Published in final edited form as:

J Mol Med. 2008 December ; 86(12): 1301–1314. doi:10.1007/s00109-008-0383-6.

Adult stem cells and their trans-differentiation potential—perspectives and therapeutic applications

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Abstract

Stem cells are self-renewing multipotent progenitors with the broadest developmental potential in a given tissue at a given time. Normal stem cells in the adult organism are responsible for renewal and repair of aged or damaged tissue. Adult stem cells are present in virtually all tissues and during most stages of development. In this review, we introduce the reader to the basic information about the field. We describe selected stem cell isolation techniques and stem cell markers for various stem cell populations. These include makers for endothelial progenitor cells (CD146/MCAM/MUC18/S-endo-1, CD34, CD133/prominin, Tie-2, Flk1/KD/VEGFR2), hematopoietic stem cells (CD34,

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CD117/c-Kit, Sca1), mesenchymal stem cells (CD146/MCAM/MUC18/S-endo-1, STRO-1, Thy-1), neural stem cells (CD133/prominin, nestin, NCAM), mammary stem cells (CD24, CD29, Sca1), and intestinal stem cells (NCAM, CD34, Thy-1, CD117/c-Kit, Flt-3). Separate section provides a concise summary of recent clinical trials involving stem cells directed towards improvement of a damaged myocardium. In the last part of the review, we reflect on the field and on future developments.

Keywords

Autoimmune disease; G-CSF; Graft vs. host reaction; Stem/progenitor cell; Trans-differentiation

Introduction—adult stem cells and their surface markers

Adult stem cells are clonogenic, self-renewing, and pluripotent cells with a plasticity to differentiate into cell types of the particular tissue in which they reside and often to trans-differentiate into different types of tissues [1]. The tremendous proliferative potential of these cells may lead to the development of cancer if the control of their differentiation, and/or proliferation, and/or apoptotic program is impaired [2]. Stem cell activity has been demonstrated in many tissues/organs, but the exact location of these adult stem cells is not always clear because of a current lack of well-defined organ-specific stem cell markers. Adult stem cells are usually located in a specific cellular niche, and niche microenvironment determine the status of stem cell activation, thus ensuring a balance between maintenance of the stem cell pool and production of progenitor cells engaged in tissue differentiation [3]. The identification and selection of stem cells within a given tissue/organ largely relies on the presence of specific cell surface markers (Table 1). Other identification methods include the ability of certain stem cells to exclude fluorescent dyes (rhodamine 123, Hoechst 33342) and DNA label retention as well as their ability to form colonies and differentiate into certain lineages as seen in mesenchymal/stromal stem cells. Dye exclusion techniques permit selection of a “side population,” and this is facilitated by the ABCG2 gene product, also named breast-cancer-resistant protein (BCRP1) [4]. This member of the family of ABC transporters has been shown to be a positive selection marker of pluripotent cells from various adult tissue sources. However, ABCG2 is neither unique to nor ubiquitously expressed in all stem/progenitor cells [5]. Another way to identify slowly cycling adult stem cells is DNA label retention. Quiescent stem cells retain the DNA label for much longer than dividing cells where the DNA label is diluted with each cell division [6]. In the following paragraphs, we will characterize at greater detail the various stem cells populations, their therapeutic potential, and existing isolation techniques.

Growth and isolation of stem cells and tests to confirm stem cell presence

The trans-differentiation potential of adult stem cells and their capacity for tissue renewal and damage repair has attracted much attention from biotechnologists and clinicians [7], and the isolation and in vitro maintenance of stem cells have immense importance in applied biology. Although flow cytometric separations of stem cells or positive and negative selections using magnetic beads tagged with antibodies targeting specific markers on the surface of stem cells are used routinely now in many applications, alternative approaches for stem cell identification have been proposed based on the specific behavior of individual stem cells. One of these approaches exploits stem cell homing characteristics [8]. These and other principles were proven to be effective in isolating stem cells for research and biological applications. Taking hematopoietic stem cells (HSCs) as an example, we will provide an overview of some of these important isolation techniques, which could also be applied to various other stem cells.

Isolation of stem cells by flow cytometry

In flow cytometry, a mixture of cells tagged by appropriate fluorochrome-labeled stem cell markers is passed through a laser beam. The cells scatter the fluorescence which provides information on the cell morphology, composition of surface proteins, DNA content, and cytoplasmic processes. The advantage of flow cytometry is the speed of the flow that allows quick processing of large population of cells. Cytometric analysis goes one step further and allows cell sorting [9], a process that breaks the fluid stream containing the cells into droplets by piezoelectric perturbation. It is then possible to deflect a selected droplet with precise timing on a charge given to the stream as it passes through an electric field. Droplets containing the (stem) cells of interest are deflected in the electric field and collected [10].

Immunomagnetic-beads-based isolation method

Immunomagnetic beads coated with specific antibodies are used either for isolation or depletion of various types of cells. Positive or negative cell isolation can be performed depending on the nature of the cell surface markers and its specific application. Positive cell isolation amends itself to any downstream application after removal of the beads. Negative cell isolation is the method of choice to ensure that cells of interest remain unaffected. Cells with multiple cell surface markers can be isolated by the combination of negative and positive cell isolation [11]. Such technologies based on immunomagnetic beads are currently in use in two major clinical applications: (1) CD34-positive stem cell isolation and (2) in vitro T cell isolation and expansion for clinical trials in novel adoptive immunotherapy [12].

Other techniques

Various other stem cell isolation techniques have been proposed and tested in recent years. Stem cell isolation by acoustic standing waves (acoustophoresis) in microfluidic channels is one of such methods where controlled acoustic waves were used within a fluid flow column with antinodes and nodes maintained in different flow layers of the channel, allowing fluid components to differentially migrate to areas of preferred acoustic interaction [13]. Acoustophoresis allows particles and cells to be driven towards the node or anti-node of a standing wave that depends on the characteristics of density and compressibility [14].

Isolation of HSCs

HSCs are highly enriched in the population characterized by low or undetectable levels of the lineage markers found on mature hematopoietic cells (B220, CD3, CD4, CD5, CD8, Mac-1, GR-1, Ter119, and NK1.1) and by high levels of c-Kit and Sca-1 [15]. Furthermore, differential expression of SLAM receptors (CD150, CD244, and CD48) on HSC and more restricted multipotent hematopoietic progenitors allows further, more distinct selection. Highly purified HSCs are $CD150^{+}$, $CD244^{-}$, and $CD48^{-}$, while multipotent hematopoietic progenitors are $CD244^{+}$, $CD150^{-}$, and $CD48^{-}$. In the course of further differentiation/commitment, they become $CD48^{+}$, $CD244^{+}$, and $CD150^{-}$ [16,17]. Thus, sorting bone-marrow-derived cells according to the above criteria is a relatively simple and practical way of stem cell enrichment. Another approach to isolating stem-cell-enriched populations is based on the ‘dye-efflux’ properties of proliferating HSCs. Due to high expression of ABCG2 transporter, HSCs rapidly efflux the DNA dye Hoechst 33342. Cells maintaining an efficient efflux of the dye can be identified by flow cytometry, and cells that efflux the dye are referred to as ‘side population’ or SP cells when visualized on dot plot [4].

Growth and validation for stem cell presence

Like other mammalian cells, stem cells are also grown and maintained at 37°C in humidified cell culture incubators under a 5% CO_2 atmosphere. The media requirements vary among individual stem cell types, and it is essential not to induce differentiation of the cultured stem

cells. HSCs are obtained from bone marrow aspirates, placental, or umbilical cord blood, and their high growth rate makes them prone to differentiation in culture. Bone marrow origin stem cells are grown in culture media supplemented with serum [18], while HSCs usually require a co-culture system containing fibroblast feeder layers that support growth and differentiation. The change of cytokine conditions in such culture systems affects the differentiation of stem cells [19]. The effects of specific culture conditions and speed of differentiation varies among different stem cell populations. Bone marrow stromal cells attach to culture dishes and continue to grow slowly for weeks before differentiation [20]. Neural stem cells from fetal or adult brain tissue can grow suspended in culture medium without any additional serum supplements [21].

Validation of the presence of stem cells in a culture system with histochemical methods, antigenic markers, and morphogenetic studies are primarily dependent on the surface morphology and immunophenotype of the stem cells of interest. For in vitro studies, cytogenetic analysis or RT-PCR-based methods are commonly applied.

Hematopoietic stem cells

HSCs are among the best characterized adult stem cells and the only stem cells being routinely used in the clinics. HSCs are able to renew themselves or differentiate into precursors which produce specialized hematopoietic cells, including lymphocytes, dendritic and natural killer cells, megakaryocytes, erythrocytes, granulocytes, and macrophages [22]. Cells in the hematopoietic hierarchy have different multi-potentiality, differentiation and self-renewal capacities, and are able to cope with the high demand for continuously producing large numbers of blood cells. The first progeny of HSCs are multipotent progenitors which retain the ability to differentiate into all hematopoietic lineages but show a lower capacity to proliferate [23]. Multipotent progenitors are more abundant than HSCs and differentiate into oligopotent progenitors, which in turn give rise to more lineage-committed precursor cells.

During sequential differentiation, stem cells present various antigenic characteristics which are associated with their properties and function. These antigens allow for the definition of stem cell subpopulations and permit clinicians to improve the outcome of HSCs transplantation by increasing the purity of HSCs product used in allo-engraftments. Human HSCs express CD34 surface antigen ($CD34^+$), which is commonly used as a marker in clinical settings to identify and quantify the population of progenitor cells to be infused [24,25]. Scientists have been trying to narrow the subset of progenitor cells by defining a set of markers that are more consistently expressed on these cells. Human HSCs are known to exhibit $CD34^+$, $Thy1^+$, $CD38^{lo/-}$, C-$kit^{-/lo}$, $CD105^+$, Lin^- phenotype [25,26]. However, there is not a general agreement on the association between any combination of these antigenic properties and function of stem cells.

Sources of HSCs

HSCs can be isolated from bone marrow (BM). BM also accommodates stromal cells, mesenchymal stem cells, and variably mature blood cells and their progenitors. HSCs constitute only a small fraction of BM population (1 in 10^4 to 1 in 10^8 of BM nucleated cells) [27]. HSCs can also be isolated from peripheral blood (PB) where they can be found in small numbers [27,28]. Stimulation with mobilizing agents, including cytokines such as G-CSF alone or in a combination with GM-CSF and/or other agents, dramatically increases the release of HSCs from BM to PB [28]. This allows collecting a high number of progenitor cell types, which constitutes an important improvement in engraftment success and effective repopulation with neutrophils and platelets [24]. Another important source of HSCs for clinical purposes is umbilical cord (UC)/placenta blood. Its therapeutic use has become popular after successful applications of UC-derived HSCs in children with Fanconi anemia [29]. A practical constraint

with HSCs collected from UC blood is their limited quantity, which is predominantly due to the low blood volume obtained from these tissues. The low UC stem cell dosage of approximately 10% of the amount of marrow transplants is adequate for transplants in children and low-weight patients but delivers too few stem cells for use in most adults with a recommended dose of more than 2.5×10^6 $CD34^+$ cells per kilogram body weight [24]. In addition to their quantity, HSCs collected from UC and PB differ in some other characteristics (Table 2). In general, HSCs obtained from tissues at earlier developmental stages have greater capacity for self-replication and long-term growth in vitro [30] and show different homing and surface properties [31]. Nonetheless, it is currently unclear whether these variations have any clinical significance. In animal models, HSCs can also be obtained from the fetal hematopoietic system including aorta–gonad–mesonephros (AGM), liver and spleen, which provide useful sources of stem cells for experimental purposes.

Heart and muscle stem cells and potential for regeneration of the heart muscle

Satellite cells, the muscle stem cells, were first described by Mauro [32] in 1961 as committed precursor cells residing in skeletal muscle. Upon an injury, the basic role of these cells is to restore skeletal muscle function. An inflicted stress may stimulate these cells to transform into myoblasts by the activation of myogenic regulatory (transcription) factors (MRF) [33]. Quiescent (satellite) cells appear to be negative for MRF, but during activation, their expression is upregulated (MyoD, Myf5, myogenin, MRF4) [33]. Moreover, some transcripts transform to alternatively spliced isoforms when satellite cells start to proliferate ($CD34^+$ from truncated to a full-length form, MNFβ to MNFα) [34]. MRF are first expressed during early embryogenesis when myoblasts are formed (prior to cardiac-specific cells), and in case of muscle injury, they appear about 6 h after injury [33]. Following proliferation, differentiation and multi-fusion, myoblast cells form new myotubes that become finally differentiated muscle fibers [35]. This potential of myoblasts and satellite cells for regeneration of heart muscle was first explored in a preclinical trial using a dog heart model of cryoinjury [36]. Myoblasts or satellite cells may give rise to both self-renewing muscle-type-specific populations of myogenic stem cells and to progenitor muscle cells.

In rodents, a stem cell population was initially identified as muscle SP that could reconstitute hematopoiesis [37]. The analysis of the transcriptional profile of this SP population obtained from skeletal muscle revealed that these cells shared transcripts with embryonic stem cells (ESC) and overlapped with bone marrow SP in almost half of all transcripts [38]. Recent studies have shown that the majority of obtained SP cells were positive for Sca1 (stem cell antigen 1), CD31, and CD45 [39]. FACS sorting of these rodent cells resulted in the identification of three subpopulations with different myogenic and hematopoietic potential and distinct proliferative and regenerative capacities.

Since the heart has a low regenerative capacity, efforts have been undertaken to restore its function after, e.g., ischemic or non-ischemic damage. Its own modest regenerative potential (possibly only by ~1% renewal per year) can be either provided by circulating multipotential stem cells [40] or cardiac progenitor stem cells residing in specific heart niches [41]. Putative human cardiac stem and progenitor cells (hCSCs) are now distinguished into four main resident cardiac cell types characterized by c-Kit, Sca1 (rodents), MDR-1, and Isl-1 markers [42]. Divided into primary and secondary heart fields (according to the heart development), these cells may reconstitute main structural elements of the myocardium such as cardiomyocytes, endothelial and conductive cells [43], and/or post-natal cardiac progenitors. First and second heart field progenitors express common mesodermal markers: Oct4, Mesp, Bry, Nkx2.5, while Isl-1 belongs exclusively to the second field characteristics giving rise to all previously mentioned cells, while the first heart field progenitors may differentiate only into cardiac

conductive and cardiac muscle cells. Attempts to multiply in vitro hCSCs for possible heart regeneration are ongoing [44]. The most ambitious aim would be to practically utilize totipotent stem cells, and preclinical studies in rodents are well advanced with application of in vitro differentiated cardiomyocytes and/or genetically pre-programmed pluripotent cells [45]. Yet it seems that at the present stage, the numbers of cells obtained in this way are low and inadequate for clinical trials [46].

Despite advances in pharmacological treatment, organ transplantation and cardiac devices (LVAD), data from the Framingham Heart Study indicate that the survival of patients who were diagnosed with heart failure is still poor [47]. Stem cells, including myoblasts, seem to ideally suit for autologous transplantation, and more than ten clinical trials involving myoblast transplantation have so far been performed (Table 3). Several features favor myoblasts as the first target for treatment of an ischemic myocardium: (1) Autologous origin excludes adverse immune responses; (2) relatively low plasticity due to their progenitor commitment reduces the risk of teratoma formation as observed with totipotent cells; (3) myoblasts have a relatively high proliferation potential, although there could still be better candidates among muscle-derived-stem cells; and (4) myoblasts are relatively resistant to ischemic conditions. Table 3 summarizes current clinical trials involving myoblast transplantation. The majority of the trials are at stage I/II; no proper control groups were assigned (except for the MAGIC trial). A vast majority of the trials were conducted at the opportunity of other surgical interventions (CABG or LVAD implantations), which makes it difficult to evaluate the beneficial effect of myoblasts alone and which resulted in the premature termination of the expensive MAGIC trial, with statistical power in over a hundred patients being compromised by multifactorial assessment of CABG operations. Nonetheless, at least three major issues overcome a general pessimistic view over the current stage of stem cells interventions in the failing heart: (a) heart hemodynamic parameters improved and correlated with higher numbers of myoblasts implanted; (b) autologous myoblasts appeared to be safe and feasible candidate cells, although the arrhythmia episodes associated in a majority of the trials required the concurrent cardioverter/defibrillator implantation [48].

Cells with myogenic (contractile) potential can be considered as the better alternative to bone-marrow-derived stem cells, which can only be harvested in low quantities, do not possess contractile properties, and only have transitory benefits [49]. Although myoblasts do not remain in the myocardium infinitely, their beneficial effect on the ejection fraction (LVEF), which begins 3–6 months after implantation and may last until almost 2 years (Kurpisz, unpublished), has been observed in most of the trials. This holds a promise for repetitive dosing of myoblasts using a low invasive percutaneous approach. Such data are already available from preclinical trials in rats and pigs [50]. The cell homing inside the myocardium seems to be sufficient when using either a percutaneous or an intramyocardial approach [51]. A long-term survival of myoblasts within the myocardium has been recently demonstrated with pro-angiogenic gene modifications, for example, with VEGF-transduced myoblasts in rats [52]. Their functionality may also be improved by connexin 43 overexpression [53]. Finally, different types of stem cells can be combined at progenitor-committed stages, thus greatly enhancing the therapeutic outcome [54] and ultimately leading to the rejuvenation of the whole organ.

Adult stem cell plasticity and the implications for regenerative medicine

Cell-based therapy may in the nearer future represent a new strategy to treat a wide array of clinical conditions. The use of adult stem cells as opposed to human embryonic stem cells for therapy avoids ethical problems and has two additional advantages: (a) Adult stem cells can be isolated from patients, and this overcomes the problem of immunological rejection and (b) the risk of tumor formation is greatly reduced as compared to the use of embryonic stem cells [55].

While pluripotency and plasticity are considered properties of early ESC, adult stem cells are traditionally thought to be restricted in their differentiation potential to the progeny of the tissue in which they reside. When parts of an organ or tissue are transplanted to a new site, the transplanted tissue maintains its original character. Similarly, when dissociated cells from an organ or tissue are cultured, they also tend to maintain their original phenotype. Despite losing some of their differentiation properties, they do not acquire differentiated characteristics of a different cell lineage. However, a remarkable plasticity in differentiation potential of stem cells derived from adult tissues was recently suggested [56] (Fig. 1). In 1998, Ferrari et al. [57] first reported that mouse bone-marrow-derived cells give rise to skeletal muscle cells when transplanted into damaged mouse muscle. Thereafter, transplanted bone marrow cells were reported to generate a wide spectrum of different cell types, including hepatocytes [58], endothelial, myocardial [59,60], neuronal, and glial cells [61]. Moreover, HSC can produce cardiac myocytes and endothelial cells [62], functional hepatocytes [63], and epithelial cells of the liver, gut, lung, and skin [64]. Mesenchymal stromal cells (MSC) of the bone marrow can generate brain astrocytes [65]. Enriched stem cells from adult mouse skeletal muscle were shown to produce blood cells [66,67]. In most of these plasticity studies, genetically marked cells from one organ of an adult mouse apparently gave rise to cell type characteristics of other organs following transplantation, suggesting that even cell types once thought to be terminally differentiated are far more plastic in their developmental potential than previously thought. A critical aspect of the observation of adult stem cell plasticity is that in order for plasticity to occur, cell injury is necessary [68]. This suggests that microenvironmental exposure to the products of injured cells may play a key role in determining the differentiated expression of marrow stem cells [69].

The events underlying stem cells plasticity could relate to a variety of mechanisms such as dedifferentiation, trans-differentiation, epigenetic changes, and/or cell fusion. Rerouting of cell fate may result from the multistep process known as dedifferentiation where cells revert to an earlier, more primitive phenotype characterized by alterations in gene expression pattern which confer an extended differentiation potential (Fig. 2). In urodele amphibians, cell dedifferentiation is a common mechanism resulting in the functional regeneration of complex body structures throughout life, including limbs, tail, and even spinal cord [70]. Recent studies on the plasticity of murine myotubes [71] and other cells derived from adult tissues suggest that dedifferentiation may also be possible in mammals [72]. At the molecular level, MSX1 has been identified as a possible factor involved in dedifferentiation processes in both urodele and human cells [71,73]. The small molecule, reversine, can induce murine myogenic lineage-committed cells to become multipotent mesenchymal progenitor cells that can proliferate and re-differentiate into bone and fat cells [74]. Epigenetic cell changes are probably involved and may be mediated by signals received from the injured cells. The identification of signals that induce dedifferentiation of somatic cells are key to elucidating the molecular mechanism of this phenomenon and may ultimately provide effective tools for the in vivo regeneration of mammalian tissues. Recent studies favor a model of continuous stem cell differentiation at the level of progenitor cells with dynamic transcriptional regulation of stem cell cycle phases and chromatin alterations associated with cell cycle transit [69]. In this model, stem cells represent a highly flexible ever-changing cell system in which the potential and characteristics of the stem cell are continually and reversibly changing with the cell cycle until a terminal differentiating stimulus is encountered at a cycle-susceptible time [69]. In this asynchronous stem cell population, there would always be a small percentage of cells receptive or primed for a specific differentiation (or de-differentiation) induction at any particular time.

Another mechanism put forward to explain stem cell switch to a novel phenotype is a process known as trans-differentiation (Fig. 2). Cells may differentiate from one cell type into another within the same tissue or develop into a completely different tissue without acquiring an intermediate recognizable, undifferentiated progenitor state. Recent studies show clearly that

bone-marrow-derived cells can colonize a wide variety of tissues in the body of a host [75, 76]. Although derived from the embryonic mesoderm, the developmental potential of bone marrow cells is not restricted to this germ layer, but these cells have also been shown to populate tissues of ectodermal and endodermal origin [77]. Both mesenchymal stem cells and bone-marrow-derived cells can give rise to a wide array of non-hematopoietic cell types such as astrocytes and neurons in the brain [61,78], cardiac myocytes in models of infarction [60], skeletal muscle [57], and hepatocytes [79]. However, the reported frequencies of colonization are low, and it is unlikely that there is much repair of organ damage by bone marrow in the normal individual.

Despite examples of trans-differentiation events of adult stem cells being reported, these findings are still controversial. Most of the reports could not be confirmed in subsequent investigations [80], and to date, trans-differentiation has never been conclusively demonstrated in any experimental setting. In every case, differentiation from a rare population of stem cells has never been excluded, or “trans-differentiation” events turned out to be misinterpretations caused by cell fusion resulting in nuclear reprogramming and changes in cell fate [81,82] (Fig. 2). It is now recognized that adult stem cells from bone marrow may fuse with cell of the target organ. So far, bone-marrow-derived cells were shown to form fusion heterokaryons with liver, skeletal muscle, cardiac muscle, and neurons [83]. There is evidence that such fused cells become mono-nucleated again, either by nuclear fusion or by elimination of supernumerary nuclei [82,84]. Fusion and nuclear transfer experiments demonstrated that genes previously silenced during development could be reactivated by cytoplasmic factors modulating the epigenetic mechanisms responsible for the maintenance of a specific state of cell differentiation. However, nuclear transfer experiments demonstrate that the capacity of cells to successfully reprogram diminishes with increasing developmental progression of the donor nuclei. Despite this limitation and the low frequency, cell fusion may be considered as a potential avenue for tissue repair.

In addition to the aforementioned phenomena of cell fate switching, the presence of a rare population of pluripotent primitive stem cells may also explain the acquisition of an unexpected phenotype. Recently, non-hematopoietic cell populations from bone marrow and umbilical cord blood were enriched by in vitro culture and demonstrate to have the potential to differentiate into derivatives of all three germline layers with meso-, endo-, and ectodermal characteristics [85,86]. Known as multipotent adult progenitor cells (MAPC), these cells contribute to most, if not all, somatic cell lineages, including brain, when injected into a mouse blastocyst [87]. Interestingly, while MAPC express Oct4, a transcription factor required for undifferentiated embryonic stem cells maintenance [88] at levels approaching those of ESC, MAPC do not express two other transcription factors known to play a major role in ESC pluripotency, Nanog and Sox2 [89]. This particular expression profile may contribute to the fact that the use of ESC, but not MAPC, carries the risk of generating tumors. Thus, MAPC are a promising source of autologous stem cells in regenerative medicine. Their low tumorigenicity, high regenerative plasticity, and optimal immunological compatibility are essential assets for the successful transplantation of MAPC-derived tissue-committed cells without immune-mediated rejection [90].

Bone marrow transplantation as a clinical example of regenerative medicine

HSCs were primarily used in the treatment of patients with hematological malignancies. During the course of treatment, patient’s cancerous cells are first destroyed by chemo/radiotherapy and subsequently replaced with BM or PB/G-CSF transplant from a human leukocyte antigen (HLA)-matched donor [91]. Allogeneic marrow transplants have also been used in the treatment of hereditary blood disorders including aplastic anemia, β-thalassemia, Wiskott–

Aldrich syndrome and SCID, as well as inborn errors of metabolism disorders such as Hunter's syndrome and Hurler's syndrome [92–96].

During the therapy of hematological malignancies, autologous PB/G-CSF HSCs are collected prior to the treatment and reinfused into the patients after the course of the aggressive chemotherapy. With a similar approach, autologous HSCs may be used to reprogram immune system and reconstitute non-autoreactive immune cells as a treatment for autoimmune disorders. The problem using auto-engrafts to rescue HSC population in cancer treatment is that patient's cancerous cells may be inadvertently collected and reinfused back into the patients along with the stem cells.

HSC transplants are also used as a therapeutic strategy against various types of solid tumors [97]. Graft-versus-tumor effect of allogeneic HSC transplants seems to be a result of an immune reaction between donor cytotoxic T cells and patient's malignant cells [98].

HSCs have the ability to generate cell types other than blood cells. Circulating HSCs are able to reside in distant tissues and participate in regeneration process by trans-differentiating into non-hematopoietic cells such as hepatocytes [15], skeletal muscle and cardiac myocytes [59, 60], neurons [99], and epithelial cells [64]. The existence of pluripotent stem cells, which are reprogrammed in the new microenvironment and differentiate into the cell types of the tissue to which they were recruited, has significant implications for regenerative medicine. However, the potential plasticity of HSCs are disputed by several reports of failure to show trans-differentiation [56] as well as other perplexing possibilities such as technical problems and the possible existence of several types of non-hematopoietic stem cells in BM [100].

Over the past two decades, HSCs have also been targeted for ex vivo gene therapy as a vehicle to transfer a modified gene in autologous settings [101]. This approach provides a promising alternative treatment for various inherent and acquired human diseases and may provide an alternative to currently still risky allogeneic HSC transplants.

Closing remarks

Since the beginning of the basic stem cell research in 1960s, scientists have been facing serious challenges with identifying true stem cells and proliferating and maintaining them in culture. For example, the scarcity of HSCs along with their morphological resemblance to other PB or marrow cell types in culture makes isolation and purification of stem cells difficult. Surface biomarkers currently used to define HSCs do not appear to be exclusively expressed on stem cells. This leads to the isolation of a heterogeneous population of cells that were mistakenly assumed as true stem cells. Self-renewal capacity of stem cells is an important property of these cells. Stem cells that maintain this property and do not differentiate into their progeny can provide an unlimited source of cells for both therapeutic and research applications.

Self-renewal divisions of stem cells are rare events in BM; HSCs replicate themselves slowly with an average turnover time of 30 days in adult BM [102]. This self-replication is also hard to induce in vitro, which altogether hinders the study of self-renewal and differentiation of stem cells, influencing factors and signaling involved in these processes and, subsequently, further targeted manipulation of these pathways. In addition to these technical difficulties, various functional assays being used by researchers make the comparison and meta-analysis of data obtained from different studies inapplicable. Furthermore, many of the studies on stem cells including in vivo experiments have been conducted in animal models, and the true relevance and reliability of the results in the human remains to be determined.

One of the major challenges with HSC transplants is failure to engraft, which is mediated by donor T cells as a result of graft-versus-host disease. The lack of assays to selectively deplete

the anti-host alloreactive T cells ex vivo and to separate graft versus host disease from beneficial effect of 'graft versus tumor' results in increased morbidity and mortality associated with HSC transplants. In addition, variability of HSC migration by mobilizing agents in different patients and the serious side effects and toxicity caused by some of them in donors require further development of methods to overcome cell dose barrier in HSC transplants [103]. Moreover, the absence of unrelated HLA-matched donors for many patients in need of HSC transplants and the high incidence of relapse of underlying diseases in transplant recipients are important challenges remained to be addressed in future attempts to improve the clinical outcome of HSCs-based therapies.

HSC/BM transplantation has been the lifesaver and a critical element of anticancer therapies, particularly in leukemias. Nowadays, however, other less toxic therapeutic options emerged. Tremendous progress has been made in the area of therapeutic antibodies [104,105]. A more integrated approach is being taken to detect new targets for the treatment of cancer and other diseases [106,107]. New proteins and peptides have recently been discovered that have cancer (semi-)selective properties [108–110]. Entirely new concepts that connect cell death, cell survival, and cell proliferation have been developed that warrant new targets [111]. Targeted therapies, with the epidermal growth factor receptor pathway as the best example gain importance [112], and finally, "traditional" chemotherapy approaches are being further developed so that new generations of chemotherapeutic drugs would become available [113].

Nevertheless, the therapeutic potential of adult stem cells as powerful tools in tissue regeneration and engineering has been recognized, and intense efforts are ongoing to harness and direct adult stem cell plasticity. Understanding the basic molecular mechanisms underlying cell fate switching of adult stem cells will be an essential contribution to ensuring their safe use in regenerative medicine. In the near future, it will most likely be possible to transplant genetically modified stem cells that carry a set of genes critical for, e.g., trans-differentiation, that are under externally regulated promoters [114] and, depending on the therapeutic requirements, direct their differentiation into desired cell populations. Even more "clinically friendly" systems may be developed where pharmacologic "small molecules" will be used to directly influence the trans- or re-differentiation potential of therapeutically applied adult stem cells both prior and after their administration into patient's body.

Acknowledgments

The authors (SHK, ML) would like to thank the Manitoba Health Research Council (MHRC) for their generous support. We apologize to those authors whose research was not cited in this review due to the reference number limitations.

Abbreviations

GM	aorta–gonad–mesonephros
BCRP1	breast cancer resistance protein1
BM	bone marrow
CABG	coronary artery bypass graft
CNS	central nervous system
CSC	cardiac stem cell
ESC	embryonic stem cells
Flk1	fetal liver kinase-1
G-CSF	granulocyte-colony stimulating factor

GM-CSF	granulocyte-macrophage-colony stimulating factor
HLA	human leukocyte antigen
HSC	hematopoietic stem cell
LVAD	left ventricular assist device
LVEF	left ventricular ejection fraction
MAPC	multipotent adult progenitor cells
MRF	myogenic regulatory factor
MSC	mesenchymal stromal cell
NCAM	neural cell adhesion molecule
NSC	neuronal stem cells
NYHA	New York Heart Association
PB	peripheral blood
Sca1	stem cell antigen 1
SP	side population
UC	umbilical cord
VEGFR2	vascular endothelial growth factor receptor 2

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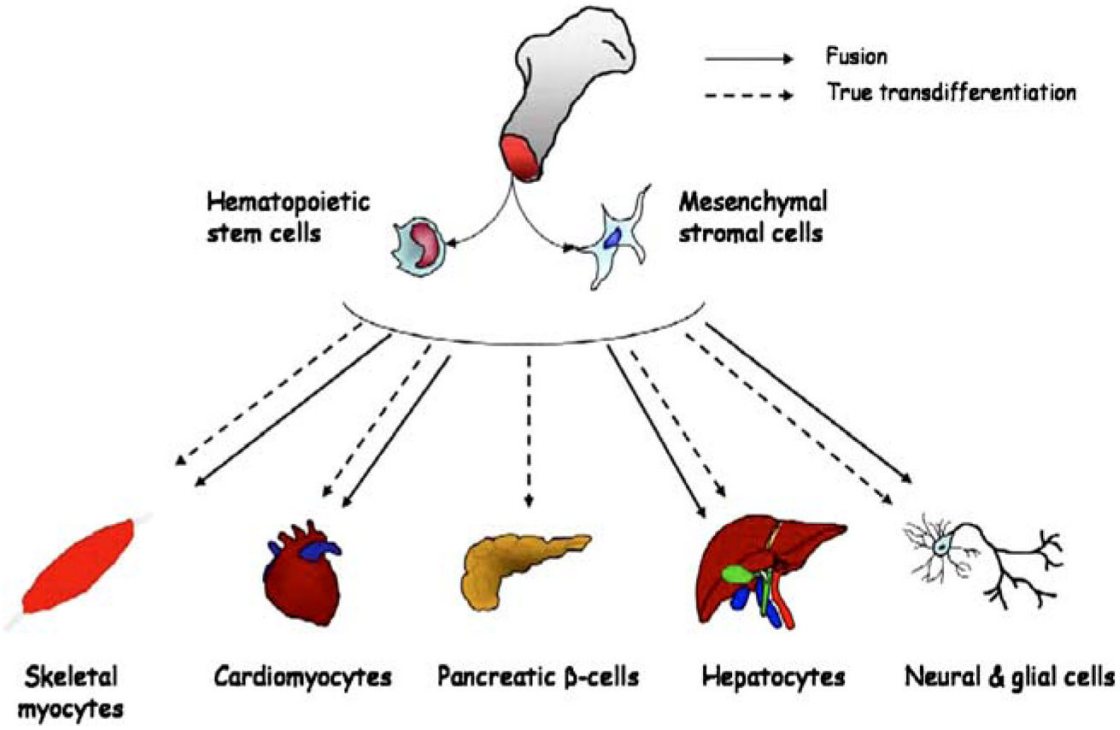

Fig. 1.
Diagram illustrating plasticity of bone-marrow-derived cells

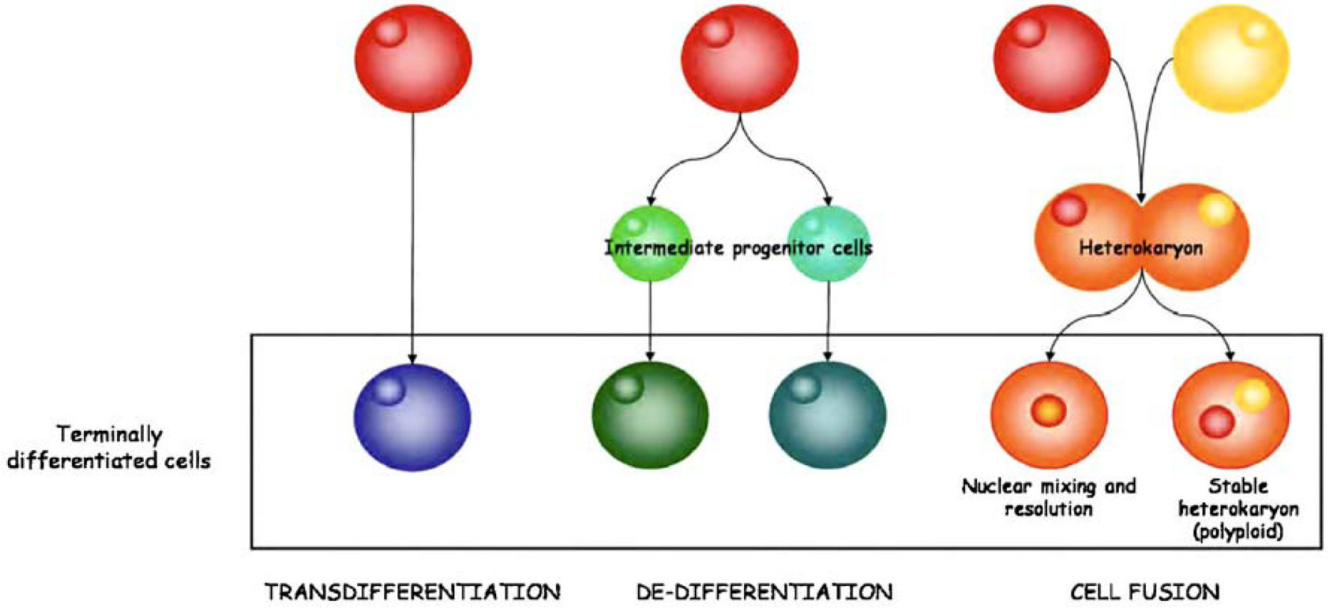

Fig. 2.
Diagram illustrating the different mechanisms of cell fate switching in adult stem cells

Table 1

Commonly used markers to identify adult stem cells in different tissues

Marker	Synonym	Significance	Literature
Endothelial progenitor cells			
CD146	MCAM, MUC18, S-endo-1	Integral membrane protein expressed on endothelial precursor cells and circulating endothelial cells	[115]
CD34		Cell surface protein on bone marrow cells, HSCs, endothelial progenitor and muscle stem cell	[115]
CD133	Prominin		[116]
Tie-2		Receptor for angiopoietin on endothelial progenitor cells and HCS	[115]
Fetal liver kinase-1	Flk1, KDR VEGFR2	Endothelial cell surface receptor protein that identifies endothelial cell progenitor	[117]
Hematopoetic stem cells			
CD34		Cell surface protein on bone marrow cells, HSCs, endothelial progenitor and muscle stem cell	[24,25]
c-Kit	CD117, YB5.B8	Cell surface receptor on bone marrow cell types that identifies HSC, hematopoietic progenitor cells and mesenchymal stem cell (MSC)	[15,25]
Stem cell antigen	Sca1, Ly-6A/E	Cell surface protein on bone marrow (BM) cell, indicative of HSC and MSC	[118]
Mesenchymal stem cells			
CD146	MCAM, MUC18, S-endo-1	Cell surface protein found on bone marrow fibroblasts	[118]
STRO-1 antigen		Cell surface glycoprotein on subsets of bone marrow stromal (mesenchymal) cells	[119]
Thy-1		Cell surface protein on HSC, MSC	[120]
Neural stem cells			
CD133	Prominin	Cell surface protein that identifies neuronal stem cells	[21]
Nestin		Marker for NSC in the CNS and in culture	[121]
Neuronal cell adhesion molecule	NCAM	Cell surface molecule that promotes cell–cell interaction; NSC migration; primitive neuroectoderm formation	[122]
Breast stem cells			
CD24 CD29		Cell surface proteins on mammary repopulating units in mice	[123]
Stem cell antigen	Sca1, Ly-6A/E	Cell surface protein on human mammary repopulating units	[124]
Intestinal stem cells			
NCAM		Marker for liver and pancreas stem cells	[125]
CD34		Marker for liver and pancreas stem cells	[126]
Thy-1		Marker for liver and pancreas stem cells	[126]
c-Kit		Marker for liver and pancreas stem cells	[126]
Flt-3		Marker for liver and pancreas stem cells	[126]

Table 2

Comparison of BM with two other sources of HSC

	PB/G-CSF	UC blood
Advantage	Easier, less aggressive collection	Easy to collect without any risk for the donor
	High number of progenitor cells	Less restriction for HLA compatibility
	Faster engraftment	Lower rate of GVHD
	Faster neutrophil, platelet, immune system recovery	Better accessibility from UC blood banks for unrelated transplantations
	Less frequency of donation-associated risks	Can be stored for years
Disadvantage	Administration of mobilizing agents is required	Limited supply
	Higher risk of chronic GVHD in allografts	Low number of progenitor cells, inadequate for transplant in adults
		Slower engraftment
		Unable to provide additional cells in case of need for second transplant

PB/G-CSF Peripheral-blood-derived HSC treated with G-CSF

Table 3

Clinical trials involving myoblast transplantation

Approach	Patient No.	Results	Reference
Intramyocardial, adjunct to CABG	10 (no controls)	LVEF increased, improved NYHA class	[127]
Intramyocardial, adjunct to LVAD implantation	5 (no controls)	Myofibers parallel to host myocardial fibers, increased blood vessel density	[128]
Intramyocardial, adjunct to CABG	10 (no controls)	LVEF and regional wall motion increased	[129]
Percutaneous	9 (no controls)	LVEF improved in 6 out of 9 cases	[130]
Intramyocardial, adjunct to CABG	18 (no controls)	LVEF regional wall motion and viability increased improved NYHA class, reduction of scar size	[131]
Intramyocardial, adjunct to CABG	11 (no controls)	LVEF, regional wall motion and viability increased	[132]
Percutaneous	5 (no controls)	LVEF and regional wall motion increased	[133]
Intramyocardial, adjunct to CABG or LVAD implantation	18 (no controls)	LVEF and viability increased, engraftment of myoblasts	[134]
Percutaneous	6 (plus controls)	LVEF increased, improved NYHA class	[135]
Intramyocardial, adjunct to CABG, allogeneic	2 (no controls)	LVEF increased	[136]
Intramyocardial, adjunct to CABG	120 (plus control)	Reduction of LV remodeling, LVEF increased at high dose	[48]