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In search of “stemness”

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Abstract

Stem cells have been identified and characterized in a variety of tissues. In this review we examine possible shared properties of stem cells. We suggest that irrespective of their lineal origin, stem cells have to respond in similar ways to regulate self-renewal and differentiation and it is likely that cell-cycle control, asymmetry/differentiation controls, cellular protective and DNA repair mechanisms, and associated apoptosis/senescence signaling pathways all might be expected to be more highly regulated in stem cells, likely by similar mechanisms. We review the literature to suggest a set of candidate stemness genes that may serve as universal stem cell markers. While we predict many similarities, we also predict that differences will exist between stem cell populations and that when transdifferentiation is considered genes expected to be both similar and different need to be examined.

Stem cells have been defined as self-renewing populations of cells that undergo symmetric and asymmetric divisions to self-renew or differentiate into multiple kinds of differentiated progeny [1–3]. It is important to note that this minimal definition of a stem cell is not one that can reliably distinguish stem cells from other dividing cells and several investigators have argued that additional, more stringent criteria are necessary.

Some investigators have suggested that the ability to self-renew over the entire lifetime of an organism and substantial contribution to a tissue should be considered prerequisite to defining a cell as a stem cell. If this more stringent definition is used, then most “so-called” stem cells would not meet this and would be better classified as progenitor cells.

Neural crest stem cells (NCSCs), for example, are a transient population [4], and blastocysts from which embryonic stem (ES) cells are derived do not exhibit prolonged self-renewal throughout the life of the organism [5]. In solid tissues, contribution of stem cells may be regionally restricted such that no single stem cell contributes to a major portion of the organ [6]. We prefer the minimal definition of stem cells rather than using the criteria of lifetime self-renewal and substantial contribution to the tissue development as has been proposed, for at the current state of knowledge this would exclude all but hematopoietic stem cells (HSCs) and perhaps germ cells from discussion. Cells fulfilling the minimal definition of “stemness” have been identified as being present in virtually all tissues and most stages of development.

Stem cells can be distinguished from each other based on the tissue from which they were harvested, their bias in differentiation ability, the stage of development at which they exist, and the genes that they express. A brief list of stem cells identified is shown in Table 1. An important emerging consensus is the observation that more than one stem cell may be

present in a particular tissue [7]. Thus, in the hematopoietic system, one can identify stem cells derived from the yolk sac [8], fetal cord blood [9], liver [10], and the adult bone marrow [11,12]. Each of these cells has prolonged self-renewal ability, can repopulate the bone marrow in transplant paradigms, and can produce multiple phenotypes in colony assays. Similarly, a veritable menagerie of stem cell populations has been identified in the nervous system present at different stages of development or in different locations in the brain [13]. While detailed information on stem cells is not available for stem cells derived from all tissues, preliminary evidence suggests a similar pattern of development with multiple types or stages of stem cells that may appear superficially similar in their differentiation ability, but can be segregated based upon their developmental history, self-renewal ability, marker expression, or positional information [14].

Despite the differences between resident stem cell populations of the same tissue, a parallel set of transdifferentiation studies have suggested that these differences may not be as significant as once thought and indeed may not be significant even among stem cells harvested from different tissues. Stem cells may be quite plastic and may be able to respond to environmental cues to alter their characteristics and mimic the properties and responses of a stem cell from any particular tissue. Thus HSCs could transdifferentiate to generate neural tissue and vice versa, and numerous studies—though not without controversy—have suggested that this is a common event.

Overall, the idea that stem cells exist in different tissues, that express specific individual characteristics, and the idea that stem cells are interchangeable has led to the concept that some properties of stem cells may be universal, thus leading to the concept of stemness. Initial attempts to identify common stem cell markers [15–17] as exemplified by the work of Ramalho-Santos et al. and Ivanova et al. have not been very successful. Indeed, a recent metaanalysis of stemness genes assessed by comparing common gene expression patterns in purified stem cell populations identified only one gene common among the three stem cell populations analyzed ([17], Fig. 1).

The failure to identify stemness genes likely reflects on the technical difficulties of the experiments rather than their absence. In general, it has been difficult to obtain pure populations of stem cells. Furthermore, stem cells undergo stochastic changes when maintained in culture and during development in vivo. Species differences in the expression of stem cell characteristics have been defined [18,19]. Comparisons therefore require isolating pure cells, with minimal culturing, at a defined state of development in sufficient quantities such that comparative methods can be performed with sufficient rigor to overcome the variability inherent in the comparison techniques itself.

Given the paucity of data and the difficulty in performing comparative experiments, we have in this review taken an alternative approach to attempt to identify “stemness” genes. We have reasoned that stem cells, irrespective of the tissue where they are harvested and their differentiation ability, face the same set of challenges. All stem cells need to regulate their cell cycle to either undergo periods of rapid division—maintain self-renewal, or enter periods of quiescence, or (in the case of stem cells that last the lifetime of an individual) avoid senescence. Stem cells, in addition, have to maintain genomic integrity to avoid the accumulation of genetic mutations and neoplastic transformation. Stem cells have to make a choice between self-renewal and differentiation, and have to respond to the extrinsic environment using growth factor receptors/extracellular matrix (ECM) molecules/gap junctional communication. Thus, cell-cycle control, asymmetry/differentiation controls, cellular protective and DNA repair mechanisms, and associated apoptosis/senescence signaling pathways are expected to be more highly regulated in stem cells, possibly by similar mechanisms (Fig. 2). We further reason that the same properties that are common

among stem cell populations will distinguish stem cells from progenitor cell populations. One would expect, for example, that regulation of senescence pathways, conservation of DNA repair mechanisms, and regulation of symmetric and asymmetric division may not be as critical for progenitor cell populations as in stem cells. In subsequent sections we describe candidate stemness genes based on this logic.

Absence of markers

Perhaps one distinguishing characteristic of stem cells is the absence of markers of differentiation. The absence of markers characteristic of differentiated cells has been used quite successfully to define and enrich for stem cells to be used for therapy (Table 2). In the HSC field this long list of markers has been concatenated as LIN⁻ (lineage-negative cells). The absence of lineage markers has been used to define other stem cell populations as well. Mesenchymal stem cells (MSCs) are LIN⁻ for osteoblast, chondrocyte, and muscle markers; neural stem cells (NSCs) are negative for astrocyte, oligodendrocyte, and neuronal markers; NCSCs are negative for Schwann cell, peripheral neuron, and mesenchymal markers; and ES cells are LIN⁻ for ectoderm, endoderm, and mesoderm markers. As with HSCs, the absence of markers can be used to select NSCs. For example, the absence of A2B5, NCAM, CD44, and CD24 can be used to enrich for NSCs [20], and personal results). Equally important, the NSC population harvested by LIN⁻ selection is identical to the population harvested by positive selection or by selection with general metabolic markers [20].

Thus, LIN⁻ is a common property of many stem cell populations. Perhaps we have been remiss in ignoring markers that are high in most differentiated cells but low or absent in stem cells that could be used to define a stem cell state. Thy1 is one such candidate molecule. It is relatively abundant in many tissues but is low or absent on stem cell populations [21]. Other candidate molecules are receptors for ECM interaction. ECM molecules expressed by stromal cells in the HSC niche [22] help to anchor growth factors and other regulators for stem cell proliferation and differentiation [23].

These LIN⁻ markers would not have been picked up in the analysis performed so far with direct comparisons of genes that are common among stem cells. However, we would predict that pair-wise comparisons performed between stem cells and differentiated cells (pooled universal RNA perhaps) would identify such LIN⁻ genes. Markers that are low or absent in stem cells but abundant in differentiated cells could be identified and a pooled set that are absent in all stem cell populations but present in most differentiated cells could be developed. Absence of such markers would allow one to identify stem cell populations. Therefore, lineage negativity should be considered a distinguishing property of stem cells (Fig. 3).

Presence of general metabolic markers

As suggested earlier, it is likely that cells from different tissues use similar strategies to maintain their stem cell state and to regulate proliferation and differentiation. These strategies are likely to be unique to stem cell populations and distinct from those in differentiated cells. Thus, it is reasonable to expect that certain metabolic parameters may be similar. Indeed, an emerging body of evidence has suggested that rhodamine and Hoechst dye efflux, forward vs side scatter properties, and the presence of the multiple drug resistance (MDR) family of transporters may be characteristic of most stem cell populations (Table 3).

Rhodamine uptake and Hoechst labeling can be used to select stem cell populations from HSCs, cord blood, MSCs, muscle, and adult brain [24–27]. The side population (SP) that is isolated by Hoechst 33342 low uptake shows the highest capability of self-renewing and is

able to differentiate into multiple types of progeny. It is likely that differences in uptake of Hoechst 33342 can be attributed to the expression of a transporter that actively effluxes dye. Indeed in HSCs and ES cells, this transporter is likely to be ABCG2 [28,29]. The expression of ABCG2 is seen at high levels and is downregulated very quickly upon differentiation. Overexpression of this transporter results in a significant increase of SP cell numbers [28]. Our results show that ABCG2 is also expressed specifically in NSCs and drops quickly upon precursor cells committing [20].

It is important to note, however, that these properties depend on the state of the cell. We have noted that rhodamine and Hoechst labeling cannot be used to select actively dividing NSC populations such as embryonic NSCs; rather, they identify quiescent populations of self-renewing progenitors [20]. However, high expression of ABCG2 can still be detected in embryonic NSCs, which is consistent with its high expression in non-SP cells obtained from murine ES cells [28]. Although the reason why ABCG2 does not efflux the Hoechst dye in these actively dividing stem cells remains unclear, its presence can certainly serve as a marker specific for stem cells.

Another general metabolic marker that has been described is aldehyde dehydrogenase (ALDH). In particular, our results using the fluorescent substrate of ALDH, Aldefluor, provide evidence that ALDH activity is high in NSCs and that this nontoxic, live labeling method can be used to identify NSCs. ALDH activity has been used to identify HSCs and cord blood progenitors [30–32]. Preliminary results suggest that Aldefluor labeling may identify other stem cell populations as well ([33], and Fig. 4, courtesy of StemCo Biomedical). This finding may provide a simple nontoxic selection procedure to isolate stem cells from any development stage.

While the data are currently incomplete, transporter expression, efflux dye, and ALDH activity can distinguish between stem cells and more differentiated cells and may be considered candidate stemness genes. It remains to be explained, however, why these markers were not picked up by comparative genomic analysis.

Communication

Stem cells are abundant in early development when organ generation is occurring. At this stage, most signaling pathways are still developing and the circulatory system is still incomplete. Nevertheless, cell populations must respond in concert to regulate overall cell number and organ size, and some communication must exist to ensure that appropriate feedback loops are present (summarized in Table 4). Gap junctions represent one such communication pathway. Indeed, gap junctions appear during early development and their expression is reduced as other signaling mechanisms come into play [34]. Gap junctions allow direct cytoplasmic exchange of small hydrophilic molecules between two adjacent cells (reviewed in [35]), and they play an important role in many normal developmental processes such as embryonic patterning, as well as in tumor progression (reviewed in [36]).

We and others have examined the expression of gap junctions on stem cells and have found that gap junctions are present on ES cells [18], NSCs [20,34], HSCs [37,38], NCSCs [39], and hepatic oval cells [40], and may be present on other stem cell populations. Appropriate gap junctional communication is necessary for NCSC migration and proliferation [39] and their active function is especially critical for NSCs as blocking gap junction signaling in NSCs induces cell death. Connexin 43 and connexin 45 proteins have been identified in ES cells [18] and functional gap junctions have been demonstrated in ES cells via dye-coupling experiments [41]. While gap junctions are not unique to stem cell populations, the components that comprise the gap junctional machinery may be common to many stem cell populations and may be functionally relevant to maintaining the stem cell state.

Indeed, α - and β -catenin, which play a pivotal role in cadherin-mediated cell adhesion, also have high expression levels in HSCs [42], NSCs [43], and other populations of stem cells [44]. Healthy neurosphere cells are attached to each other by adherens structures as detected by electromicroscopy and Western blotting [45]. N-cadherin and β -catenin, two adherens junction molecules, were found in the attachment of HSCs to their niche [46]. β -catenin as a component of both adherent junction and Wnt signaling pathway showed critical functions in control of stem cell proliferation and expansion. β -catenin can function during mammalian neuronal development and probably regulate cerebral cortical size by controlling the generation of neural precursor cells [47]. β -catenin-related transcriptional machinery, which is regulated by E-cadherin in an adhesion-independent way, may also be responsible for stimulating cell proliferation [48]. No matter which mechanism of β -catenin regulates cell proliferation, high expression of β -catenin is probably common for different populations of stem cells.

The Rho family of small GTPases, including Rho, Rac, and Cdc42, is involved in the downstream signaling pathway of adherent junctions and connected with actin cytoskeleton. Both Rac and Cdc42 colocalize with cadherins at cell membrane and positively regulate adhesive nature of cadherin through activation of p120 catenin (reviewed in [49]). N-cadherin-dependent cell contact negatively regulates the activity of Rac and Cdc42 [50]. Rho, on the other hand, has exactly opposite properties (reviewed in [51]). In addition to their function in cell adhesion, Rho GTPases also take part in cell proliferation. Rho, Rac, and Cdc42 stimulated cell-cycle progression through G1 and subsequent DNA synthesis when they were microinjected into quiescent fibroblasts. However, microinjection of dominant negative forms of Rac and Cdc42 or of the Rho inhibitor C3 transferase blocked serum-induced DNA synthesis [52]. Similarly, the transfection of dominant-negative form of Rac1 into mammalian cells will accumulate cells at G2/M stage [53]. These studies provide evidence of a role for small GTPases in cell proliferation.

Chemokine receptor CXCR4 is probably another common molecule since it is widely expressed in different stem cell populations such as HSCs and developing central nervous system. One of the α -chemokines, CXCL12 or stromal cell-derived factor (SDF-1), specifically binds to CXCR4 and plays important roles in hematopoietic stem/progenitor cell homing (reviewed in [54]). SDF-1 plays important roles in cerebellar development by involving in the movement and proliferation of cerebellar granule cells. Inactivation of CXCR4 gene results in premature migration of external granular layer cells into cerebellum [55], an identical defect seen in SDF-1 mutant mice [56]. Absence of CXCR4 reduces the number of diving cells in the dentate gyrus and causes prematurely differentiated neurons [57]. Expression of CXCR4 was observed in proliferating cells in the retina, olfactory bulb, hippocampus, cerebellum, and spinal cord [55]. SDF-1 regulates the cell proliferation probably through functioning on Sonic hedgehog (Shh) pathway by either enhancing Shh effects directly or driving cells close to the Shh source [58]. Recently Dr. Ratajczak's group detected marker expression for muscle, neural, and liver cells in circulating peripheral blood mononuclear cells as well as CXCR4 expression on their cell membrane [59]. Their data suggest that CXCR4⁺ tissue-committed cells are stem/progenitor cells residing in the bone marrow and could be mobilized into peripheral blood for organ/tissue regeneration.

Cell proliferation and cell-cycle regulation

The molecular mechanisms that regulate cell-cycle progression in eukaryotic cells are being mapped (e.g., see review [60]) and links between pathways of cell-cycle progression/apoptosis/differentiation are being revealed. From this work, it is clear that there are differences in the molecular mechanisms controlling cell division in stem cells (see reviews

[61–65]) and these differences may serve to distinguish stem cells from other populations of cells.

Clear-cut difference between how ES cells and other cells regulate proliferation have emerged. In most somatic cells in eukaryotes, proliferation rate (growth rate) is controlled by the speed at which cells proceed through the G1 phase of the growth cycle with cells spending about 70% of their time moving through G1 (see Fig. 5). Thus, the decision to enter a new round of DNA synthesis is regulated by the inactivation of retinoblastoma protein (Rb) by phosphorylation. The phosphorylation is controlled by D-type cyclins, cyclins D1–D3, and specific cyclin-dependent kinases (Cdks), Cdks 4 and 6. Mitogenic signaling is mediated by active Cdks and cyclin Ds through their action on targets such as Rb [66].

In contrast to most somatic cells, mouse D3 ES cells spend about 75% of their time in the S phase of the cell cycle [63] and it is thought that cell-cycle regulation is not regulated at the G1→S checkpoint because the D cyclins and Cdk4 and 6 are low and cyclin A2, cyclin E1, cyclin D1, cdc2, and Cdk2 are higher than more differentiated but rapidly dividing cells [67,68]. ES cells are thought to be deficient in regulation via cyclin Ds and Cdks 4 and 6 [63,69,70] and thus insensitive to regulation by p16^{Ink4a} [71,72]. All these observations are consistent with the lack of cyclin D-associated Cdks found in primitive embryos at a time when stem cells are the predominant population. Recent work by Faast et al. [72] indicates that cyclin D3 is elevated in mouse D3 ES cells. Their work indicates that the cyclin D3 complexes with Cdk6 to regulate the cell cycle in ES cells via a mechanism independent of inhibition by p16^{Ink4a}. It remains unclear, however, if these differences can be extended to all stem populations and if identifying specific genes will require additional direct comparisons. We have noted, however, low levels of p16 and p21 in NSCs and differences in the regulation of the cell cycle between NSCs and more differentiated cells [73], suggesting that it may be possible to extrapolate to other stem cell populations. In any case, these studies suggest that differences in cell-cycle control will likely exist between stem cells and more differentiated cells and these differences may be common to several stem cell populations and could be included in a list of potential stem cell genes (see Table 5).

Of the different proteins that have been shown to effect self-renewal in the adult, including Wnt, bone morphogenic protein (BMP), Shh, and the notch family, two new candidate genes have been recently identified as important stem cell markers: Bmi-1 and Pten. Lessard and Sauvageau [74] and Park et al. [75] reported that Bmi-1, a transcription repressor, is essential for HSC self-renewal. Park et al. used gene expression profiling to determine that Bmi-1 is highly expressed in HSC populations of humans and mice. When blood-producing cells from Bmi-1-deficient animals is transplanted into wild-type animals, the Bmi-1-deficient HSCs were unable to sustain the precursor pool and in six weeks, the Bmi-1-deficient HSCs were no longer detected [74,75]. This finding supports previous work showing that Bmi-1-deficient mice die of bone marrow failure within two months of birth [76]. The work from Lessard and Sauvageau suggests that Bmi-1 targets p16^{Ink4a} and p19^{Arf}. Recent work from Molofsky et al. reveals that Bmi-1 also plays a role in NSC self-renewal and indicates that p16^{Ink4a} is the important target for regulation in this stem cell system [77]. Pten (phosphatase and tensin homolog deleted from chromosome 10) is a signaling protein that acts mostly upon PIP3 or PI-3K in vivo (reviews by [78,79]). Pten loss is coupled to malignancies and Pten mutations have been detected in many human cancers. Pten-deficient mice have abnormal development patterning in ectodermal and mesodermal germ layers with enhanced proliferation of neuroepithelium and die between days E6.5 and E9.5 [80,81]. Pten affects cell motility/migration, the size of neurons, apoptosis/cell proliferation, and the size of the stem cell population (review by [78,79]). Pten-deficient ES cells form teratomas comprised of undifferentiated and neuronal cells when transplanted into

syngeneic mice. This leads to the conclusion that Pten inactivation enhances the tumorigenic potential of ES cells and alters their ability to differentiate [81]. Much of the work on Pten has focused upon neural cells, but mutations in Pten have also been linked to lymphoid neoplasms [82], suggesting that this protein plays a role in HSCs too. Thus, Pten may be involved in several different stem cell populations.

In summary, work to date indicates that protein markers such as Wnt, notch, BMP, Shh, Bmi-1, and Pten are found in several stem cell populations. As stated previously, hypothetically the themes of regulation of self-renewal (the size of the stem cell population), triggering apoptosis or differentiation, etc., are common to virtually all stem cell populations and thus, the above mentioned markers may emerge as common regulators of cell cycle or differentiation. Future work will confirm this hypothesis by determining in which types of stem cells these markers are expressed.

Symmetric and asymmetric division

A common problem faced by all stem cell populations is determining how to self-renew to both generate the appropriate number of differentiated cells and maintain an adequate pool of self-renewing stem cells (see review by [64,83–85]). Symmetric self-renewing divisions will increase the stem cell pool, while symmetric differentiation divisions will deplete the pool and asymmetric divisions will maintain the stem cell pool. Altering the number of divisions or switching between symmetric and asymmetric divisions will allow stem cells to respond to dramatic fluctuations in requirements of differentiated cells without completely depleting the stem cell pool. Since all stem cell populations that undergo prolonged self-renewal must maintain this balance, it is likely that some of these pathways will be shared among stem cell populations.

There are at least three mechanisms that may interact to regulate self-renewal/asymmetric division (see reviews by [64,84,86]). First, there are genes that are asymmetrically localized in progenitor cells prior to division, e.g., genes that are differentially segregated between daughter cells following mitosis. Second, there are intrinsic genes that regulate self-renewal/asymmetric division, e.g., stem cells can regulate the number of symmetric divisions to undergo prior to differentiation. Third, there are extrinsic signals that can trigger self-renewal/asymmetric division; for example, stem cells replace lost cells during development and loss of blood cells following irradiation [64]. A number of genes have been implicated in these three different mechanisms including Notch, numb, Miranda, tramtrack, Prospero, staufer, piwi, etc. (see Table 6). The intracellular response pathways to these polarity molecules are under investigation. For example, work from Sherley's lab [87] indicates that the p53 anti-oncogene induces asymmetric division through a guanine nucleotide-dependent mechanism. Reviewing the literature suggests that many of the molecules identified in invertebrate systems that regulate self-renewal/asymmetric division are also expressed in multiple stem cell populations in vertebrates. For example, NSCs express several Notch receptors, presenilins, numb, etc. NCSCs, HSCs, and ES cells also express these molecules, suggesting that these may regulate how stem cells divide. However, it is important to note that these are not unique to stem cell populations and may be expressed by other cells as well. Nevertheless, as a component of a list of stemness genes, their expression could confirm the presence of a stem cell in a particular system.

Long-term self-renewal markers

Many stem cell populations need to survive for the life of the organism and thus must undergo many cycles of self-renewal and differentiation. This differs from most differentiated cells, which are in permanent G0 or can undergo only limited proliferation in vivo. The ability to self-renew in vivo correlates with the ability to bypass senescence in

vitro and to bypass senescence throughout the lifespan of the individual in vivo (see reviews by [88–91]). Several pathways that are important to maintaining genomic integrity, bypassing senescence, or prolonging lifespan have been identified. These include regulation of telomere length, high levels of DNA repair enzymes, and regulation of the apoptosis pathways ([92,93]; see reviews by [94,95]). Some major components that have been identified are listed in Table 7.

It is reasonable to assume that some or all of these pathways will be active in most stem cell populations and may serve to distinguish these cells from other differentiating populations. In particular telomerase reverse transcriptase (TERT) expression and telomerase activity appear relatively specific. High TERT levels and telomerase activity have been described in multiple stem cell populations and components of the TERT pathway are present in many stem cells. With a few exceptions TERT expression and telomerase activity are low or absent in most other cells. Likewise we have noted that DNA repair machinery is high in at least two stem cell populations [18].

The corollary of long-term self-renewal is the presence of a surveillance mechanism that will identify damaged cells and target them for apoptosis. Thus, we suggest that stem cells should be sensitive to DNA damage that would direct them to undergo apoptosis. Indeed, as discussed above, most stem cells seem to be much more sensitive to radiation than other cells. After UV damage, for example, ES cells enter the p53-mediated apoptosis pathway [96–98], rather than use the p53-mediated delay at the G1/S transition point like somatic cells [99,100]. This difference probably underlies the difference in radiation sensitivity. It is important to note, however, that this is true for the proliferating stem cell population while the quiescent stem cell is likely much more resistant given the higher expression of repair, antioxidant, and antiapoptotic machinery. Thus radiation resistance, presence of TERT and telomerase activity, and high levels of DNA repair enzymes and the presence of specific antiapoptotic molecules together may help define a stem cell state.

Other candidate “stemness” genes

An important theme that has emerged from developmental biologists is how often a successful strategy is reused in multiple systems. For example, the entire cassette of regulatory genes that appear to regulate oligodendrocyte differentiation also appear to regulate pancreatic islet development [100–103]. A combination of FGF, Shh, Wnt, and BMP proteins appear important in organizing neural development, cardiac differentiation, and limb formation. It is therefore reasonable to assume that a successful strategy of proliferation, self-renewal, and differentiation may be reiterated in multiple stem cell populations and these genes could be candidate “stemness genes.” Given the paucity of data, this is necessarily speculative and we welcome a response from readers.

It is intriguing to note that in most cell populations described, FGF and insulin/IGFs appear important. ES cells, MSCs, HSCs, and NSCs all require FGF to maintain their self-renewal. Likewise, some members of the Hox gene family and the Sox (SRY-related genes containing a HMG box) family of genes are expressed in stem cell populations and are important for their self-renewal. HoxB4, for example, is important for HSC proliferation and overexpression allows cells to proliferate in culture. Sox genes are important in specifying multiple lineages including neural, endodermal, and mesodermal lineages [104–110]. Sox family genes appear to obtain specificity in signaling by interacting with POU homeodomain proteins, and it is likely that in each of these lineages specific POU domain proteins are important (summarized in Table 8).

These markers or genes are downregulated as cells differentiate and can be used to distinguish between stem and differentiated cells. Unlike genes and markers discussed in the

earlier sections, however, it is not the same gene, rather a closely related gene family member expressed, suggesting a similarity rather than identical strategies. Nevertheless, if this were verified in additional stem cell populations, one could perhaps include the presence or absence of members of particular functionally relevant families of genes as markers of the stem cell state.

Overall, we suggest that a large list of molecules common to most if not all stem cell populations can be developed. This list will include LIN⁺ markers, general metabolic markers, markers that reflect the proliferation and self-renewal characteristics of stem cells, and markers that reflect common modalities of interaction with the stem cell niche. No single marker is necessarily unique to stem cells, but a combined list of such molecules will clearly define a stem cell state and one may be reasonably confident that the absence of a significant number of these molecules is inconsistent with the presence of a self-renewing stem cell population. A subset of these markers may be sufficient to allow us to enrich for a stem cell population from any tissue, at any stage of development, and a further analysis of markers may allow us to predict the degree of self-renewal and mode of differentiation.

We would argue that such a set would constitute a molecular signature of a stem cell and developing such a list of “stemness” genes would be of theoretical interest as well as practical use. Building such a list is not technically challenging but does require effort in obtaining and directly comparing many distinct stem cell populations in the same laboratory using a variety of techniques.

Differences among stem cells

Despite predicted similarities discussed above, it is important to reiterate that we are not implying that stem cells are identical. Rather, we suggest that many similarities are predictable based on the underlying commonality of stem cell behavior. However, the same logic also predicts that multiple markers that distinguish between stem cell populations exist. Stem cell responses depend in part on the environmental niche in which they reside. Thus, their initial characteristics after being harvested from tissues will differ. Indeed, this is true as hepatic stem cells or oval cells can be readily distinguished from HSCs, MSCs, and NSCs. We suggest that other major differences will exist in X-chromosome inactivation, methylation patterns, and mitochondrial mutations which accumulate over time. Heterochronic genes that regulate appropriate timing of development will likely be different while telomere lengths are differentially regulated in different tissues (including stem cells associated with them) over time [111,112].

It is also unlikely, in our opinion, that prolonged culture will allow these underlying differences to revert to some fundamental ground stem cell state. This is perhaps best illustrated by the experiments of Verfaillie and colleagues [113–115]. While cells appear to revert to multipotent phenotypes, this reversion is not complete and differences between multipotent adult progenitor cells (MAPC) and ES cells can be identified (Table 9). These differences persist in long-term culture and while some cells acquire expression of Oct3/4, this is seen in only a fraction of the cells, and even this fraction does not appear identical to ES cells in its ability to contribute to the germ line or contribute extensively to chimeras.

Nevertheless, Verfaillie’s results and other results that have suggested transdifferentiation suggest that caution must be exercised in assessing markers predictive of reversion of phenotype. Common markers such as the ones we have described in earlier sections are reflective of common strategies of responding to the environment and are not necessarily predictors of differentiation ability. Thus we would suggest that commonality of markers like rhodamine low, Hoechst low, ABCG2 expression, telomerase activity, commonality in cytokine receptor expression, proliferation, and cell death regulation are merely indicative of

a common repertoire of responses rather than evidence of a common stem cell type. We suggest that markers unique to individual cell types are better predictors of the ability of an individual cell to differentiate into a particular cell type. Even these markers may be shared among stem cell populations that share some commonality of function. For example, both neural crest and ES cells undergo an epithelial-to-mesenchymal transition and thus likely use common strategies and will likely share a larger pool of common markers than ES cells and endodermal stem cells [116]. Likewise, HSCs and MSCs likely share a larger repertoire of common genes given the close lineal origin, but these sets of genes are very likely different from those shared between ES cells and neural crest. Thus, any two stem cell populations are likely to be more similar to each other than to differentiated cells and this similarity likely identifies common approaches to responding to the environment. We suggest that comparing two populations of stem cells is likely to provide insights into overall theme or broad mechanisms, but this type of analysis breaks down when comparisons are generalized across all types of stem cells (as was indicated by [15, 16]).

We end by offering one further caution in comparing stem cell populations. Our comparisons of ES cells across species (mouse D3 and human H1 ES cells) suggest that homologous stem cells isolated from very similar stages of development use different strategies to achieve the same output (see Table 10). Thus mouse ES cells use LIF to self-renew while human ES cells require FGF. HSCs in humans do not express Sca while rodent cells do. Occasionally the same gene may be expressed in the same pattern but different promoter/regulatory events orchestrate its pattern of expression. The promoter regions of some ES cell-specific genes, for example, show little sequence homology though they share similar patterns of expression. Thus, comparison across species should be done with caution as the comparisons may be confounded and suggest that comparisons of different stem cell populations within a single species will be a better strategy to identify common “stemness” genes.

Overall, we conclude that the idea of “stemness” is appealing but that care needs to be utilized in using this term. Stemness genes are genes that are common to one particular type of stem cell in a particular species which make this population unique and distinguishable from other cells. Some of these genes appear to be shared with other stem cell populations and will be a subset of those common to one particular population of stem cells. This subset of “stemness” genes are those that reflect common strategies utilized by particular subsets of stem cells. It is likely that there will be exceptions to their universal nature, i.e., some stem cell populations will not express one or more of these markers or when more subsets of stem cells compared, fewer stemness genes will be unique. So far, few universal markers have been identified by large-scale gene array analysis, and the ones we have discussed using an empirical approach likely represent a large cross-section of potential common stem cell markers. In particular, absence of markers of differentiation will likely be important. Identifying both common and distinct gene patterns in stem cell populations will serve in defining stem cell populations and assessing the differentiation and transdifferentiation ability.

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References

1. Lin H, Schagat T. Neuroblasts: a model for the asymmetric division of stem cells. *Trends Genet.* 1997; 13:33–39. [PubMed: 9009846]
2. Morrison SJ, Shah NM, Anderson DJ. Regulatory mechanisms in stem cell biology. *Cell.* 1997; 88:287–298. [PubMed: 9039255]
3. Burns CE, Zon LI. Portrait of a stem cell. *Dev Cell.* 2002; 3:612–613. [PubMed: 12431368]
4. Murphy M, Bartlett PF. Molecular regulation of neural crest development. *Mol Neurobiol.* 1993; 7:111–135. [PubMed: 8103990]
5. Stanton JA, Macgregor AB, Green DP. Gene expression in the mouse preimplantation embryo. *Reproduction.* 2003; 125:457–468. [PubMed: 12683917]
6. Gage FH. Mammalian neural stem cells. *Science.* 2000; 287:1433–1438. [PubMed: 10688783]
7. Hillyer CD, Wells SJ. Alternative sources of hematopoietic stem cells for bone marrow transplantation and rescue. *J Hematother.* 1993; 2:491–499. [PubMed: 8087497]
8. Palis J, Yoder MC. Yolk-sac hematopoiesis: the first blood cells of mouse and man. *Exp Hematol.* 2001; 29:927–936. [PubMed: 11495698]
9. Broxmeyer HE, Douglas GW, Hangoc G, et al. Human umbilical cord blood as a potential source of transplantable hematopoietic stem/progenitor cells. *Proc Natl Acad Sci U S A.* 1989; 86:3828–3832. [PubMed: 2566997]
10. Gale RP. Fetal liver transplants. *Bone Marrow Transplant.* 1992; 9 (Suppl 1):118–120. [PubMed: 1354519]
11. Becker AJ, McCulloch EA, Till JE. Cytological demonstration of the clonal nature of spleen colonies derived from transplanted mouse marrow cells. *Nature.* 1963; 197:452–454. [PubMed: 13970094]
12. Siminovitch L, McCulloch EA, Till JE. The distribution of colony-forming cells among spleen colonies. *J Cell Comp Physiol.* 1963; 62:327–336.
13. Pevny L, Rao MS. The stem-cell menagerie. *Trends Neurosci.* 2003; 26:351–359. [PubMed: 12850431]
14. Joshi C, Enver T. Molecular complexities of stem cells. *Curr Opin Hematol.* 2003; 10:220–228. [PubMed: 12690290]
15. Ivanova NB, Dimos JT, Schaniel C, Hackney JA, Moore KA, Lemischka IR. A stem cell molecular signature. *Science.* 2002; 298:601–604. [PubMed: 12228721]
16. Ramalho-Santos M, Yoon S, Matsuzaki Y, Mulligan RC, Melton DA. “Stemness”: transcriptional profiling of embryonic and adult stem cells. *Science.* 2002; 298:597–600. [PubMed: 12228720]
17. Fortunel NO, Otu HH, Ng HH, et al. Comment on “‘Stemness’: transcriptional profiling of embryonic and adult stem cells” and “A stem cell molecular signature”. *Science.* 2003; 302:393. author reply 393. [PubMed: 14563990]
18. Ginis I, Luo Y, Miura T, et al. Differences between human and mouse embryonic stem cells. *Dev Biol.* 2004; 269:360–380. [PubMed: 15110706]
19. Sato N, Sanjuan IM, Heke M, Uchida M, Naef F, Brivanlou AH. Molecular signature of human embryonic stem cells and its comparison with the mouse. *Dev Biol.* 2003; 260:404–413. [PubMed: 12921741]
20. Cai J, Wu Y, Mirua T, et al. Properties of a fetal multipotent neural stem cell (NEP cell). *Dev Biol.* 2002; 251:221–240. [PubMed: 12435354]
21. Heimfeld S, Weissman IL. Characterization of several classes of mouse hematopoietic progenitor cells. *Curr Top Microbiol Immunol.* 1992; 177:95–105. [PubMed: 1353436]
22. Deryugina EI, Muller-Sieburg CE. Stromal cells in long-term cultures: keys to the elucidation of hematopoietic development? *Crit Rev Immunol.* 1993; 13:115–150. [PubMed: 8352908]
23. Gupta P, Oegema TR Jr, Brazil JJ, Dudek AZ, Slungaard A, Verfaillie CM. Structurally specific heparan sulfates support primitive human hematopoiesis by formation of a multimolecular stem cell niche. *Blood.* 1998; 92:4641–4651. [PubMed: 9845530]

24. Kim M, Turnquist H, Jackson J, et al. The multidrug resistance transporter ABCG2 (breast cancer resistance protein 1) effluxes Hoechst 33342 and is overexpressed in hematopoietic stem cells. *Clin Cancer Res.* 2002; 8:22–28. [PubMed: 11801536]
25. Bhattacharya S, Jackson JD, Das AV, et al. Direct identification and enrichment of retinal stem cells/progenitors by Hoechst dye efflux assay. *Invest Ophthalmol Vis Sci.* 2003; 44:2764–2773. [PubMed: 12766085]
26. Migishima F, Oikawa A, Kondo S, et al. Full reconstitution of hematopoietic system by murine umbilical cord blood. *Transplantation.* 2003; 75:1820–1826. [PubMed: 12811240]
27. Parmar K, Sauk-Schubert C, Burdick D, Handley M, Mauch P. Sca⁺CD34[−] murine side population cells are highly enriched for primitive stem cells. *Exp Hematol.* 2003; 31:244–250. [PubMed: 12644022]
28. Zhou S, Schuetz JD, Bunting KD, et al. The ABC transporter Bcrp1/ABCG2 is expressed in a wide variety of stem cells and is a molecular determinant of the side-population phenotype. *Nat Med.* 2001; 7:1028–1034. [PubMed: 11533706]
29. Scharenberg CW, Harkey MA, Torok-Storb B. The ABCG2 transporter is an efficient Hoechst 33342 efflux pump and is preferentially expressed by immature human hematopoietic progenitors. *Blood.* 2002; 99:507–512. [PubMed: 11781231]
30. Jones RJ, Barber JP, Vala MS, et al. Assessment of aldehyde dehydrogenase in viable cells. *Blood.* 1995; 85:2742–2746. [PubMed: 7742535]
31. Kastan MB, Schlaffer E, Russo JE, Colvin OM, Civin CI, Hilton J. Direct demonstration of elevated aldehyde dehydrogenase in human hematopoietic progenitor cells. *Blood.* 1990; 75:1947–1950. [PubMed: 2337669]
32. Storms RW, Trujillo AP, Springer JB, et al. Isolation of primitive human hematopoietic progenitors on the basis of aldehyde dehydrogenase activity. *Proc Natl Acad Sci U S A.* 1999; 96:9118–9123. [PubMed: 10430905]
33. Cai J, Cheng A, Luo Y, et al. Membrane properties of rat embryonic multipotent neural stem cells. *J Neurochem.* 2004; 88:212–226. [PubMed: 14675165]
34. Rozental R, Morales M, Mehler MF, et al. Changes in the properties of gap junctions during neuronal differentiation of hippocampal progenitor cells. *J Neurosci.* 1998; 18:1753–1762. [PubMed: 9465000]
35. Alexander DB, Goldberg GS. Transfer of biologically important molecules between cells through gap junction channels. *Curr Med Chem.* 2003; 10:2045–2058. [PubMed: 12871102]
36. Levin M. Isolation and community: a review of the role of gap-junctional communication in embryonic patterning. *J Membr Biol.* 2002; 185:177–192. [PubMed: 11891576]
37. Ploemacher RE, Mayen AE, De Koning AE, Krenacs T, Rosendaal M. Hematopoiesis: Gap junction intercellular communication is likely to be involved in regulation of stroma-dependent proliferation of hemopoietic stem cells. *Hematol.* 2000; 5:133–147.
38. Durig J, Rosenthal C, Halfmeyer K, et al. Intercellular communication between bone marrow stromal cells and CD34⁺ haematopoietic progenitor cells is mediated by connexin 43-type gap junctions. *Br J Haematol.* 2000; 111:416–425. [PubMed: 11122080]
39. Bannerman P, Nichols W, Puhalla S, Oliver T, Berman M, Pleasure D. Early migratory rat neural crest cells express functional gap junctions: evidence that neural crest cell survival requires gap junction function. *J Neurosci Res.* 2000; 61:605–615. [PubMed: 10972957]
40. Zhang M, Thorgeirsson SS. Modulation of connexins during differentiation of oval cells into hepatocytes. *Exp Cell Res.* 1994; 213:37–42. [PubMed: 7517369]
41. Oyamada Y, Komatsu K, Kimura H, Mori M, Oyamada M. Differential regulation of gap junction protein (connexin) genes during cardiomyocytic differentiation of mouse embryonic stem cells in vitro. *Exp Cell Res.* 1996; 229:318–326. [PubMed: 8986615]
42. Reya T, Duncan AW, Ailles L, et al. A role for Wnt signalling in self-renewal of haematopoietic stem cells. *Nature.* 2003; 423:409–414. [PubMed: 12717450]
43. Zechner D, Fujita Y, Hulsken J, et al. β -catenin signals regulate cell growth and the balance between progenitor cell expansion and differentiation in the nervous system. *Dev Biol.* 2003; 258:406–418. [PubMed: 12798297]

44. Song X, Xie T. Wingless signaling regulates the maintenance of ovarian somatic stem cells in *Drosophila*. *Development*. 2003; 130:3259–3268. [PubMed: 12783796]
45. Lobo MV, Alonso FJ, Redondo C, et al. Cellular characterization of epidermal growth factor–expanded free-floating neurospheres. *J Histochem Cytochem*. 2003; 51:89–103. [PubMed: 12502758]
46. Zhang J, Niu C, Ye L, et al. Identification of the haematopoietic stem cell niche and control of the niche size. *Nature*. 2003; 425:836–841. [PubMed: 14574412]
47. Chenn A, Walsh CA. Regulation of cerebral cortical size by control of cell cycle exit in neural precursors. *Science*. 2002; 297:365–369. [PubMed: 12130776]
48. Stockinger A, Eger A, Wolf J, Beug H, Foisner R. E-cadherin regulates cell growth by modulating proliferation-dependent β -catenin transcriptional activity. *J Cell Biol*. 2001; 154:1185–1196. [PubMed: 11564756]
49. Anastasiadis PZ, Reynolds AB. Regulation of Rho GTPases by p120-catenin. *Curr Opin Cell Biol*. 2001; 13:604–610. [PubMed: 11544030]
50. Charrasse S, Meriane M, Comunale F, Blangy A, Gauthier-Rouviere C. N-cadherin-dependent cell-cell contact regulates Rho GTPases and beta-catenin localization in mouse C2C12 myoblasts. *J Cell Biol*. 2002; 158:953–965. [PubMed: 12213839]
51. Wheelock MJ, Johnson KR. Cadherin-mediated cellular signaling. *Curr Opin Cell Biol*. 2003; 15:509–514. [PubMed: 14519384]
52. Olson MF, Ashworth A, Hall A. An essential role for Rho, Rac, and Cdc42 GTPases in cell cycle progression through G1. *Science*. 1995; 269:1270–1272. [PubMed: 7652575]
53. Moore KA, Sethi R, Doanes AM, et al. Rac1 is required for cell proliferation and G2/M progression. *Biochem J*. 1997; 326(Pt 1):17–20. [PubMed: 9337845]
54. Nagasawa T, Tachibana K, Kishimoto T. A novel CXC chemokine PBSF/SDF-1 and its receptor CXCR4: their functions in development, hematopoiesis and HIV infection. *Semin Immunol*. 1998; 10:179–185. [PubMed: 9653044]
55. Zou YR, Kottmann AH, Kuroda M, Taniuchi I, Littman DR. Function of the chemokine receptor CXCR4 in haematopoiesis and in cerebellar development. *Nature*. 1998; 393:595–599. [PubMed: 9634238]
56. Ma Q, Jones D, Borghesani PR, et al. Impaired B-lymphopoiesis, myelopoiesis, and derailed cerebellar neuron migration in CXCR4- and SDF-1-deficient mice. *Proc Natl Acad Sci U S A*. 1998; 95:9448–9453. [PubMed: 9689100]
57. Lu M, Grove EA, Miller RJ. Abnormal development of the hippocampal dentate gyrus in mice lacking the CXCR4 chemokine receptor. *Proc Natl Acad Sci U S A*. 2002; 99:7090–7095. [PubMed: 11983855]
58. Klein RS, Rubin JB, Gibson HD, et al. SDF-1 α induces chemotaxis and enhances Sonic hedgehog-induced proliferation of cerebellar granule cells. *Development*. 2001; 128:1971–1981. [PubMed: 11493520]
59. Ratajczak MZ, Kucia M, Reza R, Majka M, Janowska-Wieczorek A, Ratajczak J. Stem cell plasticity revisited: CXCR4-positive cells expressing mRNA for early muscle, liver and neural cells “hide out” in the bone marrow. *Leukemia*. 2004; 18:29–40. [PubMed: 14586476]
60. Krylov DM, Nasmyth K, Koonin EV. Evolution of eukaryotic cell cycle regulation: stepwise addition of regulatory kinases and late advent of the CDKs. *Curr Biol*. 2003; 13:173–177. [PubMed: 12546794]
61. Ohnuma S, Harris WA. Neurogenesis and the cell cycle. *Neuron*. 2003; 40:199–208. [PubMed: 14556704]
62. D’Urso, G.; Datta, S. Cell cycle control, checkpoints, and stem cell biology. In: Marshak, DR.; Gardner, RL.; Gottlieb, D., editors. *Stem Cell Biology*. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press; 2001. p. 61–94.
63. Savatier P, Lapillonne H, Jirmanova L, Vitelli L, Samarut J. Analysis of the cell cycle in mouse embryonic stem cells. *Methods Mol Biol*. 2002; 185:27–33. [PubMed: 11768996]
64. Sommer L, Rao M. Neural stem cells and regulation of cell number. *Prog Neurobiol*. 2002; 66:1–18. [PubMed: 11897403]

65. Yoshikawa K. Cell cycle regulators in neural stem cells and postmitotic neurons. *Neurosci Res.* 2000; 37:1–14. [PubMed: 10802339]
66. Sherr CJ, Roberts JM. CDK inhibitors: positive and negative regulators of G1-phase progression. *Genes Dev.* 1999; 13:1501–1512. [PubMed: 10385618]
67. Stead E, White J, Faast R, et al. Pluripotent cell division cycles are driven by ectopic Cdk2, cyclin A/E and E2F activities. *Oncogene.* 2002; 21:8320–8333. [PubMed: 12447695]
68. Ohnuma S, Hopper S, Wang KC, Philpott A, Harris WA. Co-ordinating retinal histogenesis: early cell cycle exit enhances early cell fate determination in the *Xenopus* retina. *Development.* 2002; 129:2435–2446. [PubMed: 11973275]
69. Jirmanova L, Afanassieff M, Gobert-Gosse S, Markossian S, Savatier P. Differential contributions of ERK and PI3-kinase to the regulation of cyclin D1 expression and to the control of the G1/S transition in mouse embryonic stem cells. *Oncogene.* 2002; 21:5515–5528. [PubMed: 12165850]
70. Burdon T, Smith A, Savatier P. Signalling, cell cycle and pluripotency in embryonic stem cells. *Trends Cell Biol.* 2002; 12:432–438. [PubMed: 12220864]
71. Savatier P, Lapillonne H, van Grunsven LA, Rudkin BB, Samarut J. Withdrawal of differentiation inhibitory activity/leukemia inhibitory factor up-regulates D-type cyclins and cyclin-dependent kinase inhibitors in mouse embryonic stem cells. *Oncogene.* 1996; 12:309–322. [PubMed: 8570208]
72. Faast R, White J, Cartwright P, Crocker L, Sarcevic B, Dalton S. Cdk6-cyclin D3 activity in murine ES cells is resistant to inhibition by p16(INK4a). *Oncogene.* 2004; 23:491–502. [PubMed: 14724578]
73. Luo Y, Cai J, Liu Y, et al. Microarray analysis of selected genes in neural stem and progenitor cells. *J Neurochem.* 2002; 83:1481–1497. [PubMed: 12472902]
74. Lessard J, Sauvageau G. Bmi-1 determines the proliferative capacity of normal and leukaemic stem cells. *Nature.* 2003; 423:255–260. [PubMed: 12714970]
75. Park IK, Qian D, Kiel M, et al. Bmi-1 is required for maintenance of adult self-renewing haematopoietic stem cells. *Nature.* 2003; 423:302–305. [PubMed: 12714971]
76. van der Lugt NM, Domen J, Linders K, et al. Posterior transformation, neurological abnormalities, and severe hematopoietic defects in mice with a targeted deletion of the bmi-1 proto-oncogene. *Genes Dev.* 1994; 8:757–769. [PubMed: 7926765]
77. Molofsky AV, Pardal R, Iwashita T, Park IK, Clarke MF, Morrison SJ. Bmi-1 dependence distinguishes neural stem cell self-renewal from progenitor proliferation. *Nature.* 2003; 425:962–967. [PubMed: 14574365]
78. Morrison SJ. Pten-uating neural growth. *Nat Med.* 2002; 8:16–18. [PubMed: 11786896]
79. Li L, Liu F, Ross AH. PTEN regulation of neural development and CNS stem cells. *J Cell Biochem.* 2003; 88:24–28. [PubMed: 12461771]
80. Suzuki A, de la Pompa JL, Stambolic V, et al. High cancer susceptibility and embryonic lethality associated with mutation of the PTEN tumor suppressor gene in mice. *Curr Biol.* 1998; 8:1169–1178. [PubMed: 9799734]
81. Di Cristofano A, Pesce B, Cordon-Cardo C, Pandolfi PP. Pten is essential for embryonic development and tumour suppression. *Nat Genet.* 1998; 19:348–355. [PubMed: 9697695]
82. Sakai A, Thieblemont C, Wellmann A, Jaffe ES, Raffeld M. PTEN gene alterations in lymphoid neoplasms. *Blood.* 1998; 92:3410–3415. [PubMed: 9787181]
83. Lin H. The tao of stem cells in the germline. *Annu Rev Genet.* 1997; 31:455–491. [PubMed: 9442904]
84. Lu B, Jan L, Jan YN. Control of cell divisions in the nervous system: symmetry and asymmetry. *Annu Rev Neurosci.* 2000; 23:531–556. [PubMed: 10845074]
85. Anderson DJ. Stem cells and pattern formation in the nervous system: the possible vs the actual. *Neuron.* 2001; 30:19–35. [PubMed: 11343642]
86. Steidl U, Kronenwett R, Martin S, Haas R. Molecular biology of hematopoietic stem cells. *Vitam Horm.* 2003; 66:1–28. [PubMed: 12852251]

87. Sherley JL, Stadler PB, Johnson DR. Expression of the wild-type p53 antioncogene induces guanine nucleotide-dependent stem cell division kinetics. *Proc Natl Acad Sci U S A*. 1995; 92:136–140. [PubMed: 7816803]
88. Rubin H. The disparity between human cell senescence in vitro and lifelong replication in vivo. *Nat Biotechnol*. 2002; 20:675–681. [PubMed: 12089551]
89. Yaswen P, Stampfer MR. Molecular changes accompanying senescence and immortalization of cultured human mammary epithelial cells. *Int J Biochem Cell Biol*. 2002; 34:1382–1394. [PubMed: 12200033]
90. Bree RT, Stenson-Cox C, Greal M, Byrnes L, Gorman AM, Samali A. Cellular longevity: role of apoptosis and replicative senescence. *Biogerontology*. 2002; 3:195–206. [PubMed: 12232501]
91. Rao MS, Mattson MP. Stem cells and aging: expanding the possibilities. *Mech Ageing Dev*. 2001; 122:713–734. [PubMed: 11322994]
92. Van Sloun PP, Jansen JG, Weeda G, et al. The role of nucleotide excision repair in protecting embryonic stem cells from genotoxic effects of UV-induced DNA damage. *Nucleic Acids Res*. 1999; 27:3276–3282. [PubMed: 10454634]
93. Smith LL, Collier HA, Roberts JM. Telomerase modulates expression of growth-controlling genes and enhances cell proliferation. *Nat Cell Biol*. 2003; 5:474–479. [PubMed: 12717449]
94. Prost S, Bellamy CO, Clarke AR, Wyllie AH, Harrison DJ. p53-independent DNA repair and cell cycle arrest in embryonic stem cells. *FEBS Lett*. 1998; 425:499–504. [PubMed: 9563521]
95. Gorbunova V, Seluanov A, Pereira-Smith OM. Expression of human telomerase (hTERT) does not prevent stress-induced senescence in normal human fibroblasts but protects the cells from stress-induced apoptosis and necrosis. *J Biol Chem*. 2002; 277:38540–38549. [PubMed: 12140282]
96. Corbet SW, Clarke AR, Gledhill S, Wyllie AH. p53-dependent and -independent links between DNA-damage, apoptosis and mutation frequency in ES cells. *Oncogene*. 1999; 18:1537–1544. [PubMed: 10102623]
97. Sabapathy K, Klemm M, Jaenisch R, Wagner EF. Regulation of ES cell differentiation by functional and conformational modulation of p53. *EMBO J*. 1997; 16:6217–6229. [PubMed: 9321401]
98. Ziegler A, Jonason AS, Leffell DJ, et al. Sunburn and p53 in the onset of skin cancer. *Nature*. 1994; 372:773–776. [PubMed: 7997263]
99. Aladjem MI, Spike BT, Rodewald LW, et al. ES cells do not activate p53-dependent stress responses and undergo p53-independent apoptosis in response to DNA damage. *Curr Biol*. 1998; 8:145–155. [PubMed: 9443911]
100. Lee JC, Mayer-Proschel M, Rao MS. Gliogenesis in the central nervous system. *Glia*. 2000; 30:105–121. [PubMed: 10719353]
101. Campagnoni CW, Landry CF, Pribyl TM, et al. Identification of genes in the oligodendrocyte lineage through the analysis of conditionally immortalized cell lines. *Dev Neurosci*. 2001; 23:452–463. [PubMed: 11872946]
102. Germain M, Shore GC. Cellular distribution of *bcl-2* family proteins. *Sci STKE*. 2003;10.
103. Norgaard GA, Jensen JN, Jensen J. FGF10 signaling maintains the pancreatic progenitor cell state revealing a novel role of Notch in organ development. *Dev Biol*. 2003; 264:323–338. [PubMed: 14651921]
104. Prior HM, Walter MA. SOX genes: architects of development. *Mol Med*. 1996; 2:405–412. [PubMed: 8827711]
105. Pevny LH, Lovell-Badge R. Sox genes find their feet. *Curr Opin Genet Dev*. 1997; 7:338–344. [PubMed: 9229109]
106. Azuma T, Seki N, Yoshikawa T, Saito T, Masuho Y, Muramatsu M. cDNA cloning, tissue expression, and chromosome mapping of human homolog of SOX18. *J Hum Genet*. 2000; 45:192–195. [PubMed: 10807548]
107. Cheung M, Abu-Elmagd M, Clevers H, Scotting PJ. Roles of Sox4 in central nervous system development. *Brain Res Mol Brain Res*. 2000; 79:180–191. [PubMed: 10925158]
108. Takash W, Canizares J, Bonneaud N, et al. SOX7 transcription factor: sequence, chromosomal localisation, expression, transactivation and interference with Wnt signalling. *Nucleic Acids Res*. 2001; 29:4274–4283. [PubMed: 11691915]

109. Katoh M. Expression of human SOX7 in normal tissues and tumors. *Int J Mol Med*. 2002; 9:363–368. [PubMed: 11891528]
110. Saitoh T, Katoh M. Expression of human SOX18 in normal tissues and tumors. *Int J Mol Med*. 2002; 10:339–344. [PubMed: 12165811]
111. Friedrich U, Griesse E, Schwab M, Fritz P, Thon K, Klotz U. Telomere length in different tissues of elderly patients. *Mech Ageing Dev*. 2000; 119:89–99. [PubMed: 11080530]
112. Nakamura K, Izumiyama-Shimomura N, Sawabe M, et al. Comparative analysis of telomere lengths and erosion with age in human epidermis and lingual epithelium. *J Invest Dermatol*. 2002; 119:1014–1019. [PubMed: 12445186]
113. Jiang Y, Vaessen B, Lenvik T, Blackstad M, Reyes M, Verfaillie CM. Multipotent progenitor cells can be isolated from postnatal murine bone marrow, muscle, and brain. *Exp Hematol*. 2002; 30:896–904. [PubMed: 12160841]
114. Jiang Y, Jahagirdar BN, Reinhardt RL, et al. Pluripotency of mesenchymal stem cells derived from adult marrow. *Nature*. 2002; 418:41–49. [PubMed: 12077603]
115. Reyes M, Lund T, Lenvik T, Aguiar D, Koodie L, Verfaillie CM. Purification and ex vivo expansion of postnatal human marrow mesodermal progenitor cells. *Blood*. 2001; 98:2615–2625. [PubMed: 11675329]
116. Tucker RP. Neural crest cells: a model for invasive behavior. *Int J Biochem Cell Biol*. 2004; 36:173–177. [PubMed: 14643882]
117. Orkin SH, Morrison SJ. Stem-cell competition. *Nature*. 2002; 418:25–27. [PubMed: 12097889]
118. Brandenberger R, Wei H, Zhang S, et al. Transcriptome characterization elucidates signaling networks that control human ES cell growth and differentiation. *Nat Biotechnol*. In press.

Comparison of Stem Cells

Figure 1. Comparing stem cell common genes with microarray analysis by different groups. Three independent studies revealed only one gene is common among three stem cell populations: ESC, NSC, and HSC.

Figure 2.

Possible stem cell division pathways. Stem cells can remain quiescent or enter cell cycling in three ways: asymmetric division gives rise to a stem cell daughter and a precursor daughter; two kinds of symmetric division will generate twin daughter cells of either stem cell or progenitor properties.

Figure 3.

Isolating neural stem cells from negative selection of lineage markers. (A,B): Rat E14.5 spinal cord neural cells were stained with anti-NCAM and A2B5 and sorted on a fluorescence-activated cell sorting machine. A2B5⁻/NCAM⁻ (Lin⁻) cells were collected from M1 gate and applied for clonal analysis. (C): A sample of Lin⁻ clones was induced to differentiate and triple-labeled with three lineage markers: GalC (green), βIII tubulin (red), and GFAP (blue) after culture of 24 days.

Figure 4. One sample of human mobilized peripheral blood stem/progenitor cells positive for the green ALDH reaction product and Hoechst 33342 nuclear stain (63 $\times$). Human mobilized peripheral blood cells were stained with Aldefluor, a fluorescent substrate of ALDH. ALDH⁺ cells were sorted and comprised 0.5% of the live cells as gated by FSC and SSC. The confocal microscopy was done by Dr. Sandra Foster of StemCo Biomedical and Dr. Robert Zucker of the US Environmental Protection Agency. (Photo courtesy StemCo Biomedical Inc.)

Cell cycle regulation in stem cells

Figure 5.

Cell cycle regulation of stem cells. The cell cycle in somatic cells is controlled by the G1/S checkpoint. In contrast, ES cells move rapidly through the cell cycle and are regulated at the S/G2 checkpoint. Once ES cells differentiate, the cell cycle rate slows and is regulated like that of somatic cells. One notable exception is that most somatic cells delay at the G1/S checkpoint in response to DNA damage to effect repair. In contrast, stem cell populations are very sensitive to UV damage and enter apoptosis. Signals that trigger self-renewal divisions vs asymmetric divisions also affect cell-cycle regulation. Some of these differentiation signals are intrinsic via polar segregation of genes during mitosis or via expression of asymmetry genes. The linkage between cell-cycle control and self-renewal/differentiation is not well understood. In stem cells, the cell-cycle controls that delay progression of cycle vs enter apoptosis are weighted towards apoptosis. As stem cells differentiate to more specialized cells, the weighting shifts toward cell-cycle delay/DNA repair. Depending upon the tissue, stem cells can remain quiescent in G1- or G0-like state for long periods and then may be reactivated. Growth factors/mitogens affect stem cells in vitro and may play a role in reactivation in vivo. In other tissues, stem cells are continuously active (testes, hematopoietic system, skin, olfactory sensory epithelium). In other tissues, most stem cells are quiescent throughout life (nervous system, ovary). The long-term control mechanisms are not well known.

Table 1

Tissue-specific stem cells

Ectoderm

Epidermal stem cells

Neural stem cells

Neural crest stem cells

Hair follicle stem cells

Mesoderm

Cardiac muscle stem cells

Skeletal muscle stem cells

Umbilical cord blood stem cells

Mesenchymal stem cells

Hematopoietic stem cells

Umbilical cord matrix stem cells

Multipotent adult precursor cells

Endoderm

Pancreatic islet stem cells

Hepatic oval stem cells

Germ cellsPrimordial germ cells

Table 2

Absence of lineage markers (Samples of lineage-negative markers that can be used to isolate different populations of stem cells are listed)

Neural stem cells	Neuron	N-CAM, β -III tubulin
	Astrocyte	A2B5, CD44, S100 β
	Oligodendrocyte	GalC, O4
Hematopoietic stem cells	Erythroid	TER-119, CD71
	B cell	B220, CD10, CD19, CD20, CD56
	Granulocyte	Gr-1
	T cell	CD3, CD4, CD8
Embryonic stem cells	Trophoectoderm	Mash2
	Endoderm	Actc1,
	Mesoderm	Brachyury, Myf5, Pdx1, HNF3b
	Ectoderm	Msx1, Islet1, Krt1-15
Mesenchymal stem cells	Adipocyte	Lipoprotein lipase and peroxisome proliferator-activated receptor gamma2
	Chondrocyte	Collagen types II, IX and X, and aggrecan
	Osteoblast	Alkaline phosphatase, collagen type I, osteopontin and osteocalcin

General metabolic markers

Table 3

Marker	ES	NSC	HSC	MSC	Cord blood stem cell	MAPC	Pancreatic stem cell
Growth factor requirement	LIF, FGF	EGF, FGF	FGF	FGF	EGF, FGF	EGF, LIF, PDGFBB	FGF
ALDH activity	?	+	+	+	?	?	?
Hoescht 33342/SP	?	+	+	+	?	?	+
ABCG-2 expression	+	+	+	+	+	?	+
Rhodamine 123 exclusion	?	-	+	?	+	?	?
Connexin expression	+	+	+	+	?	?	?
Unique set of markers	+	+	+	+	+	+	+
Lack of lineage markers (Lin ⁻)	+	+	+	+	+	+	+
Potential markers							
FGFR	+	+	+	+	+	?	+
TERT/Telomerase activity	+	+	+	+	+/-	?	?
Wnt pathway	+	+	+	+	?	?	+
Sox expression	+	+	+	?	?	?	+
Notch pathway	+	+	+	+	+	?	+

Table 4

Communication markers

Marker	ES	NSC	MSC	HSC
Connexin 43	+	+/-	?	?
Connexin 45	+	+	+	?
Functional gap junctions (dye coupling)	+	+	+	+
Cadherin and catenin	+	+	+	+
Rho family (Rho, Rac, Cdc42)	+			
CXCR4	+	+		+
Shh	+			

Table 5

Cell-cycle-related pathways

Pathways	Comments
Intrinsic and extrinsic signaling	Growth factors, cytokines, etc. can regulate stem cell number and trigger expansion of the stem cell pool/production of progenitors.
DNA repair and apoptosis pathways	Stem cells resist DNA damage due to upregulation of DNA repair pathways. When DNA damage is detected, e.g., UV radiation, ES cells undergo apoptosis rather than delay at the G1/S checkpoint.
Regulating cell cycle	ES cells are regulated at the S/G2 checkpoint, somatic cells and differentiating cells are regulated at G1/S checkpoint.
Symmetric/asymmetric division	The controls of differentiation vs cell growth (symmetric division) are not well known. Factors such as piwi, Pten, Bmi-1, Notch, etc. have been identified as playing a role.

Table 6List of *Drosophila* genes regulating symmetric and asymmetric divisions

		Genes	Possible functions
Symmetric division	NSC	Notch	Maintenance of neural stem cell
		Presenilin	
	GSC	Piwi	Maintain the germline stem cell population and control the cell division rate
	SSC	Hedgehog	Required for proliferation of somatic stem cells
Asymmetric division	Both GSC and SSC	Yb	Upregulator of Piwi and hedgehog
	Protein determinants	Numb	Basal proteins to direct localization to the apical cell cortex of neuroblast
		Miranda	
		Pon	
		Tramtrack	
		Prospero	
		Staufen	
		Par-6	
		Bazooka	
		aPKC	
	Transcription factors	Snail	Regulating genes involved in asymmetry and cell division of neuroblasts
		Worniu	
		Escargot	

Table 7**Pathways regulating senescence**

Pathways	Comments
Maintaining telomere length	Telomerase activity tends to be high in stem cell populations and it is likely that specific components of this pathway will be high in stem cell populations.
DNA repair and apoptosis pathways	Stem cells are radiation sensitive but often resistant to other apoptotic signals.
Regulating cell cycle	The ability to undergo prolonged periods of quiescence and then reenter the cell cycle suggests that cell-cycle regulation will differ from most other cell populations.
Mitochondrial and oxidative stress pathways	Prolonged lifespan requires special mechanisms to regulate mitochondrial stability and response to oxidative stress.
Immortality genes	Genes that prolong lifespan or bypass senescence have been identified. These include Mortalins, and MORF's.
Igf/akt/PTEN	The igf/akt/PTEN pathway has been shown to be important in regulating cell size and proliferation as well as lifespan. It is likely that components of this pathway will be shared by stem cell populations.

Several pathways that regulate prolonged self-renewal have been identified. These include the telomerase pathway required to maintain intact telomeres, the p53/the rb pathway, the igf/akt/PTEN pathway, the mitochondrial/oxidative stress pathway. In addition, immortality-associated genes have been identified. It is likely that cells such as stem cells which need to self-renew throughout the lifespan of the individual will express these genes.

Table 8

Common growth factors and transcription factors

Potentially Shared Markers
FGFs
Shh
Wnts
BMPs
Insulin/IGF family members
Hox-gene family members
SRY family/SOX family members
Notch signaling-related molecules

Table 9

ES cells vs MAPCs

Property	ES cells	MAPCs
Growth potential	indefinite	indefinite
Contributes to germ cells?	yes	?
Common growth factors	yes (mouse)	yes (mouse)
Differentiates to most cell types	yes	yes (lower efficiency)
Expression of telomerase	yes	?
Expression of Oct-4	yes	low levels
Expression of Rex-1	yes	yes
Expression of SSEA-1	yes	yes (subset)
MAPCs lineage markers	absent	low levels Flk-1, Flt-1, AC133, CD44, CDw90, KDR, B2-microglobulin, higher levels CD13, CD49b absent
ES cell lineage markers	Aldehyde dehydrogenase, Forward/side scatter/SP, Hoescht/rhodamine, alkaline phosphatase, CXCR-4, ABCG-2 transporter, SOX-2, UTF-1, FoxD3, FGFR-4, connexins, glucose transporter GLUT1, Mash2/Hash2, eomesodermin, TRA-1-81/TRA-1-60	

Modified from [117].

Table 10

Distinguishing features of mouse and human ES cells: Major differences between mouse and human ES cells based on work from our laboratory ([18,118] are summarized)

Marker	Mouse	Human
Morphology	More diverse	Rounded with sharp boundaries
SSEA1	Present	Absent
SSEA4	Absent	Present
Vimentin	Absent	Present
Trophoectoderm markers	Absent	Present
β -III tubulin	Present	β -5 tubulin present
LIFR	High	Low/variable
Gp130	High	Low/variable
FGF4	High	Absent/undetectable
HRASP	Required	pseudogene
E-hox	Required	No orthologue present
Fox-D3	Present/required	Low/absent