapitation, planaria (e.g., a flatworm) will regenerate a new head within 5 days. Hydra, a small tubular freshwater animal that spends its life clinging to rock, is able to produce two new organisms

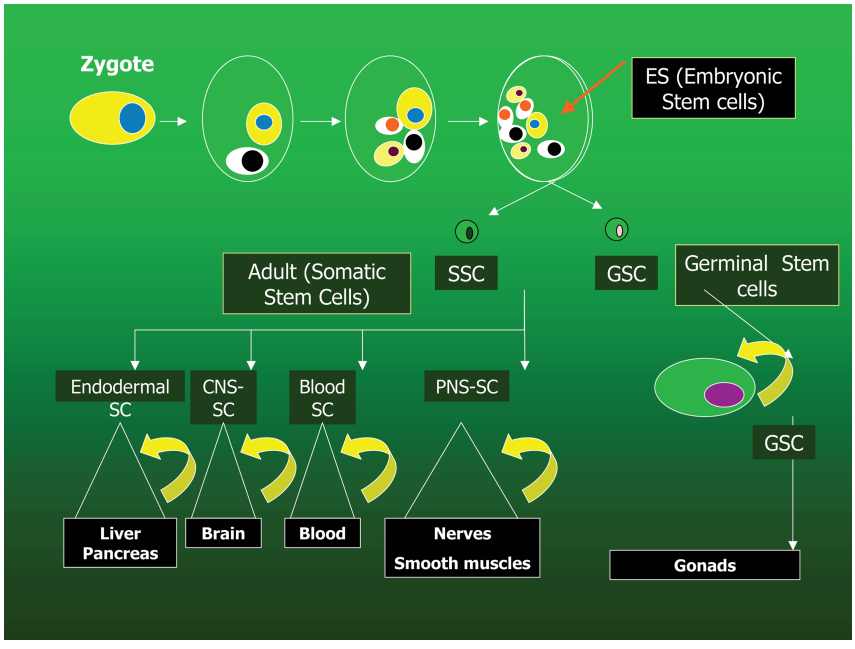

Figure 1.2 Embryonic stem cells (ES) are derived from 5- to 7-day-old embryos and are pluripotent. Pluripotent stem cells can also be derived from germinal stem cells (GSC) and possibly from some somatic (adult) stem cells (SSC). During embryonic development, tissue-specific stem cells (SC) give rise to the mature, differentiated cell types that constitute the specific organs with special functions.

within 7–10 days when its body is halved. After losing a leg or the tail to a predator, a salamander will recover with a new limb or tail within a matter of days.

Mammals pay a high price for climbing up the evolutionary ladder, and have lost comparable regenerative power. Those animals with staggering regenerative potentials are either in possession of an abundance of stem cells, or they can convert specialized cells into stem cells on demand. For example, it has been estimated that some 20 % of the planaria consists of stem cells, while hydra is a "kind of permanent embryo" [6]. Salamanders use a completely different mechanism; when they need a new limb or tail, they convert an adult differentiated cell back to an embryonic undifferentiated one. These cells then gather at the site of a severed organ and form a blastema, which regenerates the missing part. An understanding of the cues and molecules that enable the stem cells to initiate self-renewal, divide, proliferate, and then differentiate to rejuvenate damaged tissue might be the key to regenerative medicine.

To a limited extent, humans can rejuvenate some types of tissue, such as the skin and the bone marrow, but are nowhere near as proficient. The regenerative power is associated with an adequate presence of stem cells in these organs – that

is, epidermal stem cells in the skin and hematopoietic stem cells (HSCs) in the bone marrow (Fig. 1.2). Moreover, regenerative potential of the skin and marrow declines with age [7, 8]. An understanding of how ESCs differentiate into various tissues and how adult stem cells can be coaxed to replace damaged tissue could therefore hold promise for cell replacement of tissue repair in many age-related degenerative disorders.

1.4 Adult and Embryonic Stem Cells

In 1998, the group of James Thomson reported on the establishment of human ESC lines. Human ESCs used for research have been extracted form embryos created by *in-vitro* fertilization. Some 40 cells forming the inner cell mass at day 5–7 after fertilization are transferred to a culture dish lined with feeder cells. After culturing and replating for several months, these cells might maintain their self-renewing ability without differentiating into specialized cells, and give rise to ESC lines that could, in theory, replicate for ever [9–11]. Thus, ESCs have the potential to form most – if not all – cell types of the adult body over almost unlimited periods.

As mentioned above, the adult body has a small number of adult or somatic stem cells in some tissues and organs [12–14]. Such adult stem cells (ASCs) have been known to possess the ability to regenerate the corresponding tissue from which they are derived. Hematopoietic stem cells (HSCs), for example, continuously regenerate the circulating blood cells and cells of the immune system during the life span of the organism. Based on animal models, many studies have recently claimed that ASCs might exhibit developmental potentials comparable to those exhibited by ESCs [14]. More recent reports, however, have severely challenged the interpretation of the initial results, suggesting the "plasticity potential" or "trans-differentiation" of ASCs [15–18]. Hence, ASCs have the ability to regenerate the tissue from which they are derived over the lifespan of the individual, while ESCs have the potential to form most, if not all, cell types of the adult body over very long periods of *in-vitro* cultivation. ESCs seem to demonstrate unlimited potential for growth and differentiation. The use of ES-derived cells for transplantation, however, is associated with hazards and ethical controversies. In animal studies, undifferentiated ESCs can induce teratocarcinomas after transplantation, and they have been shown to be epigenetically instable. Pre-culturing of immature ESCs in conditions that induce differentiation along a specific pathway might reduce the risk of tumor genesis. Animal studies have also shown that only donor ESCs after a specific differentiation stage would be accepted by a fully grown animal. ESCs must be primed towards a predefined differentiation pathway before transplantation. Such cultures are likely to contain a variety of cells at different stages of development, as well as undifferentiated ESCs. Purification of the cell preparation is necessary before clinical use could be considered.

1.5
In the Beginning was the Hematopoietic Stem Cell

The concept of stem cells was introduced by Alexander Maximow in 1909 as the common ancestors of different cellular elements of blood [19]. It took, however, almost another 60 years – that is, in 1963 – before McCullough and his coworkers provided unequivocal evidence for the existence of stem cells in the bone marrow [20, 21]. In a murine model, their series of experiments demonstrated that, first of all, cells from the bone marrow could reconstitute hematopoiesis and hence rescue lethally irradiated recipient animals. Second, by serial transplantations, they have established the self-renewal ability of these cells. When cells from the spleen colonies in the recipients were harvested and re-transplanted into other animals that received a lethal dose of irradiation, colonies of white and red blood corpuscles were again found in the secondary recipients. Based on these experiments, HSCs were defined as cells with the abilities of self-renewal as well as multilineage differentiation. This discovery marked the beginning of modern-day stem cell research. Only in recent years have other somatic stem cells been identified in tissues with a more limited regenerative capacity, such as the liver and the brain [22, 23].

The first *successful* attempts of using bone marrow transplantation as a treatment strategy for patients with hereditary immunodeficiency or acute leukemias were performed during the late 1960s [24–27]. The original idea was to replace the diseased bone marrow with a healthy one after myeloablation. Without the benefits of present-day knowledge of immunology and supportive care, morbidity and mortality rates associated with the treatment procedure were then high [27]. Nevertheless, the results were considered encouraging as compared to those obtained with conventional treatment options. Bone marrow transplantation has in

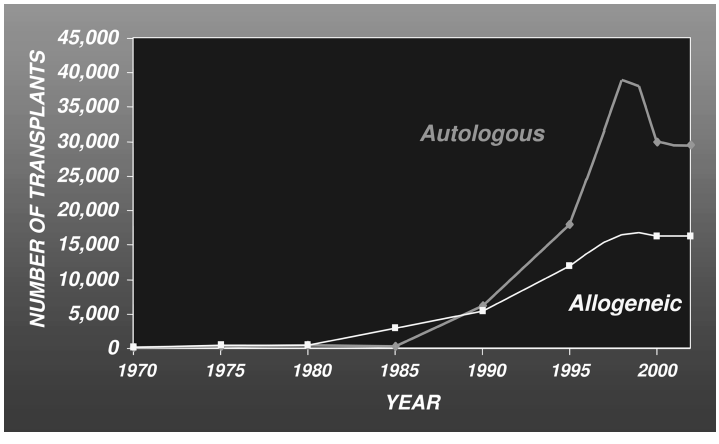

Figure 1.3 Annual numbers of blood and bone marrow transplants worldwide (1970 to 2002), as registered by the International Bone Marrow Transplant Registry.

the meantime been proven to be the only chance of cure for some patients with leukemia and some hereditary diseases [28]. Its success was due to the presence of HSCs in the marrow graft, which were able to reconstitute the blood and immune systems after myeloablation. Although initially identified in the marrow, HSCs could also be found in the peripheral blood upon stimulation, such as during the recovery phase after myelosuppressive therapy [29] or after the administration of cytokines [30, 31]. Such HSCs obtained from the peripheral blood or isolated CD34+ cell populations have been used successfully in lieu of bone marrow to reconstitute hematopoietic and immune functions in the recipients [32, 33]. According to the International Bone Marrow Transplantation Registry, blood stem cell transplantation now offers chances of durable cure for at least some 27 000 patients each year as a treatment strategy for various cancers, marrow failure, or hereditary diseases (Fig. 1.3) [34].

1.6 Trans-Differentiation of ASCs

Parallel to encouraging developments in ESC research, numerous studies have reported that ASCs might exhibit developmental potentials comparable to those exhibited by ESCs. In one of the first studies in murine models, Ferraris et al. reported that unmanipulated bone marrow cells were found to participate in the muscle regeneration process when injected into skeletal muscle that was chemically induced to undergo regeneration [35]. Furthermore, bone marrow cells that have engrafted in the muscle were also involved in the repair process if muscle injury was experimentally induced again at a later time. Since then, many authors have reported that stem cells within the marrow of mice possessed the ability to form differentiated skeletal muscle fibers, and that even cardiac muscle cells were able to regenerate by recruiting circulating marrow-derived stem cells. Eglitis and Mezey [36] showed that bone marrow cells were able to differentiate into microglia, astroglia and neurons within the central nervous system. Stem cells from the rat bone marrow have been shown to give rise to hepatocytes in recipients with artificially induced hepatic injury [37]. Other authors have confirmed that bone marrow-derived stem cells probably participated in hepatocyte restoration [38]. Multi-organ, multi-lineage engraftment by a single bone marrow-derived stem cell with HSC phenotype has been reported by Krause et al. [39]; indeed, their data have provided one of the few indications that multiple tissues could develop from a single hematopoietic tissue-derived stem cell. The magnitude of engraftment was, however, minuscule such that the biological relevance has been questioned.

ASCs from several nonhematopoietic tissues have also been reported to produce cell types other than those from the tissue in which they reside. Bjornson et al. showed that neural stem cells could produce a variety of blood cell types after transplantation into irradiated hosts [40]. The observation that adult neural stem cells might have a broader developmental potential has also been reported

by Clarke et al. [41]. The latter group showed that neural stem cells from the adult mouse brain could contribute to the formation of chimeric chick and mouse embryos. These adult neural stem cells gave rise to cells of all germ layers.

1.7 The Plasticity of ASCs: All Hype and no Hope?

More recent reports, however, have severely challenged the interpretation of the initial results suggesting the "trans-differentiation" of ASCs [15–18]. For example, in the experiments described by Bjornson et al., the cells from neurospheres that were dissociated and transplanted were passaged 12 to 35 times in the presence of growth factors prior to transplantation [40]. One possible explanation for the loss of specificity of neural stem cells is that they were transformed during their *in-vitro* passaging. It has long been established that cells growing in culture, even of defined, permanent cell lines, can spontaneously change their gene expression pattern and state of differentiation, giving rise to clonally stable "trans-differentiated" sub-lines [42]. ASCs in culture, when exposed to extreme pressures to trans-differentiate, might generate cells with genetic instability and with features of unrelated cell types. Efforts to repeat this experiment has been reported by Morshead and coworkers. The latter group confirmed that transformation of primary neural stem cells did occur during *in-vitro* passaging, but they could under no circumstances observe any contribution of neural cells to the blood cell lineage [18]. These authors concluded that trans-differentiation could not be proven. Studies conducted by Ying et al. and Terada et al. then provided evidence that cell fusion between somatic stem cells (SSCs) and ESCs occurred spontaneously upon coculturing *in vitro* [16, 17]. Both groups cautioned that such hybrid cells with tetraploid nuclei and characteristics of both SSCs and ESCs could account for the proclaimed plasticity potentials of ASCs. To verify the trans-differentiation potential of hematopoietic stem cells (HSC), Wagers et al. have generated chimeric animals by transplantation of a single green fluorescent protein (GFP)-marked HSC into lethally irradiated nontransgenic recipients. Single HSCs robustly reconstituted peripheral blood leukocytes in these animals, but did not contribute to any nonhematopoietic tissues, including brain, kidney, gut, liver, and muscle. These data indicated that "trans-differentiation" of circulating HSCs and/or their progeny is an extremely rare event, if it occurred at all [43]. Schmittwolf et al. demonstrated that only through modifications of DNA and chromatin could they establish long-term, stable and trans-differentiated hematopoietic cells from neurosphere cells [44]. Almeida-Porada et al., however, have provided new evidence that trans-differentiation did occur without cell fusion, especially under physiological conditions of the developing fetus, albeit at much lower frequencies then previously claimed [45] (see also Chapter 8).

Most of the experiments performed thus far have focused on the dramatic changes in the destiny that is, differentiation program of ASCs. Trans-differentiation, or in some rare examples plasticity, seemed indeed possible under highly se-

lective pressure from the microenvironment. There is, however, an absolute paucity of data on the cellular and molecular processes involved in the complex cascade of (trans-) differentiation. The first step, which is migration of the ASCs towards their niche and communication with the surrounding cells in the microenvironment, has not been elucidated adequately. Evidences at cellular and molecular levels show that re-programming along a different differentiation pathway are lacking. It is also not known how the newly acquired differentiation program can be maintained. Indeed, until these processes are known, it is premature to translate the observations in animal models into clinical trials.

1.8 The Battle of Two Cultures: ESCs versus ASCs

In both self-renewing as well as differentiation potentials, ASCs have been proven to be far inferior to ESCs. In injury models, ASCs from an allogeneic donor (e.g., from bone marrow), might be responsible for some of the reconstituted cells in the recipient's organs of another ontogenetic derivative. Cell and nuclear fusions might be largely responsible for this phenomenon. When trans-differentiated cells within evidence of fusion could be identified, they were of such minuscule amount as to be of no clinical relevance. Thus, many have come to the conclusion that only ESCs could hold promise for the future (Fig. 1.4).

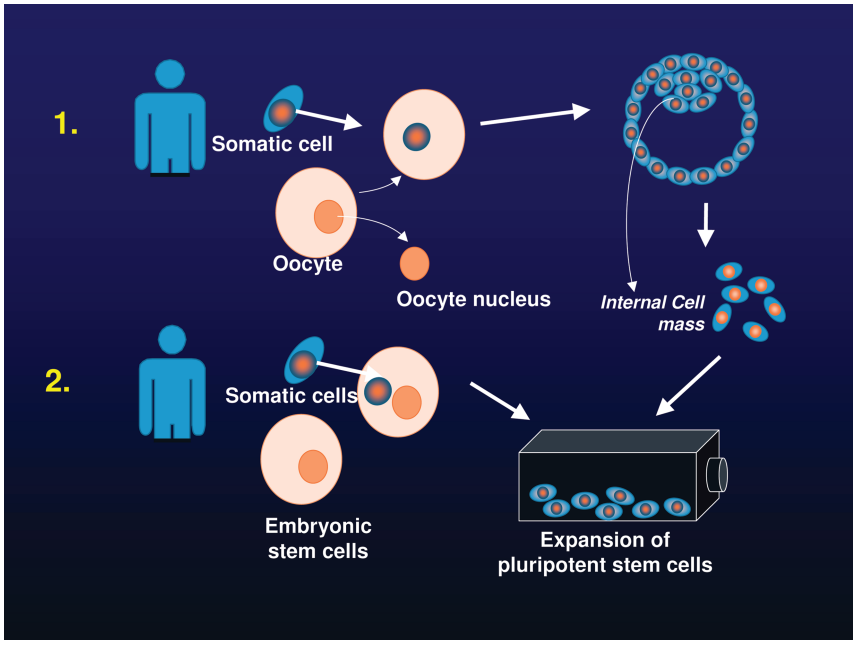

Figure 1.4 The different methods for therapeutic cloning.

Although a number of countries have since permitted the use of public funding for ESC research, opponents of this approach regard cells derived from sacrificed embryos as being close to cannibalism. On the other hand, advocates of ESC research pointed out that unwanted embryos derived from *in-vitro* fertilization clinics are continuously destined for disposal worldwide. If parents agree to donate embryos, it would not be ethical to deny their use for research purposes that target at identifying novel strategies to treat incurable diseases. Another critical challenge for the clinical use of ESCs or cell preparations derived thereof is the development of tumors, especially teratocarcinoma. The therapeutic potential of ESCs is also hampered by the threat of contamination from serum products and live feeder cells of animal origin. Serum-free and feeder layer-free systems have been used successfully by some groups, but the results have yet to be reproduced and confirmed. Thus, the debate pro and contra ESC research goes on from state to state, from country to country. Within the European Union, no consensus could thus far be reached. Whereas ESC research is strictly regulated in Germany and Austria, the U.K., the Netherlands, Belgium, Spain and Italy – and recently also Switzerland – took a much more liberal stance and have permitted ESC research under specific criteria. The U.K. has also been one of the first countries to have permitted therapeutic cloning.

1.9 The Challenges for Stem Cell Technology

One of the major challenges for the application of ESC and ASC technology is the establishment of standards and definition of stem cell preparations. The heterogeneity of the starting population renders comparison of results between different groups difficult, and this might account for the lack of reproducibility of some of the initially reports using ASCs. The significance of establishing standards and guidelines for clinical applications can best be demonstrated by the evolution of bone marrow or blood stem cell transplantation from a highly experimental procedure to the standard strategy that it is today [14, 28]. With the significance of hematopoietic tissue transplantation as curative treatment for hematologic malignancies and marrow failure, the need for *in-vitro* assays to identify human hematopoietic progenitors has increased. However, in order to infer that any *in-vitro* assay measures stem cells, the properties of the cells analyzed *in vitro* must be compared with those of repopulating units tested *in vivo* [14, 46]. Repopulating units were estimated in transplantation models and could be performed only in animals (for a review, see [13, 46]). Colony assays, including those for long-term initiating cell (LTC-IC) and myeloid-lymphoid initiating cell (ML-IC), have been developed that might serve as surrogate markers for the repopulating potentials of the stem cells present in a given population. Surface markers, such as CD34, CD133, Thy-1, HLA-DR have been shown to be associated with the "stemness" of cell preparations, while CD38 plus a whole range of surface markers have been associated with lineage commitment. Hence, many groups have

used the CD34+/CD38– and lineage-negative population as representative for primitive progenitor cells for hematopoiesis.

Despite all of the efforts made throughout the past 40 years, no *in-vitro* assay has ever been considered adequate for the identification of HSCs [13, 14, 46]. Hence, there is no appropriate substitute for the repopulation assay in murine transplantation model after a lethal dose of irradiation [20, 21]. Clearly, this experimental approach cannot be used to estimate human HSCs. However, the immunocompromised mouse model (i.e., SCID mouse model and variations thereof), or the *in-utero* sheep transplantation model at a time when the animal is tolerant to human HSCs, have been proposed for estimating the repopulating potentials of human HSCs [47]. Preparative protocols for acquisition, *in-vitro* cultivation, expansion and differentiation along specific pathways of ESCs or ASCs have thus far been extremely heterogeneous. A precise characterization and standardization of ESCs as well as ASC preparations and the progeny cells derived thereof represents a *conditio sine qua non* for future development and for comparing the results from different research groups. Hence, there is an urgent need for establishing robust standards and developing a catalogue of marker profiles for the definition of stem cells and of their differentiation products. Such efforts will be described in Chapters 3 and 7.

1.10 Regulation of Self-Renewal versus Differentiation, Asymmetric Divisions

A hallmark of stem cell activity is the dual capacity to self-renew and to differentiate into cells of multiple lineages. Thus, the ability to divide asymmetrically might be regarded as a unique feature of stem cells. A central question in developmental biology is how a single cell can divide to produce two progeny cells that adopt different fates. Different daughter cells with different functions can, in theory, arise by uneven distribution of determinants upon cell division (i.e., due to intrinsic factors) or become different upon subsequent exposure to environmental signals (i.e., due to extrinsic factors) [47]. Recent advances in the understanding of asymmetric division of stem cells in *Drosophila* and murine models have provided some insight into human stem cell development.

Evidence from the development of neuroblasts from neuroepiderm in *Drosophila* and in the mouse model supports the idea that asymmetric divisions are defined mostly by cell-autonomous (i.e., intrinsic) information. For example, *Drosophila* neuroblasts (NBs) – which are precursors of the central nervous system – arise from polarized epithelial cells during development [48]. The NBs enlarge and delaminate from the ventral ectoderm, forming a subepithelial array of neural stem cells. The plane of cleavage upon division is orientated parallel to their apical-basal axis, resulting in symmetric division. The NBs then undergo a series of asymmetric divisions and, while maintaining the axis of apical-basal polarity, adjust their cleavage plane such that it is perpendicular to this axis, and produce NBs to renew themselves and smaller ganglion mother cells (GMC-1). Asym-

metric division is orientated intrinsically and autonomously. GMC production is followed by a single division, generating two post-mitotic neurons or glial precursors. During early embryonic development, asymmetric divisions therefore provide a mechanism for positioning specific cell types at defined sites. An axis of polarity is established in the mother cell, and this coordinates with the general body plan. Cell-fate determinants are distributed asymmetrically along this axis. During mitosis the spindle is also orientated along this axis so that cytokinesis creates two daughter cells containing different concentrations of these determinants [49–53]. In *Drosophila*, for example, homologues of PAR-3, atypical protein kinase C and PAR-6 mediate polarity and they direct epithelial cell polarity and mediate both spindle orientation and localization of cell-fate determinants in NBs [54, 55]. For subsequent development, intracellular or extrinsic mechanisms

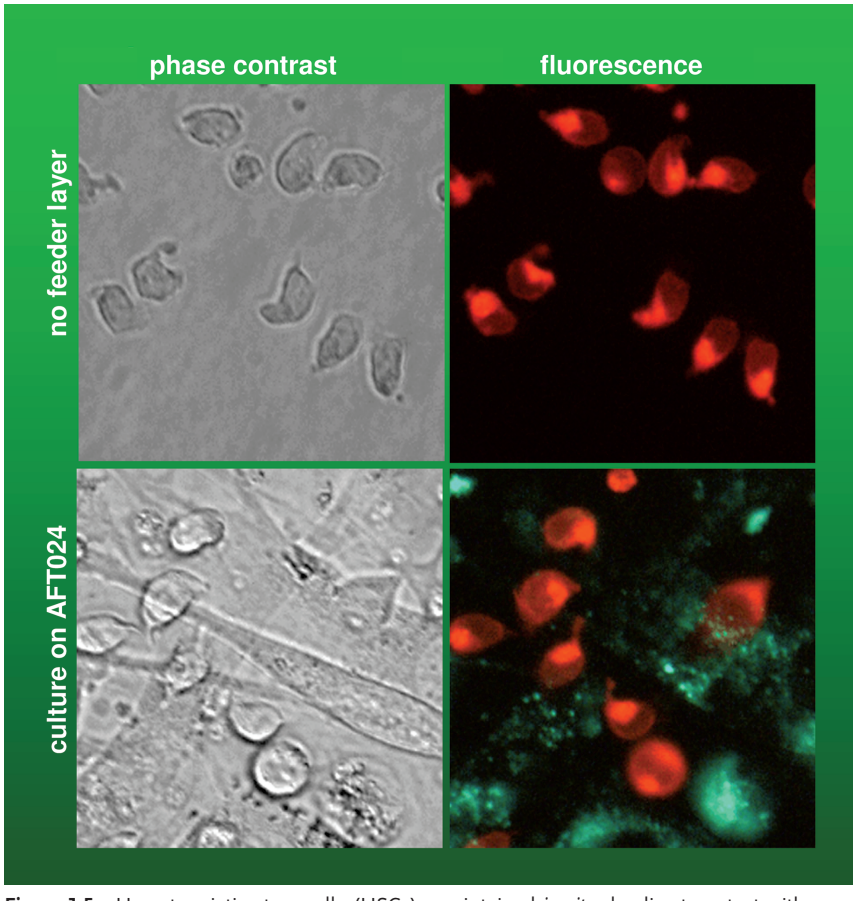

Figure 1.5 Hematopoietic stem cells (HSCs) can be enriched in the CD34+/CD38– fraction. The fluorescent membrane dye PKH26 can be used to label these cells red. The cells can be maintained *in vitro* by direct contact with a supportive cell layer that provides the appropriate microenvironment (e.g., the AFT024 feeder layer; green).

(as a consequence of communication of the daughter cells with each other or with surrounding cells) play then a major role and extrinsic signals might be involved in instructing the asymmetric fates of the daughter cells [56, 57]. Kiger et al. [58] and Tulina and Matunis [59], for example, have defined the molecular nature and spatial organization of the signaling pathway that governs asymmetric divisions of stem cells in the *Drosophila* testis. In the latter, germline cells and SSCs attach to a cluster of support cells called the "hub". Upon division of a germline stem cell, the daughter cell in direct contact with the hub retains the self-renewal potential, whereas the other daughter cell was destined to differentiate into a gonioblast and subsequently into spermatogonia. Evidence was provided that *Unpaired*, a ligand which activates the JAK-STAT signaling cascade and is expressed by the apical hub cells in the testis, causes stem cells to retain their self-renewal potential. Analogous to this finding, the maintenance of mammalian ESCs has been shown to require a similar JAK-STAT signaling, which is counterbalanced by the requirement for MAP kinase activation, and the latter in turn promotes ESC differentiation [60]. In another recent publication, Yamashita et al. demonstrated that germline stem cells were anchored to the hub through localized adherens junctions. Interactions between DE-cadherin on the surface of hub cells and germline stem cells could stabilize a localized binding site for beta-catenin and Apc2 at the germline stem cell (GSC) cortex [61]. The cadherin-catenin and the associated cytoskeletal system seem to be key players in this context.

For HSCs, our group has demonstrated that only contact of primitive CD34+/CD38– cells with a stem cell-supporting microenvironment (AFT024) increased asymmetric divisions of both primitive and committed progenitors by recruiting significant numbers of primitive cells into the cell cycle [62] (Fig. 1.5). This phenomenon of recruitment, as well as the shift in asymmetric division, could not be induced by cytokines [63]. Thus, dormant cells that are usually in G_0 can be recruited to cycle without loss of primitive function after cell–cell contact with AFT024. Only direct contact with cellular elements of the niche could increase the absolute number of cells undergoing asymmetric division. The stem cell niche thus provides the cues to regulate self-renewing divisions and subsequently to control cell numbers.

1.11
Genotype and Expression Profiles of Primitive HSCs

As the slow-dividing fraction (SDF) of HSCs is associated with primitive function and self-renewal, while the fast-dividing fraction (FDF) predominantly proceeds to differentiation, we have separated the $CD34^+/CD38^-$ cells according to their divisional kinetics as a functional parameter for the isolation of primitive stem cells [64]. We then performed a genotypic analysis of these two populations (FDF versus SDF) using genome-wide analysis. Genome-wide gene expression analysis of these populations was determined using a Human Transcriptome Microarray containing 51 145 cDNA clones of the Unigene Set-RZPD3 [65]. In addition, gene expression profiles of $CD34^+/CD38^-$ cells were compared with those of

$CD34^+/CD38^+$ cells. Among the genes showing the highest expression levels in the SDF were the following: CD133, erg, cyclin g2, MDR1, osteopontin, clqr1, ifi16, jak3, fzd3 and hoxa9, a pattern compatible with their primitive function and self-renewal capacity. We have also demonstrated that the SDF of $CD34^+/CD38^-$ cells displayed significantly more podia formation and migratory activity as compared to the more committed progenitor cells found among the FDF [65].

Several other attempts have been made to identify the gene expression profiles of stem cells using microarray technology. In most of these studies, the target population was separated from their native stem cell niche before analysis [66–69]. In a meta-analysis of our own data and the data of three other studies on HSCs, we have shown that, despite the use of different starting materials, derivation from different species, applying very different platforms and methods of analysis, an interesting overlap of genes that are overexpressed in the primitive subsets of HSCs was found [65]. This included *fzd6, mdr1,* RNA-binding protein with multiple splicing (*rbpms*), *jak3, and hoxa9*. Other studies have focused on the specific molecular make-up of the HSC niche. Hackney et al. have analyzed the gene expression profiles of AFT024 cells in comparison to other fetal liver-derived lines of varying stem cell support [69]. A number of genes that potentially influenced stem cell function were highly expressed in AFT024 cells, underscoring the hypothesis that many pathways might be involved in supporting stem cell function [70, 71].

1.12 Maintaining Stemness: Interactions between HSCs and the Cellular Microenvironment

Our group, as well as other investigators, has shown that direct contact with the cellular microenvironment was able to maintain the stem cell function of $CD34^+/CD38^-$ cells to increase the number of asymmetric divisions, and recruit more $CD34^+/CD38^-$ cells into cell cycle compared to those exposed to cytokines alone [63,72–74]. In order to define the essential cellular and molecular mechanisms involved in the interaction between HSCs and the stroma feeder layer, we have studied the impact of cocultivation on the behavior and gene expression of HSCs [75]. We have shown that HSCs developed directed migratory activity towards stroma cells, indicating that HSCs migrated towards signals secreted by the supportive stroma cells [76]. The HSCs subsequently established stable contact to stroma cells by means of a uropod, on which CD44 and CD34 were colocalized. CD44 is known to bind fibronectin and hyaluronic acid, and is essential for the homing and proliferation of HSCs [77–79].

Using a human genome cDNA microarray developed in our group, we have subsequently analyzed the gene expression profiles of $CD34^+/CD38^-$ cells upon cultivation with or without stroma for 16, 20, 48, or 72 h. Several genes that play a role in cell adhesion, the re-organization of the cytoskeleton system, the maintenance of methylation patterns, stabilization of DNA during proliferation

and repair were up-regulated within the first 72 h upon exposure. The overexpression of genes coding for tubulin α, tubulin β, and ezrin was indicative of the significant role of reorganization of the cytoskeleton system upon interaction with the cellular microenvironment. This was also compatible with the increase in motility and adhesion, as described previously [76]. Other genes that were up-regulated included proliferating cell nuclear antigen (*pcna*), which is involved in the control of DNA replication, and DNA (cytosine-5)-methyltransferase (*dnmt1*), which is responsible for maintaining methylation patterns during embryonic development [80–82]. A few genes characteristic for primitive HPC were again overexpressed, which included the receptor for the complement component molecule C1q (*c1qr1*) and *HLA-DR* [65]. Among the gene sequences that were down-regulated were various hemoglobin genes [76]. Our previous experiments also showed that hemoglobin genes were expressed more highly in the more committed progenitors, and these results indicate that HSCs cultivated without stroma showed an intrinsic propensity to differentiate along the erythrocyte lineage [65].

1.13 Mesenchymal Stem Cells

Mesenchymal stem cells (MSCs) represent another archetype of multipotent SSC that give rise to a variety of cell types including osteocytes, chondrocytes, adipocytes and other kinds of connective tissue cells such as those in tendons. Recent studies have indicated that, given the appropriate microenvironment, MSCs could also differentiate into cardiomyocytes or even cells of nonmesodermal derivation, including hepatocytes and neurons. MSCs have been used within clinical trials for regenerative medicine. These multipotent stem cells might hold promise for the following reasons:

- In contrast to most other SSCs, they can be isolated from a diverse set of tissues that are readily accessible, such as bone marrow, fat tissues and umbilical cord blood.
- These cells could be expanded *in vitro* without losing their "stemness" or self-renewal capacity [83, 84].
- MSCs have been shown to differentiate *in vitro* into bone, cartilage, muscle, tendon, and fat, and possibly also into cardiomyocytes and hepatocytes [85–91].
- In conjunction with HSCs, allogeneic MSCs have been transplanted without graft rejection or major toxicities [92] (Fig. 1.6).

Verfaille and coworkers have described the derivation of multipotent adult progenitor cells (MAPCs) from murine and human bone marrow [93, 94] (see also Chapter 11). These MAPCs were able to differentiate into functional hepatocyte-like cells and were probably related to the MSCs. Similar multipotent pro-

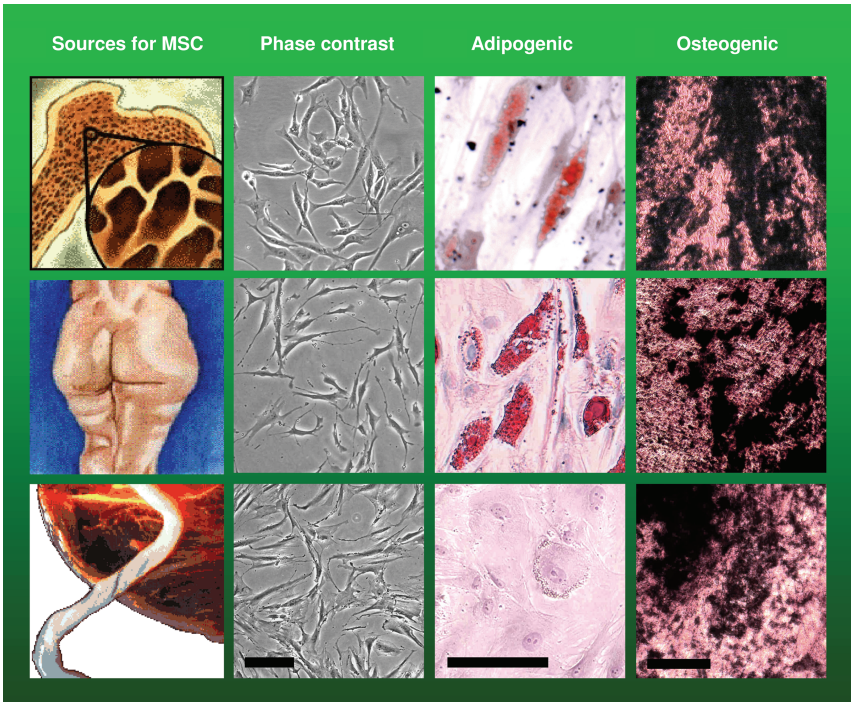

Figure 1.6 Mesenchymal stem cells (MSCs) can be isolated from various tissues, including bone marrow, adipose tissue and from umbilical cord blood. MSCs are plastic adherent with a spindle-shaped morphology. Adipogenic and osteogenic differentiation can be induced by appropriate culture conditions, as examined by Oil Red-O staining or von Kossa staining. Scale bar: 100 μm.

genitors, "unrestricted somatic stem cells" (USSCs), derived from umbilical cord blood, have recently been described by Kögler et al. [95] (see also Chapter 2).

The clinical relevance of all these multipotent stem cells of mesenchymal origin is highly controversial for the following reasons. The prerequisite of prolonged *in-vitro* culture prior to the emergence of MSCs, MAPCs or USSCs raises the question as to whether such cells exist naturally in postnatal tissues. The precise definition of these MSCs, MAPCs or USSCs – and especially their precise cellular and molecular characterization – have remained elusive. Under the culture conditions for the propagation of MSCs, MAPCs or USSCs, these cells might become epigenetically unstable. Further expansion or trans-differentiation for specific maturation pathways *in vitro* might render them more so, and pre-malignant transformation of cells cannot be completely excluded at this juncture. Last, but not least, a sophisticated analysis of self-renewal and differentiation on a single cell basis has to date proved elusive in MSCs, and serial transplantations have not

been performed. Current preclinical research on the trans-differentiation potentials of ASCs (including MSCs) has focused mainly on descriptive phenomena such as emergence of differentiation markers, but lacks the solid fundamentals of cell biology. Almost no data exist on the cellular and molecular processes involved in the complex cascade of differentiation into specific pathways such as from HSCs or MSCs into cardiomyocytes or hepatocytes. Further characterization of MSCs requires the development of robust phenotypic and functional markers, and the demonstration of multipotentiality [A, B]. Further basic cell biology research, based especially on precise knowledge of molecular and genetic mechanisms, is urgently needed to provide a safe background for the use of cultured stem cells within the clinical setting, irrespective of their origin – that is, embryonic cells or adult tissues.

1.14 Preliminary Clinical Studies

With the exception of HSCs, the application of stem cell preparations or other cell products thereof as replacement therapy for organ failure, though tantalizing, is yet far from clinical practice. Nevertheless, a few clinical studies have suggested benefits for the use of marrow-derived progenitor cells for cardiovascular diseases, and for the use of liver cell preparations for hepatic failure. Stamm et al., for example, have demonstrated the feasibility and safety of administering progenitor cells derived from autologous bone marrow to the infarcted myocardium of patients with ischemic heart disease who undergo a coronary artery bypass surgery [96] (see also Chapter 14). In an ongoing controlled clinical trial, the same authors have also provided evidence of a pronounced effect of cell therapy on the blood supply to ischemic tissue, associated with an improvement of contractile function. Thus, the scientific basis for the use of ASCs or ESCs for regenerative medicine has remained controversial. As shown by the adverse events associated with gene therapy during the past years [97, 98], clinical trials without any precise scientific foundation might in the long run jeopardize scientific progress and public trust. Issues of concern for the application of stem cell technology in regenerative medicine include the reproducibility for the early trans-differentiation experiments, a definition of the starting cell population and cell products, the standardization of expansion or differentiation processes, and the toxicology and functional properties of the differentiated cell products compared to the target tissue.

1.15
Concluding Remarks and Future Perspectives

At present, it is unclear whether ASCs can match the ESC's capacity to differentiate into cells of almost any organ. Whereas most studies in the past have focused on dramatic changes in long-term fate, such as the conversion into tissues of another germinal derivation, little is known about the mechanisms of the initial steps leading to a different maturation pathway. Neither has the hierarchy of molecular changes involved in switching to another differentiation program been defined. Cross-talk with the microenvironment probably determines the long-term fate, both in terms of the differentiation program as well as in terms of the balance between self-renewal *versus* differentiation.

During the past 40 years, we have learned that stem cell research requires intensive resources and scientific environment that is conducive to innovation. In the case of blood stem cell transplantation, some 20 years of continuous improvements in clinical research has contributed to the establishment of this procedure as a standard and curative treatment for specific diseases. During this time, many groups have attempted to expand HSC use *ex vivo*, though attempts by others to reproduce the initial reports of the expansion of HSCs have not proved successful. Similar concerns also apply to the use of other ASCs that need to be expanded *in vitro*. It is absolutely essential that the initial population is well characterized and the subsequent expansion procedure standardized. Other than morphology, immunophenotyping and alternative methods of characterizing the stem cell population (e.g., division history, molecular markers, genotypic and proteomic analysis) might be required in order to define specific stem cell populations. Importantly, these experiments are currently under way.

Research into the trans-differentiation potentials of ASCs has thus far focused mainly on descriptive phenomena such as the emergence of differentiation markers, but lacks the solid fundaments of cell biology. Very few data exist on the cellular and molecular processes involved in the complex process of trans-differentiation. For example, the molecular mechanism behind the dramatic change in cell fate from HSCs or MSCs into progenitors of cardiomyocytes or hepatocytes is totally unknown. Indeed, the cues and mechanisms governing the decision processes of self-renewal versus differentiation, as well as differentiation along specific pathways are, at best, sketchy. Specific soluble regulatory molecules and direct contact with the cellular microenvironment might play a role in the regulation of self-renewal versus differentiation, as well as the adoption of a specific differentiation program. Today, an understanding of the basic principles which govern stem cell fate is more important than demonstrating dramatic changes therein. Consequently, there is an urgent need for basic cell biological research, especially based on precise knowledge of molecular and genetic mechanisms, in order to provide a safe background for the use of cultured stem cells in the clinical setting, whether of embryonic or adult origin.

Given the present status in stem cell research, it is essential that we keep all options open with regard to investigations into both ESCs and ASCs in order

to appreciate the complexity of their differentiation pathways and of their developmental processes. Only with a thorough understanding of the molecular mechanisms involved might we acquire the power to manipulate the destiny of stem cells.

Abbreviations/Acronyms

ASC	adult stem cell
CNS	central nervous system
ESC	embryonic stem cell
FDF	fast-dividing fraction
GFP	green fluorescent protein
GMC	ganglion mother cell
GSC	germline stem cell
HSC	hematopoietic stem cell
LTC-IC	long-term initiating cell
MAPC	multipotent adult progenitor cell
ML-IC	myeloid-lymphoid initiating cell
MSC	mesenchymal stem cell
NB	neuroblast
PNS	peripheral nervous system
SDF	slow-dividing fraction
SSC	somatic stem cell
USSC	unrestricted somatic stem cell

References

1. Wilmut I, Schnieke AE, McWhir J, Kind AJ, Campbell KH (1997). Viable offspring derived from fetal and adult mammalian cells. *Nature* 385: 810.
2. Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, Jones JM (1998). Embryonic stem cell lines derived from human blastocysts. *Science* 282: 1145.
3. Fuchs E, Segre JA (2000). Stem cells: a new lease on life. *Cell* 100: 143.
4. Carlson BM (1996). *Patten's Foundations of Embryology*. 6th edn. New York, McGraw-Hill.
5. Sadler TW (2002). *Langman's Medical Embryology*, 8th edn. Philadelphia, Lippincott Williams and Wilkins.
6. Ho AD, Wagner W, Mahlknecht U. (2005). Stem cells and ageing. *EMBO Rep* 6: S35–S38.
7. Stenderup K, Justesen J, Clausen C, Kassem M. (2003). Aging is associated with decreased maximal life span and accelerated senescence of bone marrow stromal cells. *Bone* 33: 919–926.
8. Figueroa R, Lindenmaier H, Hergenhahn M, Nielsen KV, Boukamp P. (2000). Telomere erosion varies during in vitro aging of normal human fibroblasts from young and adult donors. *Cancer Res* 60(11): 2770–2774. Erratum in: *Cancer Res* 60(15): 4301.
9. Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall

VS, Jones JM (1998). Embryonic stem cell lines derived from human blastocysts. *Science* 282, 1145–1147.

10. Richards M, Fong CY, Chan WK, Wong PC, Bongso A. (2002). Human feeders support prolonged undifferentiated growth of human inner cell masses and embryonic stem cells. *Nat Biotechnol* 20: 933–936.

11. Amit M, Shariki C, Margulets V, Itskovitz-Eldor J (2004). Feeder layer- and serum-free culture of human embryonic stem cells. *Biol Reprod* 70: 837–845.

12. Gage FH (2000). Mammalian neural stem cells. *Science* 287, 1433–1438.

13. Weissman I L (2000). Translating stem and progenitor cell biology to the clinic: barriers and opportunities. *Science* 287, 1442–1446.

14. Ho AD, Punzel M (2003). Hematopoietic stem cells: can old cells learn new tricks? *J Leukocyte Biol* 73: 547–555.

15. Wagers AJ, Christensen JL, Weissman IL (2002). Cell fate determination from stem cells. *Gene Ther* 9: 606–612.

16. Terada N, Hamazaki T, Oka M, Hoki M, Mastalerz DM, Nakano Y, Meyer EM, Morel L, Petersen BE, Scott EW (2002). Bone marrow cells adopt the phenotype of other cells by spontaneous cell fusion. *Nature* 416: 542–545.

17. Ying QL, Nichols J, Evans EP, Smith AG (2002). Changing potency by spontaneous fusion. *Nature* 416: 545–548.

18. Morshead CM, Benveniste P, Iscove NN, van der Kooy D (2002). Hematopoietic competence is a rare property of neural stem cells that may depend on genetic and epigenetic alterations. *Nat Med* 8: 268–273.

19. Maximow A (1909). Der Lymphozyt als gemeinsame Stammzelle der verschiedenen Blutelemente in der embryonalen Entwicklung und im postfetalen Leber der Säugetiere. *Folia Haematol (Leipz)* 8: 125–141.

20. Siminovitch L, McCulloch EA, Till JE (1963). The distribution of colony-forming cells among spleen colonies. *J Cell Comp Physiol* 62: 327.

21. Becker A, McCulloch EA, Till JE (1963). Cytological demonstration of the clonal nature of spleen colonies derived from transplanted mouse marrow cells. *Nature* 197: 452.

22. Gage FH (1998). Stem cells of the central nervous system. [Review]. *Curr Opin Neurobiol* 8: 671.

23. Block GD, Locker J, Bowen WC, Petersen BE, Katyal S, Strom SC, Riley T, Howard TA, Michalopoulos GK (1996). Population expansion, clonal growth, and specific differentiation patterns in primary cultures of hepatocytes induced by HGF/SF, EGF and TGFa in a chemically defined (HGM) medium. *J Biol Chem* 132: 1133.

24. Bach FH, Albertini RJ, Joo P, Anderson JL, Bortin MM (1968). Bone-marrow transplantation in a patient with the Wiskott-Aldrich syndrome. *Lancet* 2: 1364.

25. Gatti RA, Meuwissen HJ, Allen HD, Hong R, Good RA (1968). Immunological reconstitution of sex-linked lymphopenic immunological deficiency. *Lancet* 2: 1366.

26. de Koning J, van Bekkum DW, Dicke KA, Dooren LJ, Radl J, van Rood JJ (1969). Transplantation of bone-marrow cells and fetal thymus in an infant with lymphopenic immunological deficiency. *Lancet* 1: 1223.

27. Thomas ED, Bryant JI, Buckner CD, et al. (1971). Allogeneic marrow grafting using HL-A matched donor-recipient sibling pairs. *Trans Assoc Am Physicians* 84: 248.

28. Thomas ED, Flournoy N, Buckner CD, et al. (1977). Cure of leukaemia by marrow transplantation. *Leukemia Res* 1: 67–70.

29. Körbling M, Dörken B, Ho AD, Pezzutto A, Hunstein W, Fliedner TM (1986). Autologous transplantation of blood-derived hemopoietic stem cells after myeloablative therapy in a patient with Burkitt's lymphoma. *Blood* 67: 529.

30. Haas R, Ho AD, Bredthauer W, Cayeux S, Egerer G, Knauf W, Hunstein W (1990). Successful autologous transplantation of blood stem cells mobilized with recombinant human granulocyte-macrophage colony-stimulating factor. *Exp Hematol* 18: 94.

31. Lane TA, Law P, Maruyama M, Young D, Burgess J, Mullen M, Mealiffe M, Ter-

stappen LWMM, Hardwick A, Moubayed M, Oldham F, Corringham RET, Ho AD (1995). Harvesting and enrichment of hematopoietic progenitor cells mobilized into the peripheral blood of normal donors by granulocyte macrophage-colony stimulating factor (GM-CSF) or G-CSF: Potential role in allogeneic marrow transplantation. *Blood* 85: 275.

32. Juttner CA, To HO, Ho JQ, Bardy PG, Dyson PG, Haylock DN, Kimber RJ (1988). Early lymphoematopoietic recovery after autografting using peripheral blood stem cells in acute non-lymphoblastic leukemia. *Transplant Proc* 20: 40.

33. Corringham RET, Ho AD (1995). Rapid and sustained allogeneic transplantation using immunoselected CD34+-selected peripheral blood progenitor cells mobilized by recombinant granulocyte- and granulocyte-macrophage colony-stimulating factors. *Blood* 86: 2052.

34. *International Bone Marrow Transplant Registry*, Annual Report, 2004.

35. Ferrari G, Cusella-De Angelis G, Coletta M, Paolucci E, Stornaiuolo A, Cossu G, Mavilio F (1998). Muscle regeneration by bone marrow-derived myogenic progenitors. *Science* 279: 1528.

36. Eglitis MA, Mezey E (1997). Hematopoietic cells differentiate into both microgliaand macroglia in the brains of adult mice. *Proc Natl Acad Sci USA* 94: 4080.

37. Petersen BE, Bowen WC, Patrene KD, Mars WM, Sullivan AK, Murase N, Boggs SS, Greenberger JS, Goff JP (1999). Bone marrow as a potential source of hepatic oval cells. *Science* 284: 1168.

38. Theise ND, Badve S, Saxena R, Henegariu O, Sell S, Crawford JM, Krause DS (2000). Derivation of hepatocytes from bone marrow cells of mice after radiation-induced myeloablation. *Hepatology* 31: 235.

39. Krause DS, Theise ND, Collector MI, Henegariu SH, Gardner R, Neutzel S, Sharkis SJ (2001). Multi-organ, multilineage engraftment by a single bone marrow-derived stem cell. *Cell* 105: 369.

40. Bjornson CR, Rietze RL, Reynolds BA, Magli MC, Vescovi AL (1999). Turning brain into blood: a hematopoietic fate adopted by adult neural stem cells *in vivo*. *Science* 283: 534.

41. Clarke DL, Johansson CB, Wilbertz J, Veress B, Nilsson E, Karlstrom H, Lendahl U, Frisen J (2000). Generalized potential of adult neural stem cells. *Science* 288: 1660.

42. Knapp AC, Franke WW (1989). Spontaneous losses of control of cytokeratin gene expression in transformed, non-epithelial human cells occurring at different levels of regulation. *Cell* 59: 67–79.

43. Wagers AJ, Sherwood RI, Christensen JL, Weissman IL (2002). Little evidence for developmental plasticity of adult hematopoietic stem cells. *Science* 297: 2256.

44. Schmittwolf C, Kirchhof N, Jauch A, Dürr M, Harder F, Zenke M, Müller AM (2005). *In vivo* haematopoietic activity is induced in neurosphere cells by chromatin-modifying agents. *EMBO J* 24: 554–566.

45. Almeida-Porada G, Porada, CD, Chamberlain J, Torabi A, Zanjani ED (2004). Formation of human hepatocytes by human hematopoietic stem cells in sheep. *Blood* 104: 2582–2590.

46. Ho AD, Haas R, Champlin R (Eds.) (2000). *Hematopoietic stem cell transplantation*. Marcel Dekker, New York, p. 604.

47. Zanjani ED, Pallavicini MG, Ascensao JL, Flake AW, Langlois RG, Reitsma M, MacKintosh FR, Stutes D, Harrison MR, Tavassoli M (1992). Engraftment and long-term expression of human fetal hematopoietic stem cells in sheep following transplantation in utero. *J Clin Invest* 89: 1178.

48. Lin H, Schagat T (1997). Neuroblasts: a model for the asymmetric division of stem cells. *Trends Genet* 13(1): 33.

49. Hirate J, Nakagoshi H, Nabeshima Y, Matsuzaki F (1995). Asymmetric segregation of the homeodomain protein Prospero during *Drosophila* development. *Nature* 377: 627–630.

50. Knoblich JA, Jan LY, Jan YN (1995). Asymmetric segregation of Numb and Prospero during cell division. *Nature* 377: 624–627.

51. Spana EP, Doe CQ (1995). The Prospero transcription factor is asymmetrically localized to the cell cortex during neuro-

blast mitosis in *Drosophila*. *Development* 121: 3187–3195.

52. Li P, Yang X, Wasser M, Cai Y, Chia W (1997). Inscuteable and Staufen mediate asymmetric localization and segregation of Prospero RNA during *Drosophila* neuroblast cell divisions. *Cell* 90: 437–447.

53. Ikeshima-Kataoka H, Skeath JB, Nabeshima Y, Doe CQ, Matsuzaki F (1997). Miranda directs Prospero to a daughter cell during *Drosophila* asymmetric divisions. *Nature* 390: 625–629.

54. Joberty G, Petersen C, Gao L, Macara IG (2000). The cell polarity protein Par6 links Par3 and atypical protein kinase C to CDc42. *Nature Cell Biol* 2: 531–539.

55. Lin D, et al. (2000). A mammalian PAR-3-PAR-6 complex implicated in CDc42/Rac1 and aPKC signalling and cell polarity. *Nature Cell Biol* 2: 540–547.

56. Sherley JL (2002). Asymmetric cell kindetics genes: The key to expansion of adult stem cells in culture. *Stem Cells* 20: 561–572.

57. Lee, HS, Crane GG, Merok JR, Tunstead JR, Hatch NL, Panchalingam K, Powers MJ, Griffith LG, Sherley JL (2003). Clonal expansion of adult rat hepatic stem cell lines by suppression of asymmetric cell kinetics (Sack). *Wiley Periodicals* 760–771.

58. Kiger AA, Jones DL, Schulz C, Rogers MB, Fuller MT (2001). Stem cell self-renewal specified by JAK-STAT activation in response to a support cell cue. *Science* 294: 2542.

59. Tulina N, Matunis E (2001). Control of stem cell self-renewal in *Drosophila* spermatogenesis by JAK-STAT signaling. *Science* 294: 2546.

60. Matsuda T, Nakamura T, Nakao K, Arai T, Katsuki M, Heike T, Yokota T (1999). STAT3 activation is sufficient to maintain an undifferentiated state of mouse embryonic cells *EMBO J* 18: 4261.

61. Yamashita QM, Jones DL, Fuller MG (2003). Orientation of asymmetric stem cell division by the APC tumor suppressor and centrosome. *Science* 301: 1547–1550.

62. Punzel M, Liu D, Zhang T, Eckstein V, Miesala K, Ho AD (2003). The symmetry of initial divisions of human hematopoietic progenitors is altered only by the cellular microenvironment. *Exp Hematol* 31: 339–347.

63. Huang S, Law P, Francis K, Palsson BO, Ho AD (1999). Symmetry of initial cell divisions among primitive hematopoietic progenitors is independent of ontogenic age and regulatory molecules. *Blood* 94: 2595–2604.

64. Punzel M, Zhang T, Liu D, Eckstein V, Ho AD. Functional analysis of initial cell divisions defines the subsequent fate of individual human CD34+CD38– cells. *Exp Hematol* 30: 464–472.

65. Wagner W, Ansorge A, Wirkner U, Eckstein V, Schwager J, Miesala K, Selig J, Saffrich R, Ansorge W, Ho AD (2004). Molecular evidence for stem cell function of the slow-dividing fraction among human hematopoietic progenitor cells by genome-wide analysis. *Blood* 104: 675–686.

66. Terskikh AV, Miyamoto T, Chang C, et al. (2003). Gene expression analysis of purified hematopoietic stem cells and committed progenitors. *Blood* 102: 94–101.

67. Ivanova NB, Dimos JT, Schaniel C, et al. (2002). A stem cell molecular signature. *Science* 298: 601–604.

68. Ramalho-Santos M, Yoon s, Matsuzaki Y, et al. (2002). 'Stemness': transcriptional profiling of embryonic and adult stem cells. *Science* 298: 597–600.

69. Hackney JA, Charbord P, Brunk BP, et al. (2002). A molecular profile of a hematopoietic stem cell niche. *Proc Natl Acad Sci USA* 99: 13061–13066.

70. Pazianos G, Uqoezwa M, Reya T (2003). The elements of stem cell self-renewal: a genetic perspective. *Biotechniques* 35: 1240–1247.

71. Lauzurica P, Sancho D, Torres M, et al. (2000). Phenotypic and functional characteristics of hematopoietic cell lineages in CD69-deficient mice. *Blood* 95: 2312–2320.

72. Thiemann FT, Moore KA, Smogorzewska EM, et al. (1998). The murine stromal cell line AFT024 acts specifically on human CD34+ CD38– progenitors to maintain primitive function and immunophenotype in vitro. *Exp Hematol* 26: 612–619.

73. Prosper F, Verfaillie CM (2001). Regulation of hematopoiesis through adhesion receptors. *J Leukocyte Biol* 69: 307–316.

74. Punzel M, Gupta P, Verfaillie CM (2002). The microenvironment of AFT024 cells maintains primitive human hematopoiesis by counteracting contact mediated inhibition of proliferation. *Cell Commun Adhes* 9: 149–159.

75. Fruehauf S, Srbic K, Seggewiss R, et al. (2002). Functional characterization of podia formation in normal and malignant hematopoeitic cells. *J Leukocyte Biol* 71: 425–432.

76. Wagner W, Saffrich R, Wirkner U, Eckstein V, Blake J, Ansorge A, Schwager C, Wein F, Miesala K, Ansorge W, Ho AD (2005). Hematopoietic progenitor cells and cellular microenvironment: Behavioral and molecular changes upon interaction. *Stem Cells* 23: 1180–1191.

77. Francis K, Ramakrishna R, Holloway W, et al. (1998). Two new pseudopod morphologies displayed by the human hematopoietic KG1a progenitor cell line and by primary human CD34+ cells. *Blood* 92: 3616–3623.

78. Zhang J, Niu C, Ye L, et al. (2003). Identification of the haematopoietic stem cell niche and control of the niche size. *Nature* 425: 836–841.

79. Calvi LM, Adams GB, Weibrecht KW, et al. (2003). Osteoblastic cell regulate the haematopoietic stem cell niche. *Nature* 425: 841–846.

80. Reik W, Dean W, Walter J (2001). Epigenetic reprogramming in mammalian development. *Science* 293: 1089–1093.

81. Espada J, Ballestar E, Fraga MF, et al. (2004). Human DNA methyltransferase 1 is required for maintenance of the histone H3 modification pattern. *J Biol Chem* 279: 37175–37184.

82. Bakin AV, Curran T (1999). Role of DNA 5-methylcytosine transferase in cell transformation by fos. *Science* 283: 387–390.

83. Caplan AI (2000). Mesenchymal stem cells and gene therapy. *Clin Orthop Rel Res* 379 (Suppl.): S67–S70.

84. Bianco P, Gehron Robey P (2000). Marrow stromal stem cells. *J Clin Invest* 105: 1663–1668.

85. Pereira RF, Halford KW, O'Hara MD, Leeper DB, Sokolov BP, Pollard MD, Bagasra O, Prockop DJ (1995). Cultured adherent cells from marrow can serve as long-lasting precursor cells for bone, cartilage, and lung in irradiated mice. *Proc Natl Acad Sci USA* 92: 4857–4861.

86. Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S, Marshak DR (1999). Multilineage potential of adult human mesenchymal stem cells. *Science* 284: 143–147.

87. Conget PA, Minguell JJ (1999). Phenotypical and functional properties of human bone marrow mesenchymal progenitor cells. *J Cell Physiol* 181: 67–73.

88. Dennis JE, Merriam A, Awadallah A, Yoo JU, Johnstone B, Caplan AI (1999). A quadripotential mesenchymal progenitor cell isolated from the marrow of an adult mouse. *J Bone Miner Res* 14: 700–709.

89. Fukuda K (2000). [Generation of cardiomyocytes from mesenchymal stem cells]. *Tanpakushitsu Kakusan Koso* 45: 2078–2084.

90. Lee KD, Kuo TK, Whang-Peng J, Chung YF, Lin CT, Chou SH, Chen JR, Chen YP, Lee OK (2004). In vitro hepatic differentiation of human mesenchymal stem cells. *Hepatology* 40: 1275–1284.

91. Remy-Martin JP, Marandin A, Challier B, Bernard G, Deschaseaux M, Herve P, Wei Y, Tsuji T, Auerbach R, Dennis JE, et al. (1999). Vascular smooth muscle differentiation of murine stroma: a sequential model. *Exp Hematol* 27: 1782–1795.

92. Le Blanc K, Rasmusson I, Sundberg B, Gotherstrom C, Hassan M, Uzunel M, Ringden O (2004). Treatment of severe acute graft-versus-host disease with third party haploidentical mesenchymal stem cells. *Lancet* 363: 1439–1441.

93. Reyes M, Lund T, Lenvik T, Aguiar D, Koodie L, Verfaillie CM (2001). Purification and ex vivo expansion of postnatal human marrow mesodermal progenitor cells. *Blood* 98: 2615–2625.

94. Jiang Y, Jahagirdar BN, Reinhardt RL, Schwartz RE, Keene CD, Ortiz-Gonzalez XR, Reyes M, Lenvik T, Lund T, Blackstad M, et al. (2002). Pluripotency of me-

senchymal stem cells derived from adult marrow. *Nature* 418: 41–49.

95. Kogler G, Sensken S, Airey JA, Trapp T, Muschen M, Feldhahn N, Liedtke S, Sorg RV, Fischer J, Rosenbaum C, et al. (2004). A new human somatic stem cell from placental cord blood with intrinsic pluripotent differentiation potential. *J Exp Med* 200: 123–135.

96. Stamm C, Westphal B, Kleine HD, Petzsch M, Kittner C, Klinge H, Schumichen C, Nienaber CA, Freund M, Steinhoff G (2003). Autologous bone-marrow stem-cell transplantation for myocardial regeneration. *Lancet* 361: 45–46.

97. Tiberghien P, Ferrand C, Lioure B, Milpied N, Angonin R, Deconinck E, Certoux JM, Robinet E, Saas P, Petracca B, et al. (2001). Administration of herpes simplex-thymidine kinase-expressing donor T cells with a T-cell-depleted allogeneic marrow graft. *Blood* 97: 63–72.

98. Hacein-Bey-Abina S, von Kalle C, Schmidt M, Le Deist F, Wulffraat N, McIntyre E, Radford I, Villeval JL, Fraser CC, Cavazzana-Calvo M, Fischer A (2003). A serious adverse event after successful gene therapy for X-linked severe combined immunodeficiency. *N Engl J Med* 348: 255–256.

A. Wagner W, Feldman RE, Jr., Seckinger A, Maurer MH, Wein F, Blake J, Krause U, Kalenka A, Burgers HF, Saffrich R, Wuchter P, Kuschinsky W, and Ho AD (2006). The heterogeneity of human mesenchymal stem cell preparations – Evidence from simultaneous analysis of proteomes and transcriptomes. *Exp Hematol* 34:536–548.

B. Wagner W, Wein F, Seckinger A, Frankhauser M, Wirkner U, Krause U, Blake J, Schwager C, Eckstein V, Ansorge W, and Ho AD (2005). Comparative characteristics of mesenchymal stem cells from human bone marrow, adipose tissue, and umbilical cord blood. *Exp Hematol* 33:1402–1416.