Adult stem cells: assessing the case for pluripotency

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It has been known for decades that stem cells with limited differentiation potential are present in post-natal tissues of mammals, and adult stem cells are already used clinically. For instance, hematopoietic stem cells can reestablish the hematopoietic system following myeloablation, and stem cells are being used to regenerate corneal and skin tissue. But recent studies report that adult tissues might contain cells with pluripotent characteristics. These have evoked significant excitement, given the medical implications, but have also met with much skepticism. Indeed, most studies still await independent confirmation, there is a low frequency with which the apparent lineage switching occurs, and importantly such lineage switching defies established developmental biology and stem cell principles. Here, I critically review the published data indicating that postnatal stem cells persist that have greater differentiation potential than previously thought.

Published online: 26 September 2002

Stem cells have been defined in many different ways. However, the main principles include (1) self-renewal, or the ability to generate at least one daughter cell with characteristics similar to the initiating cell; (2) multi-lineage differentiation of a single cell; and (3) and *in vivo* functional reconstitution of a given tissue [1]. The embryonic stem (ES) cell, first obtained from mouse [2,3], and more recently from non-human primates and human blastocysts [4,5], fulfills all these principles (Fig. 1). Both mouse and human ES cell-lines have been maintained continuously in culture for more than 300–400 cell doublings. ES cells differentiate into all somatic cell types when injected into a blastocyst and form mature progeny cells of all three embryonic germ layers *in vitro* (embryoid bodies) or *in vivo* (teratomas). Finally, all differentiated progeny of ES cells are functional cells as mice generated by tetraploid embryo complementation are viable [6].

Most adult stem cells in theory also fulfill these criteria, even though the degree of self renewal and differentiation potential is less than that seen for ES cells. The best studied adult stem cell, the hematopoietic stem cell (HSC) [7–9], undergoes, at least *in vivo*, self-renewing cell divisions, differentiates at the single cell level into all mature blood elements and functionally repopulates the hematopoietic system of a myeloablated animal or human. Other adult stem cells have been more recently defined and are therefore less well studied. However, neural stem cells (NSCs) [10,11], mesenchymal stem cells (MSCs) [12–16] and epidermal stem cells [17] all

fulfill these basic criteria. Other cells also termed stem cells, such as corneal stem cells [18] and angioblasts or endothelial stem cells [19] fulfill all criteria except that they differentiate into a single type of differentiated cell only.

'Stem cell plasticity' is a new term that has been used to describe the recent observations that greater potential might persist in post-natal adult stem cells than previously thought. There is yet no consensus on what the definition for such plasticity should be. However, to strictly define a stem cell with greater plasticity, simple application of the conventional stem cell definition should suffice. Then, minimal criteria for a stem cell with plasticity should be (1) self-renewal ability; (2) differentiation of a single cell into cells of the tissue of origin and into at least one cell type different from the tissue of origin; and (3) functional differentiation *in vivo* into cells of the tissue of origin and at least one cell type of a tissue other than the tissue of origin. In this review, we will critically assess studies describing stem cell plasticity in light of this definition.

Studies describing stem cell plasticity

Over the past five years, at least 50 manuscripts have been published indicating that cells from a given tissue might be capable of differentiating into cells of a different tissue, and these have therefore been placed under the umbrella of stem cell plasticity (Fig. 2). The majority of studies were done in rodent models, while fewer used human cell sources. Most studies were based on *in vivo* transplantation of either sex-mismatched cells or genetically marked cells, and detection of donor cells was based on presence of the Y-chromosome or the marker gene. There are excellent reviews of the pitfalls involved in detection of donor cells using either marking system [20,21]. The majority of studies depended on phenotypic characteristics to define differentiation into cells different from the tissue of origin, but have yet to demonstrate that the cells of the second tissue have functional characteristics of that lineage. Finally, in the majority of studies, non-purified populations of cells or cells purified to partial homogeneity were transplanted, therefore making it impossible to assess the clonal origin of differentiated cells of the tissue of origin and of cells with characteristics of a second tissue. The following sections will discuss the evidence for stem cell plasticity.

Plasticity of hematopoietic bone marrow cells

More than 80% of studies reporting adult stem cell plasticity to date have been performed using bone marrow (BM), or BM or peripheral blood (PB) enriched for HSC. Differentiation not only into hematopoietic cells but also cells with characteristics of skeletal muscle [22,23], cardiac muscle [24,25], endothelium [26], neuroectoderm [27,28], skin epithelium [29] and endodermal cells [29–31], including hepatocytes, gastrointestinal epithelium and lung epithelium, has been described.

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Fig. 1. Stem cell definition. Stem cells are defined as cells that have: (a) self-renewal ability; (b) multi-lineage differentiation ability of a single cell; and (c) show *in vivo* functional reconstitution of a tissue. This is exemplified here by hematopoietic stem cells (HSCs). HSCs can undergo sequential self-renewing cell divisions, even though this is not unlimited. Single HSCs can differentiate into neutrophils, lymphocytes, red cells and platelets, even though it has been believed that they do not differentiate into cells outside of the hematopoietic system. HSCs can also reconstitute all hematopoietic cell types when transplanted into lethally irradiated mice or humans.

In all these studies, fresh BM or PB cells were transplanted without prior *in vitro* culture, so that the question of whether the cell with plasticity can undergo self-renewal could not be assessed. Furthermore, grafted cells were not re-transplanted in secondary recipients precluding assessment of self-renewal of the cell with plasticity *in vivo*. Exceptions are the studies by Krause *et al.* [29] and Grant *et al.* [26], in which single cells reestablished hematopoiesis, suggesting self-renewal of the HSC *in vivo*. Furthermore, in the study by Grant *et al.* [26], serial transplantations were performed to demonstrate self-renewal of the HSC.

Most studies transplanted mixed cell populations from wild-type animals, or animals transgenic for the β -galactosidase gene, which allows detection by X-Gal staining, or the enhanced green-fluorescent protein (eGFP) marker gene. Even in studies in which enriched HSC gave rise to both hematopoietic cells and, for instance, liver epithelium [32], multiple cells were grafted. Therefore, the second criterion – single cell multi-lineage differentiation – could not be assessed. This was not the case for the report by Krause *et al.* [29], in which single 'homed' CD34⁺Sca1⁺ cells were transferred to secondary recipients, and differentiation into hematopoietic and epithelial cells was shown. Likewise, in the study by Grant *et al.* [26], single HSCs were transplanted, and cells within endothelium of the retina and hematopoietic cells were shown to be derived from a single progenitor.

Most studies defined plasticity based on the acquisition of morphologic and phenotypic characteristics of the non-hematopoietic tissue. Because the degree of engraftment in non-hematopoietic tissues was usually less than a few percent, acquisition of phenotypic characteristics alone, such as dystrophin expression and albumin expression, cannot be used to prove functionality of the individual grafted cells. Therefore, most studies also did not fulfill the third criterion of stem cells, namely

functional differentiation *in vivo*. Notable exceptions are the study by Lagasse *et al.* [31] demonstrating functional replacement of hepatocytes following systemic injection of murine BM cells enriched for HSC. The animal model used was the hereditary tyrosinemia model, caused by deletion of the gene encoding fumarylacetoacetate hydrolase (FAH). This mutation is lethal, although animals – like humans – can be kept alive by administration of 2-(2-nitro-4-fluoromethylbenzoyl)-1,3-cyclohexanedione (NTBC), which inhibits 4-hydroxyphenylpyruvate dioxygenase, upstream of FAH. The only other therapy is replacement of hepatocytes by wild-type hepatocytes [32]. Lagasse *et al.* showed that animals transplanted with wild-type BM or wild-type BM enriched for HSC could be weaned off NTBC, and that donor BM and HSC differentiated into cells with phenotypic characteristics of hepatocytes that must be functional as they rescued the otherwise-lethal phenotype. In a study by Orlic *et al.* [24], the investigators demonstrated in a murine model of myocardial ischemia, that grafting of enriched HSC in the infarcted area leads to engraftment of BM-derived cells that acquire characteristics of immature cardiomyoblasts. In addition, significant improvement in cardiac function was shown. Finally, in a study by Grant *et al.* [26], endothelial cells generated from single transplanted HSC were functional as new capillaries were formed following radiation-induced lesions of the retina.

Stem cell plasticity of mesenchymal bone marrow cells
Aside from HSCs, BM also contains MSCs. MSCs can be cultured *ex vivo* for several passages and differentiate at the single-cell level into limb-bud mesodermal cell types, including osteoblasts, chondroblasts, adipocytes, fibroblasts and skeletal myoblasts [12–16]. When introduced *in vivo*, MSCs differentiate into the same array of cell types [33]. Several recent reports have shown that MSCs might acquire characteristics of cells outside the limb-bud mesoderm, including endothelium [34,35], neuroectoderm [35–37] and endoderm [35,38]. Because all studies using MSCs use cells cultured *in vitro* that have been passaged several times (for >100 cell doublings) [13,15,16,35,38,39], they demonstrate that the cell with plasticity has self-renewal activity. The study by Jiang *et al.* [35] also showed that cells collected from initial transplant recipients could upon transfer to secondary recipients give rise to multiple cell types, suggesting *in vivo* self renewal behavior.

To address the clonal derivation of differentiated progeny, some studies have depended on ring-cloning to isolate progeny of single cells [15,16]. However, this approach does not fully prove that single cells give rise to multiple progeny as MSCs are very motile in culture and several individual cells might therefore have contributed to the colony that is isolated in a given ring. Other studies have used more foolproof single-cell sorting strategies [14] or retroviral

Fig. 2. Stem cell plasticity. Stem cell plasticity has been described as the unexpected potential of cells from bone marrow, muscle or brain to give rise to cells of tissues other than the tissue of origin. Examples include bone-marrow-derived cells or even purified hematopoietic stem cells that can give rise to endothelium, skeletal muscle, cardiac muscle or hepatocytes.

marking strategies [35,38,39] to demonstrate multi-lineage differentiation from single cells.

Only a few studies have assessed function of differentiated cells other than limb bud mesoderm lineage cells. Reyes *et al.* [34] showed that multipotent adult progenitor cells (MAPCs) differentiate in the presence of vascular endothelial growth factor into cells with endothelial morphology and phenotype and function like endothelial cells *in vitro* and *in vivo*. Schwartz *et al.* [38] showed that MAPCs, induced to differentiate to hepatocyte-like cells with fibroblast growth factor 4 and hepatocyte growth factor, secrete albumin and urea and have phenobarbital-inducible cytochrome-p450 activity, all functional characteristics of hepatocytes. Even though several groups showed that MSCs can differentiate into cells with morphological and phenotypic characteristics of glia and neurons [35–37], they have yet to show that these cells have electrophysiological characteristics of neurons. None of the studies has shown that MSCs functionally repopulate damaged tissue outside of limb-bud mesodermal tissues following transplantation *in vivo*.

Plasticity of skeletal muscle cells

Aside from BM cell plasticity, plasticity has been reported for cells derived from other organs. Jackson *et al.* [40] reported that transplantation of muscle cells or muscle side population cells (Sp), selected based on their ability to extrude Hoechst dye and cultured *ex vivo* for 2–3 days, could give rise to hematopoietic cells following lethal irradiation, and could compete with BM-derived HSCs, a criterion commonly used to demonstrate the presence of functional HSCs [41]. However, not shown in this initial study was that the same cell that gives rise to hematopoietic cells also gives rise to myoblasts. Likewise, Gussoni *et al.* showed that muscle Sp cells could reconstitute the hematopoietic system in lethally irradiated mice [23]. However, a subsequent study by McKinney-Freeman *et al.* [42] and Kawada *et al.* [43]

showed that this apparent plasticity might be caused by HSCs present in muscle tissue. Thus, these studies do not demonstrate stem cell plasticity, even though a single tissue is capable of generating two different cell types – a phenomenon that could be termed tissue plasticity.

Plasticity of neural cells

Bjornson *et al.* [44] reported that murine NSCs cultured *ex vivo* for several population doublings could differentiate into hematopoietic cells. Similar results were reported for human NSCs by Shih *et al.* [45]. Both studies used *ex vivo* cultured cells that had undergone several cell doublings and therefore fulfill the first criterion of stem cells. Both used cells generated from single neural spheres, which are thought to be derived from a single NSC. However, they did not use retroviral marking or single cell sorting to fully prove that the cells within a neural sphere were single-cell derived. Finally, although a large proportion of the hematopoietic cells were derived from the neural spheres, neither study demonstrated that these cells could rescue animals from aplasia, nor did they use competitive repopulation assays to demonstrate the function of hematopoietic cells *in vivo*. However, a third study, reported that the frequency with which this lineage switch can occur for murine NSCs is at best extremely low [46].

Clarke *et al.* also assessed the plasticity of NSCs [47]. Progeny of single brain-derived neural spheres were introduced either in the chick embryo or in the murine blastocyst. In both assays, they demonstrated that NSC progeny could contribute to multiple tissues, even though some tissues appeared not to be derived from the NSCs, including, interestingly, the hematopoietic system. Like Bjornson *et al.* [44] and Shih *et al.* [45], Clarke *et al.* used *ex vivo* cultured cells that had undergone several cell doublings, and the cell with plasticity therefore fulfills the first criterion of stem cells. Single neural sphere progeny were used that were, however, not retrovirally marked or generated from single sorted cells to fully prove that the cells within a neural sphere were single-cell derived. Finally, none of the chimeric mice was born, which precludes assessment of the functional differentiation of the cells *in vivo*.

Plasticity of other tissues

Toma *et al.* described that cells isolated from dermis of mouse and man can be cultured *ex vivo* for several months and can differentiate into cells with phenotypic characteristics of neurons and glial cells, as well as adipocytes and smooth muscle, *in vitro* [48]. Single sorted cells were used to initiate the cultures. However, the differentiated cells were not functionally characterized, and *in vivo* repopulation studies were not performed.

Finally, there are reports of plasticity within the endodermal lineage. Shen *et al.* [49] showed that a

Fig. 3. Possible explanations for the perceived plasticity. (a) Stem cells for a given tissue might exist in an unrelated organ. (b) Perceived plasticity might be caused by the transplanted cells fusing with a host cell of a different lineage, leading to transfer of the genetic information of the transplanted cell to the host-derived cell. (c) Plasticity might occur via de- and re-differentiation, as is seen in cloning or in limb regeneration in amphibians. (d) Cells with pluripotent characteristics might persist even after the initial steps of embryological development.

human pancreatic cell line as well as fetal pancreatic tissue can be induced to acquire phenotypic characteristics of hepatocytes *in vitro* by treatment with dexamethasone or introduction of C/EBP β . This lineage switch appeared to occur without interim cell division. However, no functional characterization was performed on the hepatocyte-like cells, and no proof exists that these hepatocyte-like cells functionally repopulate the liver *in vivo*. Yang *et al.* showed that oval cells, the progenitor for hepatocytes and biliary epithelium, can be cultured *ex vivo* for prolonged periods of time, and can be induced to express insulin. These cells reverse glucose intolerance when transplanted *in vivo* in a streptozotocin-treated mouse [50]. However, as the transplanted tissue was not removed to demonstrate that the reversal in insulin dependence was caused by the graft rather than incomplete ablation of endogenous β cells, additional studies will be required to fully prove that the lineage switch generated functional β cells.

Possible explanations for perceived plasticity

At least four different explanations exist for the perceived plasticity described in the studies discussed above (Fig. 3). First, it is clear that stem cells for a given tissue might exist in unrelated tissues (Fig. 3a). This has been elegantly shown for HSCs in muscle [42,43], and also has been shown for oval cells in BM [51]. In this instance, the apparent lineage switch is not caused by a single stem cell that can alter its differentiation behavior; rather, the presence of multiple stem cells gives the impression of plasticity.

Second, the perceived plasticity might be caused by the transplanted cells fusing with a host cell of a

different lineage, leading to transfer of the genetic information of the transplanted cell to the host-derived cell (Fig. 3b). This phenomenon underlying the heterokaryon technique was described many decades ago [52] and was more recently demonstrated to occur between ES cells and somatic cells. In two independent studies, coculture of ES cells with cells derived from adult tissue resulted in fusion of the ES cell with an adult cell, in which the genetic information in the ES cell is transferred to the adult cell [53,54]. This phenomenon was rare ($1/10^5$ – 10^6 cells), occurred in the presence of significant selectable pressure, and the resulting cells were tetraploid. However, no published study dealing with plasticity has proven that the observed *trans*-differentiation is not caused by this phenomenon. Circumstances where this mechanism might be more likely include conditions where extensive selectable pressure is needed to see the plasticity of stem cells, where plasticity is seen in tissues that 'tolerate' tetraploidy, such as muscle, hepatocytes, Purkinje cells and others, and where the frequency of *trans*-differentiation is low. However, it is important to keep in mind that, although the studies by Ying *et al.* and Terada *et al.* reminded the scientific community of this possibility [53,54], neither study showed that such a cell fusion phenomenon occurs *in vivo*, where most of the plasticity phenomena have been described.

Third, since the cloning of Dolly [55], a large body of evidence has been created indicating that an adult cell from many mammalian species [56,57] can be reprogrammed to acquire a pluripotent fate when the nucleus is introduced into the cytoplasm of a non-fertilized egg. De-differentiation and re-differentiation is also thought to underlie the ability of amphibians and fish to regenerate limbs spontaneously. Therefore, the perceived plasticity might occur via de- and re-differentiation or via nuclear reprogramming (Fig. 3c). Under this scenario, stem cells, progenitor cells or even more-differentiated cells would undergo genetic reprogramming when removed from their usual 'niche' and introduced into a different microenvironmental niche. Factors in egg cytoplasm that reprogram the nucleus of the somatic donor cell during nuclear transplantation are not known. Insights into the molecular mechanisms underlying nuclear reprogramming might therefore help our understanding of adult stem cell plasticity and might be exploited in the future to induce lineage switching even without nuclear transplantation. Likewise, the exact mechanisms that underlie limb regeneration in amphibians and fish are not fully understood. It is believed that, upon loss of a limb, 'terminally differentiated' cells in the blastema de-differentiate and then re-differentiate to generate the many different cell types required for creation of a new limb. Signaling via retinoic acid receptors [58] and Sonic Hedgehog (Shh) [59] is thought to play a role in the genetic reprogramming required for this phenomenon to occur. Specific homeobox genes have

been characterized that play a role in this de- and re-differentiation event [60]. For example, *Msx1* can induce a similar de-differentiation event when introduced in mammalian cell lines [61]. Whether any of the de- and re-differentiation pathways that have been identified in fish and amphibians play a role in higher mammalian stem cell plasticity will need to be defined.

Finally, cells with pluripotent characteristics might persist even after the initial steps of embryological development (Fig. 3d). Pluripotent ES cells are characterized at the molecular level by the transcription factor oct-4 [62–64], which is expressed in the pre-gastrulation embryo, early cleavage-stage embryo, cells of the inner cell mass of the blastocyst and in embryonic carcinoma cells. Oct-4 is downregulated when cells are induced to differentiate *in vitro* [65], and, in the adult animal, oct-4 is found only in germ cells. Several studies have shown that Oct-4 is required for maintaining the undifferentiated phenotype of ES cells and plays a major role in determining early steps in embryogenesis and differentiation [62,66]. Another hallmark of ES cells is the presence of telomerase [67,68], which allows ES cells to undergo unlimited cell divisions. One might therefore conclude that, for a stem cell to be pluripotent, some or all of these characteristics should be present. Except for MAPCs described in the study by Jiang *et al.* [35], who showed that murine MAPCs express – albeit at low levels – oct-4 and the stage-specific embryonic antigen SSEA-1, no adult somatic cell has been described that expresses these characteristics. However, as it remains to be determined whether MAPCs exist as such *in vivo* or are the result of de-differentiation of an MSC-like cell into a cell with greater potential, there is currently no definitive proof that true pluripotent stem cells exist *in vivo* during post-natal life. Studies aimed at proving or disproving their existence are not only of scientific importance but will have far-reaching implications for use of the plasticity phenomenon in clinical medicine.

Can adult stem cells be used for therapeutic purposes? Hematopoietic stem cells from BM, blood or umbilical cord blood have been used clinically to re-establish the hematopoietic system following radiation and/or chemotherapy for the past three decades. More recently, use of epidermal stem cells and corneal stem cells is being explored in the clinic, and a large number of studies have evaluated, in pre-clinical models, the use of NSCs to correct defects of the central nervous system and spinal cord.

Several studies have assessed whether BM or peripheral blood cells might contribute to tissues other than the hematopoietic system in the clinical setting. Results from these studies are mixed. Even in the 1980s, several groups tested whether non-hematopoietic stromal cells within the BM were donor derived after BM transplantation. Some

studies suggested that stromal cells were donor in origin [69], whereas others contradicted this notion [70], and a more recent study evaluating patients more than 10 years after transplant concluded that stromal cells might not be replaced by donor cells [78]. Along the same lines, Horwitz *et al.* [71] reported that donor marrow transplanted into patients with osteogenesis imperfecta might ameliorate bone brittleness, presumably because mesenchymal cells engrafted. As no evidence was provided that donor osteoblasts were present, it remains to be determined whether clinical improvement was due to replacement of existing osteoblasts with donor cells.

‘Although stem cell plasticity is not fully proven, there is sufficient evidence to warrant continued efforts to prove or disprove that some adult stem cells might be more pluripotent...’

Subsequently, several studies have shown that human BM cell or cells circulating in the blood might contribute to many different tissues. Theise *et al.* [72] reported that donor-derived cells that appear to have differentiated into hepatocytes can be detected in the liver of patients who develop liver damage following BM transplantation. Likewise, in patients that underwent orthoptic liver transplantation, host-derived cells with apparent hepatocyte features could be detected in the donor liver. Korbli *et al.* [73] reported that up to 7% of hepatocytes and epithelial cells of the skin and gastrointestinal tract in patients that had undergone allogeneic sex-mismatched peripheral blood grafts might be derived from the graft. Of note, in both studies, donor-derived epithelial cells were usually found as single cells – and not as clusters of cells, as one might expect for donor cells *trans*-differentiating into epithelial stem cells that then proliferate locally and differentiate to form a colony of donor cells. This notion is consistent with the study by Hematti *et al.* [79], who showed that, although up to 14% of keratinocytes in the skin of patients that underwent donor HSC transplantations are donor derived, keratinocyte stem cells remain host in origin. This study indicates therefore that further studies will be needed to determine whether the phenomenon of adult stem cell plasticity will be applicable to regenerative medicine as this will require *trans*-differentiation to a stem cell phenotype and not to a mature cell phenotype.

Quaini *et al.* [74] reported that between 12% and 16% of cardiac myocytes might be host derived in patients undergoing orthoptic heart transplantation. However, three subsequent studies [75–77] have suggested that cardiomyocytes of noncardiac origin in transplanted hearts are at best extremely rare (in the

range of 0.1–0.01%), even in the setting of significant cardiac damage in the setting of rejection. Such levels of contribution would not be clinically useful.

Although the discussion above indicates that stem cell plasticity is not proven, there is sufficient evidence to warrant continued efforts to prove or disprove that some adult stem cells might be more pluripotent as this would open the door to the use of stem cells from one organ, such as bone marrow or circulating in the blood, to correct genetic or degenerative disorders of a second organ from which stem cells are more difficult to isolate, such as brain.

One question to be addressed will be whether the mechanism underlying the perceived plasticity matters when considering clinical application of adult stem cells. One might argue that even if liver regeneration from BM [30,31] were to be derived from a hepatic stem cell or oval cell co-existing with HSC in BM, BM cells might still be an excellent source of cells to treat liver failure as long as robust and persistent engraftment could be shown. This would however not qualify as stem cell plasticity. Perhaps, if perceived plasticity was due to fusion of a donor cell with an existing tissue cell, this might still be useful to treat tissues consisting of multinucleated cells, such as skeletal muscle. Then, if BM cells could be induced to fuse in high frequency with skeletal myoblasts, this might form a novel gene-therapy approach for muscular dystrophy, provided that the fused progeny were otherwise normal.

However, for adult stem cell plasticity to be clinically useful on a large scale, a ready supply of well-characterized 'pluripotent' adult stem cells will be required. If pluripotency is acquired by *ex vivo* manipulation which caused de-differentiation, then defining the molecular mechanism(s) underlying the lineage switching will be required to enhance the frequency and fidelity with which this event occurs. One caveat that will need to be addressed is whether the process of de- and re-differentiation always occurs without causing unwanted genetic abnormalities that might lead to aberrant differentiation or might even be oncogenic. If pluripotent stem cells indeed exist *in vivo*, better characterization of the phenotype of such cells, their natural environment and proliferation and/or differentiation behavior, and their ability to be recruited to areas of injury, will be important to

exploit their clinical usefulness. In addition, it will behoove scientists and clinicians alike to test whether re-activation of presumed quiescent pluripotent stem cells by *ex vivo* culture or by *in vivo* manipulation will not cause uncontrollable proliferation and/or differentiation similar to teratomas, seen when ES cells are transplanted *in vivo*.

Some would argue that one of the major advantages of adult stem cells is that they might be harvested from the patient and avoid unwanted immune responses. However, because the mechanism(s) underlying a variety of degenerative disorders are currently not known, it remains to be determined whether similar defects might be present in stem cells or their progeny from an individual afflicted by these disorders. Second, although autologous stem cell therapy might well be suitable to treat chronic disorders, such as Parkinson's disease, such therapy might not be available in a timely manner to correct acute injuries, such as myocardial infarcts, cerebral ischemia and spinal cord injury, if preclinical models demonstrate that the therapy is most efficacious when given shortly after the injury.

Concluding remarks

Despite the ever-growing number of studies suggesting that pluripotent stem cells can be obtained from adult tissues and that certain cells might have the capacity to acquire a more pluripotent state, the field of stem cell plasticity is in its infancy. Indeed, the evidence that pluripotency exists or can be re-induced is not yet conclusive. Because some initial reports have since been reinterpreted, as the majority of studies still await independent confirmation and as the possible mechanisms underlying the perceived plasticity are not understood, the scientific community has looked upon this field with healthy skepticism. Investigators studying plasticity will need to assess findings of plasticity in light of the definition commonly accepted to define stem cells – that is, demonstrate that single cells derived from adult tissue differentiate into multiple lineages characterized not only based on phenotype but also on function and support robust, sustained and functional multilineage engraftment *in vivo*. Notwithstanding the skepticism, this is an exciting time to be involved in stem cell research, with great challenges and also great expectations ahead.

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