



In search of “stemness”

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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. © 2004 International Society for Experimental Hematology. Published by Elsevier Inc.

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 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 in 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

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Table 1. Tissue-specific stem cells

79	Ectoderm
80	Epidermal stem cells
81	Neural stem cells
82	Neural crest stem cells
83	Hair follicle stem cells
84	Mesoderm
85	Cardiac muscle stem cells
86	Skeletal muscle stem cells
87	Umbilical cord blood stem cells
88	Mesenchymal stem cells
89	Hematopoietic stem cells
90	Umbilical cord matrix stem cells
91	Multipotent adult precursor cells
92	Endoderm
93	Pancreatic islet stem cells
94	Hepatic oval stem cells
95	Germ cells
96	Primordial germ cells

97 studies have suggested that these differences may not be
98 as significant as once thought and indeed may not be signifi-
99 cant even among stem cells harvested from different tissues.
100 Stem cells may be quite plastic and may be able to respond
101 to environmental cues to alter their characteristics and mimic
102 the properties and responses of a stem cell from any particu-
103 lar tissue. Thus HSCs could transdifferentiate to generate
104 neural tissue and vice versa, and numerous studies—though
105 not without controversy—have suggested that this is a
106 common event.

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

119 The failure to identify stemness genes likely reflects on
120 the technical difficulties of the experiments rather than their
121 absence. In general, it has been difficult to obtain pure popu-
122 lations of stem cells. Furthermore, stem cells undergo sto-
123 chastic changes when maintained in culture and during
124 development in vivo. Species differences in the expression
125 of stem cell characteristics have been defined [18,19]. Com-
126 parisons therefore require isolating pure cells, with minimal
127 culturing, at a defined state of development in sufficient
128 quantities such that comparative methods can be performed
129 with sufficient rigor to overcome the variability inherent in
130 the comparison techniques itself.

131 Given the paucity of data and the difficulty in performing
132 comparative experiments, we have in this review taken an

Comparison of Stem Cells

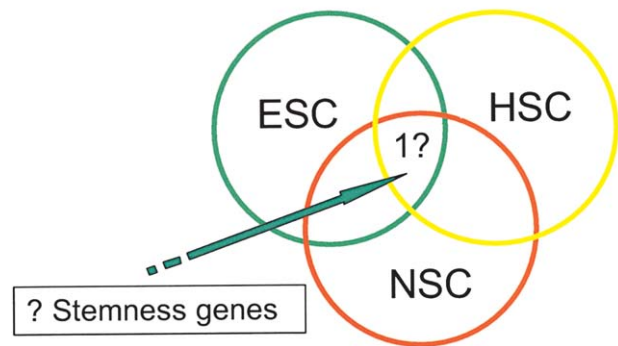
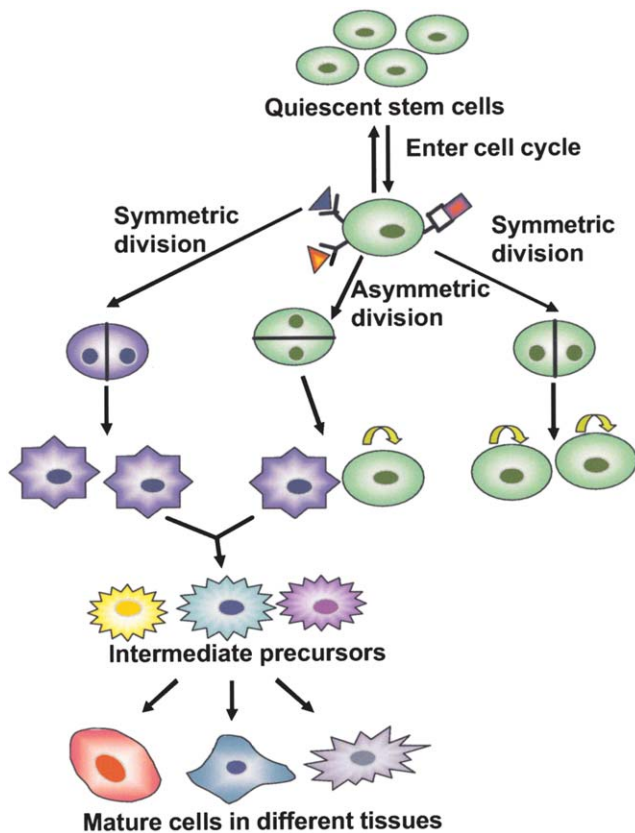


Figure 1. Comparing stem cell common genes with microarray analysis
by different groups. Three independent studies revealed only one gene
is common among three stem cell populations: ESC, NSC, and HSC.

136 alternative approach to attempt to identify “stemness” genes.
137 We have reasoned that stem cells, irrespective of the tissue
138 where they are harvested and their differentiation ability,
139 face the same set of challenges. All stem cells need to
140 regulate their cell cycle to either undergo periods of rapid
141 division—maintain self-renewal, or enter periods of quies-
142 cence, or (in the case of stem cells that last the lifetime of
143 an individual) avoid senescence. Stem cells, in addition, have
144 to maintain genomic integrity to avoid the accumulation of
145 genetic mutations and neoplastic transformation. Stem cells
146 have to make a choice between self-renewal and differentia-
147 tion, and have to respond to the extrinsic environment using
148 growth factor receptors/extracellular matrix (ECM) mole-
149 cules/gap junctional communication. Thus, cell-cycle con-
150 trol, asymmetry/differentiation controls, cellular protective
151 and DNA repair mechanisms, and associated apoptosis/
152 senescence signaling pathways are expected to be more
153 highly regulated in stem cells, possibly by similar mecha-
154 nisms (Fig. 2). We further reason that the same properties
155 that are common among stem cell populations will distin-
156 guish stem cells from progenitor cell populations. One would
157 expect, for example, that regulation of senescence pathways,
158 conservation of DNA repair mechanisms, and regulation of
159 symmetric and asymmetric division may not be as critical
160 for progenitor cell populations as in stem cells. In subsequent
161 sections we describe candidate stemness genes based on
162 this logic.

Absence of markers

163 Perhaps one distinguishing characteristic of stem cells is the
164 absence of markers of differentiation. The absence of mark-
165 ers characteristic of differentiated cells has been used quite
166 successfully to define and enrich for stem cells to be used
167 for therapy (Table 2). In the HSC field this long list of
168 markers has been concatenated as LIN⁻ (lineage-negative
169



170 **Figure 2.** Possible stem cell division pathways. Stem cells can remain
 171 quiescent or enter cell cycling in three ways: asymmetric division gives
 172 rise to a stem cell daughter and a precursor daughter; two kinds of symmetric
 173 division will generate twin daughter cells of either stem cell or progenitor
 174 properties.

175 cells). The absence of lineage markers has been used to
 176 define other stem cell populations as well. Mesenchymal
 177 stem cells (MSCs) are LIN^- for osteoblast, chondrocyte,
 178 and muscle markers; neural stem cells (NSCs) are negative for
 179 astrocyte, oligodendrocyte, and neuronal markers; NCSCs
 180 are negative for Schwann cell, peripheral neuron, and mesen-

chymal markers; and ES cells are LIN^- for ectoderm, endo- 196
 197 derm, and mesoderm markers. As with HSCs, the absence of
 198 markers can be used to select NSCs. For example, the absence
 199 of A2B5, NCAM, CD44, and CD24 can be used to enrich
 200 for NSCs ([20], and personal results). Equally important,
 201 the NSC population harvested by LIN^- selection is identical
 202 to the population harvested by positive selection or by
 203 selection with general metabolic markers [20].

204 Thus, LIN^- is a common property of many stem cell
 205 populations. Perhaps we have been remiss in ignoring mark-
 206 ers that are high in most differentiated cells but low or absent
 207 in stem cells that could be used to define a stem cell state.
 208 *Thy1* is one such candidate molecule. It is relatively abun-
 209 dant in many tissues but is low or absent on stem cell
 210 populations [21]. Other candidate molecules are receptors
 211 for ECM interaction. ECM molecules expressed by stromal
 212 cells in the HSC niche [22] help to anchor growth factors
 213 and other regulators for stem cell proliferation and differen-
 214 tiation [23].

215 These LIN^- markers would not have been picked up in
 216 the analysis performed so far with direct comparisons of
 217 genes that are common among stem cells. However, we
 218 would predict that pair-wise comparisons performed
 219 between stem cells and differentiated cells (pooled universal
 220 RNA perhaps) would identify such LIN^- genes. Markers
 221 that are low or absent in stem cells but abundant in differen-
 222 tated cells could be identified and a pooled set that are absent
 223 in all stem cell populations but present in most differentiated
 224 cells could be developed. Absence of such markers would
 225 allow one to identify stem cell populations. Therefore, line-
 226 age negativity should be considered a distinguishing prop-
 227 erty of stem cells (Fig. 3).

228 Presence of general metabolic markers

229 As suggested earlier, it is likely that cells from different
 230 tissues use similar strategies to maintain their stem cell
 231 state and to regulate proliferation and differentiation. These
 232 strategies are likely to be unique to stem cell populations

181 **Table 2.** Absence of lineage markers (Samples of lineage-negative markers that can be used to isolate different populations of stem cells are listed)

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

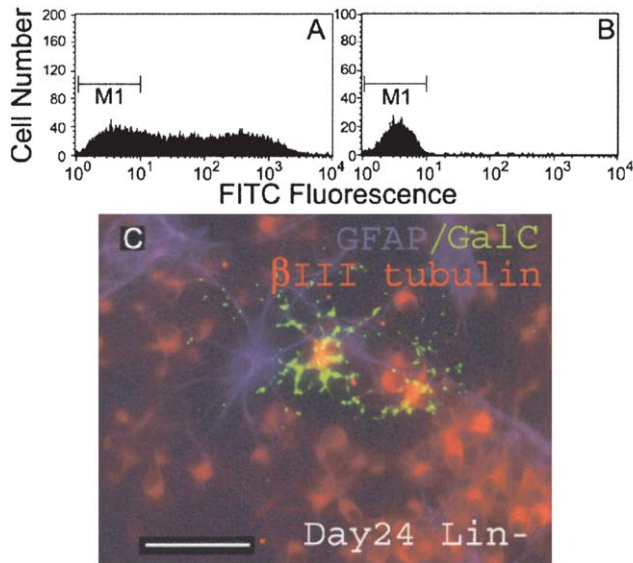


Figure 3. Isolating neural stem cells from negative selection of lineage markers. (A,B): Rat E14.5 spinal cord neural cells were stained with anti-NCAM and A2B5 and sorted on a fluorescence-activated cell sorting machine. A2B5⁻/NCAM⁻ (Lin⁻) cells were collected from M1 gate and applied for clonal analysis. (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.

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].

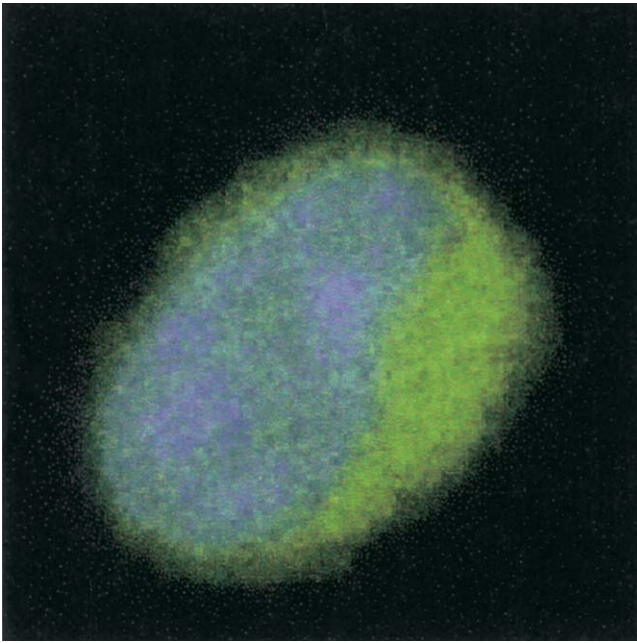
It is important to note, however, that these properties depend on the state of the cell. We have noted that rhodamine and Hoechst labeling cannot be used to select actively dividing NSC populations such as embryonic NSCs; rather, they identify quiescent populations of self-renewing progenitors [20]. 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.

Table 3. General metabolic markers

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							
FGFR	+	+	+	+	+	?	+
TERT/Telomerase activity	+	+	+	+	+/-	?	?
Wnt pathway	+	+	+	+	?	?	+
Sox expression	+	+	+	?	?	?	+
Notch pathway	+	+	+	+	+	?	+



307 **Figure 4.** One sample of human mobilized peripheral blood stem/progeni-
 308 tor cells positive for the green ALDH reaction product and Hoechst 33342
 309 nuclear stain (63×). Human mobilized peripheral blood cells were stained
 310 with Aldefluor, a fluorescent substrate of ALDH. ALDH⁺ cells were
 311 sorted and comprised 0.5% of the live cells as gated by FSC and SSC.
 312 The confocal microscopy was done by Dr. Sandra Foster of StemCo Bio-
 313 medical and Dr. Robert Zucker of the US Environmental Protection Agency.
 314 (Photo courtesy StemCo Biomedical Inc.)

315 Communication

316 Stem cells are abundant in early development when organ
 317 generation is occurring. At this stage, most signaling path-
 318 ways are still developing and the circulatory system is still
 319 incomplete. Nevertheless, cell populations must respond in
 320 concert to regulate overall cell number and organ size, and
 321 some communication must exist to ensure that appropriate
 322 feedback loops are present (summarized in Table 4). Gap
 323 junctions represent one such communication pathway.
 324 Indeed, gap junctions appear during early development and
 325 their expression is reduced as other signaling mechanisms
 326 come into play [34]. Gap junctions allow direct cytoplasmic
 327 exchange of small hydrophilic molecules between two adja-
 328 cent cells (reviewed in [35]), and they play an important role
 329 in many normal developmental processes such as embryonic

330 **Table 4.** Communication markers

331 Marker	ES	NSC	MSC	HSC
332 Connexin 43	+	+/-	?	?
333 Connexin 45	+	+	+	?
334 Functional gap junctions (dye coupling)	+	+	+	+
335 Cadherin and catenin	+	+	+	+
336 Rho family (Rho, Rac, Cdc42)	+			
337 CXCR4	+	+		+
338 Shh	+			

339 patterning, as well as in tumor progression (reviewed in
 340 [36]).

341 We and others have examined the expression of gap junc-
 342 tions on stem cells and have found that gap junctions are
 343 present on ES cells [18], NSCs [20,34], HSCs [37,38],
 344 NCSCs [39], and hepatic oval cells [40], and may be present
 345 on other stem cell populations. Appropriate gap junctional
 346 communication is necessary for NCSC migration and prolif-
 347 eration [39] and their active function is especially critical
 348 for NSCs as blocking gap junction signaling in NSCs induces
 349 cell death. Connexin 43 and connexin 45 proteins have been
 350 identified in ES cells [18] and functional gap junctions have
 351 been demonstrated in ES cells via dye-coupling experiments
 352 [41]. While gap junctions are not unique to stem cell popula-
 353 tions, the components that comprise the gap junctional
 354 machinery may be common to many stem cell populations
 355 and may be functionally relevant to maintaining the stem
 356 cell state.

357 Indeed, α - and β -catenin, which play a pivotal role in
 358 cadherin-mediated cell adhesion, also have high expres-
 359 sion levels in HSCs [42], NSCs [43], and other populations
 360 of stem cells [44]. Healthy neurosphere cells are attached
 361 to each other by adherens structures as detected by electromi-
 362 croscopy and Western blotting [45]. N-cadherin and β -ca-
 363 tenin, two adherens junction molecules, were found in the
 364 attachment of HSCs to their niche [46]. β -catenin as a
 365 component of both adherent junction and Wnt signaling pathway
 366 showed critical functions in control of stem cell proliferation
 367 and expansion. β -catenin can function during mammalian
 368 neuronal development and probably regulate cerebral cortical
 369 size by controlling the generation of neural precursor
 370 cells [47]. β -catenin-related transcriptional machinery,
 371 which is regulated by E-cadherin in an adhesion-independent
 372 way, may also be responsible for stimulating cell prolifera-
 373 tion [48]. No matter which mechanism of β -catenin regulates
 374 cell proliferation, high expression of β -catenin is probably
 375 common for different populations of stem cells.

376 The Rho family of small GTPases, including Rho, Rac,
 377 and Cdc42, is involved in the downstream signaling pathway
 378 of adherent junctions and connected with actin cytoskeleton.
 379 Both Rac and Cdc42 colocalize with cadherins at cell mem-
 380 brane and positively regulate adhesive nature of cadherin
 381 through activation of p120 catenin (reviewed in [49]). N-
 382 cadherin-dependent cell contact negatively regulates the ac-
 383 tivity of Rac and Cdc42 [50]. Rho, on the other hand, has
 384 exactly opposite properties (reviewed in [51]). In addition
 385 to their function in cell adhesion, Rho GTPases also take
 386 part in cell proliferation. Rho, Rac, and Cdc42 stimulated
 387 cell-cycle progression through G1 and subsequent DNA syn-
 388 thesis when they were microinjected into quiescent fibro-
 389 blasts. However, microinjection of dominant negative forms
 390 of Rac and Cdc42 or of the Rho inhibitor C3 transferase
 391 blocked serum-induced DNA synthesis [52]. Similarly, the
 392 transfection of dominant-negative form of Rac1 into mam-
 393 malian cells will accumulate cells at G2/M stage [53]. These

394 studies provide evidence of a role for small GTPases in
395 cell proliferation.

396 Chemokine receptor CXCR4 is probably another
397 common molecule since it is widely expressed in different
398 stem cell populations such as HSCs and developing central
399 nervous system. One of the α -chemokines, CXCL12 or
400 stromal cell-derived factor (SDF-1), specifically binds to
401 CXCR4 and plays important roles in hematopoietic stem/
402 progenitor cell homing (reviewed in [54]). SDF-1 plays
403 important roles in cerebellar development by involving in
404 the movement and proliferation of cerebellar granule cells.
405 Inactivation of CXCR4 gene results in premature migration of
406 external granular layer cells into cerebellum [55], an identical
407 defect seen in SDF-1 mutant mice [56]. Absence of CXCR4
408 reduces the number of diving cells in the dentate gyrus and
409 causes prematurely differentiated neurons [57]. Expression
410 of CXCR4 was observed in proliferating cells in the retina,
411 olfactory bulb, hippocampus, cerebellum, and spinal cord
412 [55]. SDF-1 regulates the cell proliferation probably through
413 functioning on Sonic hedgehog (Shh) pathway by either
414 enhancing Shh effects directly or driving cells close to the
415 Shh source [58]. Recently Dr. Ratajczak's group detected
416 marker expression for muscle, neural, and liver cells in circu-
417 lating peripheral blood mononuclear cells as well as CXCR4
418 expression on their cell membrane [59]. Their data suggest
419 that CXCR4⁺ tissue-committed cells are stem/progenitor
420 cells residing in the bone marrow and could be mobilized
421 into peripheral blood for organ/tissue regeneration.

422 Cell proliferation and cell-cycle regulation

423 The molecular mechanisms that regulate cell-cycle progres-
424 sion in eukaryotic cells are being mapped (e.g., see review
425 [60]) and links between pathways of cell-cycle progression/
426 apoptosis/differentiation are being revealed. From this work,
427 it is clear that there are differences in the molecular mecha-
428 nisms controlling cell division in stem cells (see reviews
429 [61–65]) and these differences may serve to distinguish stem
430 cells from other populations of cells.

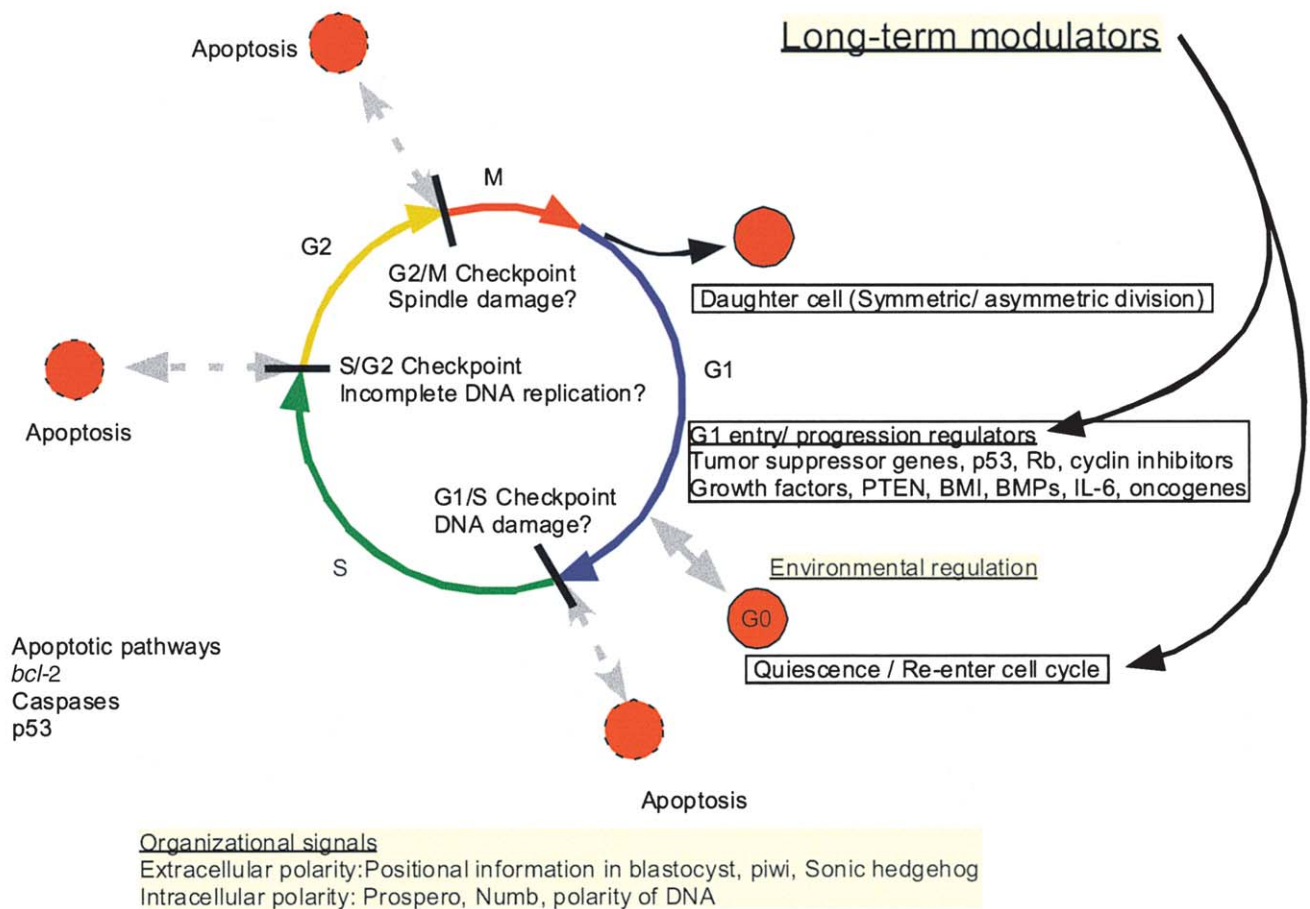
431 Clear-cut difference between how ES cells and other cells
432 regulate proliferation have emerged. In most somatic cells in
433 eukaryotes, proliferation rate (growth rate) is controlled by
434 the speed at which cells proceed through the G1 phase
435 of the growth cycle with cells spending about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

444 In contrast to most somatic cells, mouse D3 ES cells spend
445 about 75% of their time in the S phase of the cell cycle [63]
446 and it is thought that cell-cycle regulation is not regulated

447 at the G1→S checkpoint because the D cyclins and Cdk4
448 and 6 are low and cyclin A2, cyclin E1, cyclin D1, cdc2, and
449 Cdk2 are higher than more differentiated but rapidly dividing
450 cells [67,68]. ES cells are thought to be deficient in regulation
451 via cyclin Ds and Cdks 4 and 6 [63,69,70] and thus insensi-
452 tive to regulation by p16^{Ink4a} [71,72]. All these observations
453 are consistent with the lack of cyclin D-associated Cdks
454 found in primitive embryos at a time when stem cells are
455 the predominant population. Recent work by Faast et al.
456 [72] indicates that cyclin D3 is elevated in mouse D3 ES
457 cells. Their work indicates that the cyclin D3 complexes with
458 Cdk6 to regulate the cell cycle in ES cells via a mechanism
459 independent of inhibition by p16^{Ink4a}. It remains unclear,
460 however, if these differences can be extended to all stem
461 populations and if identifying specific genes will require
462 additional direct comparisons. We have noted, however, low
463 levels of p16 and p21 in NSCs and differences in the regula-
464 tion of the cell cycle between NSCs and more differentiated
465 cells [73], suggesting that it may be possible to extrapolate
466 to other stem cell populations. In any case, these studies
467 suggest that differences in cell-cycle control will likely exist
468 between stem cells and more differentiated cells and these
469 differences may be common to several stem cell populations
470 and could be included in a list of potential stem cell genes (see
471 Table 5).

472 Of the different proteins that have been shown to effect
473 self-renewal in the adult, including Wnt, bone morphogenic
474 protein (BMP), Shh, and the notch family, two new candidate
475 genes have been recently identified as important stem cell
476 markers: Bmi-1 and Pten. Lessard and Sauvageau [74] and
477 Park et al. [75] reported that Bmi-1, a transcription repressor,
478 is essential for HSC self-renewal. Park et al. used gene
479 expression profiling to determine that Bmi-1 is highly ex-
480 pressed in HSC populations of humans and mice. When
481 blood-producing cells from Bmi-1-deficient animals is trans-
482 planted into wild-type animals, the Bmi-1-deficient HSCs
483 were unable to sustain the precursor pool and in six weeks, the
484 Bmi-1-deficient HSCs were no longer detected [74,75]. This
485 finding supports previous work showing that Bmi-1-deficient
486 mice die of bone marrow failure within two months of birth
487 [76]. The work from Lessard and Sauvageau suggests that
488 Bmi-1 targets p16^{Ink4a} and p19^{Arf}. Recent work from Molof-
489 sky et al. reveals that Bmi-1 also plays a role in NSC
490 self-renewal and indicates that p16^{Ink4a} is the important target
491 for regulation in this stem cell system [77]. Pten (phosphatase
492 and tensin homolog deleted from chromosome 10) is a sig-
493 naling protein that acts mostly upon PIP3 or PI-3K in vivo
494 (reviews by [78,79]). Pten loss is coupled to malignancies
495 and Pten mutations have been detected in many human can-
496 cers. Pten-deficient mice have abnormal development pat-
497 terning in ectodermal and mesodermal germ layers with
498 enhanced proliferation of neuroepithelium and die between
499 days E6.5 and E9.5 [80,81]. Pten affects cell motility/migra-
500 tion, the size of neurons, apoptosis/cell proliferation, and the

Cell cycle regulation in stem cells



501 **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
502 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
503 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
504 populations are very sensitive to UV damage and enter apoptosis. Signals that trigger self-renewal divisions vs asymmetric divisions also affect cell-cycle
505 regulation. Some of these differentiation signals are intrinsic via polar segregation of genes during mitosis or via expression of asymmetry genes.
506 The linkage between cell-cycle control and self-renewal/differentiation is not well understood. In stem cells, the cell-cycle controls that delay progression
507 of cycle vs enter apoptosis are weighted towards apoptosis. As stem cells differentiate to more specialized cells, the weighting shifts toward cell-cycle
508 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
509 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
510 system, skin, olfactory sensory epithelium). In other tissues, most stem cells are quiescent throughout life (nervous system, ovary). The long-term control
511 mechanisms are not well known.

512 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
513 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
514 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
515 be involved in several different stem cell populations.

522 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.

532 **Table 5.** Cell-cycle-related pathways

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

542 **Symmetric and asymmetric division**

543 A common problem faced by all stem cell populations is
 544 determining how to self-renew to both generate the appropri-
 545 ate number of differentiated cells and maintain an adequate
 546 pool of self-renewing stem cells (see review by [64,83–
 547 85]). Symmetric self-renewing divisions will increase the
 548 stem cell pool, while symmetric differentiation divisions will
 549 deplete the pool and asymmetric divisions will maintain the
 550 stem cell pool. Altering the number of divisions or switching
 551 between symmetric and asymmetric divisions will allow
 552 stem cells to respond to dramatic fluctuations in requirements
 553 of differentiated cells without completely depleting the stem
 554 cell pool. Since all stem cell populations that undergo pro-
 555 longed self-renewal must maintain this balance, it is likely
 556 that some of these pathways will be shared among stem
 557 cell populations.

558 There are at least three mechanisms that may interact to
 559 regulate self-renewal/asymmetric division (see reviews by
 560 [64,84,86]). First, there are genes that are asymmetrically lo-
 561 calized in progenitor cells prior to division, e.g., genes that
 562 are differentially segregated between daughter cells follow-
 563 ing mitosis. Second, there are intrinsic genes that regulate

self-renewal/asymmetric division, e.g., stem cells can regu- 583
 late the number of symmetric divisions to undergo prior to 584
 differentiation. Third, there are extrinsic signals that can 585
 trigger self-renewal/asymmetric division; for example, stem 586
 cells replace lost cells during development and loss of blood 587
 cells following irradiation [64]. A number of genes have 588
 been implicated in these three different mechanisms includ- 589
 ing Notch, numb, Miranda, tramtrack, Prospero, staufer, 590
 piwi, etc. (see Table 6). The intracellular response pathways 591
 to these polarity molecules are under investigation. For ex- 592
 ample, work from Sherley's lab [87] indicates that the p53 593
 anti-oncogene induces asymmetric division through a gua- 594
 anine nucleotide-dependent mechanism. Reviewing the liter- 595
 ature suggests that many of the molecules identified in 596
 invertebrate systems that regulate self-renewal/asymmetric 597
 division are also expressed in multiple stem cell populations 598
 in vertebrates. For example, NSCs express several Notch 599
 receptors, presenilins, numb, etc. NCSCs, HSCs, and ES 600
 cells also express these molecules, suggesting that these may 601
 regulate how stem cells divide. However, it is important to 602
 note that these are not unique to stem cell populations and 603
 may be expressed by other cells as well. Nevertheless, as a 604

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

565	Genes		Possible functions	
566	Symmetric division	NSC	Maintenance of neural stem cell	
567				
568		GSC	Maintain the germline stem cell population and control the cell division rate	
569		SSC	Required for proliferation of somatic stem cells	
570		Both GSC and SSC	Upregulator of Piwi and hedgehog	
571	Asymmetric division	Protein determinants	Basal proteins to direct localization to the apical cell cortex of neuroblast	
572			Numb	
573			Miranda	
574			Pon	
575			Tramtrack	
576			Prospero	
577			Staufen	
578			Par-6	Apical proteins to direct localization to the basal cell cortex of neuroblast
579			Bazooka	
580			aPKC	
581		Transcription factors	Regulating genes involved in asymmetry and cell division of neuroblasts	
582		Snail		
		Wormiu		
		Escargot		

605 component of a list of stemness genes, their expression could
606 confirm the presence of a stem cell in a particular system.

607 Long-term self-renewal markers

608 Many stem cell populations need to survive for the life
609 of the organism and thus must undergo many cycles of self-
610 renewal and differentiation. This differs from most differenti-
611 ated cells, which are in permanent G0 or can undergo only
612 limited proliferation in vivo. The ability to self-renew in
613 vivo correlates with the ability to bypass senescence in vitro
614 and to bypass senescence throughout the lifespan of the
615 individual in vivo (see reviews by [88–91]). Several path-
616 ways that are important to maintaining genomic integrity,
617 bypassing senescence, or prolonging lifespan have been
618 identified. These include regulation of telomere length, high
619 levels of DNA repair enzymes, and regulation of the apoptosis
620 pathways ([92,93]; see reviews by [94,95]). Some major
621 components that have been identified are listed in Table 7.

622 It is reasonable to assume that some or all of these path-
623 ways will be active in most stem cell populations and may
624 serve to distinguish these cells from other differentiating
625 populations. In particular telomerase reverse transcriptase
626 (TERT) expression and telomerase activity appear relatively
627 specific. High TERT levels and telomerase activity have
628 been described in multiple stem cell populations and compo-
629 nents of the TERT pathway are present in many stem cells.
630 With a few exceptions TERT expression and telomerase
631 activity are low or absent in most other cells. Likewise
632 we have noted that DNA repair machinery is high in at least
633 two stem cell populations [18].

634 The corollary of long-term self-renewal is the presence
635 of a surveillance mechanism that will identify damaged cells
636 and target them for apoptosis. Thus, we suggest that stem
637 cells should be sensitive to DNA damage that would direct
638 them to undergo apoptosis. Indeed, as discussed above,
639 most stem cells seem to be much more sensitive to radiation

than other cells. After UV damage, for example, ES cells
656 enter the p53-mediated apoptosis pathway [96–98], rather
657 than use the p53-mediated delay at the G1.S transition point
658 like somatic cells [99,100]. This difference probably under-
659 lies the difference in radiation sensitivity. It is important to
660 note, however, that this is true for the proliferating stem cell
661 population while the quiescent stem cell is likely much more
662 resistant given the higher expression of repair, antioxidant,
663 and antiapoptotic machinery. Thus radiation resistance, pres-
664 ence of TERT and telomerase activity, and high levels of
665 DNA repair enzymes and the presence of specific antiapop-
666 totic molecules together may help define a stem cell state. 667

668 Other candidate “stemness” genes

669 An important theme that has emerged from developmental
670 biologists is how often a successful strategy is reused in
671 multiple systems. For example, the entire cassette of regula-
672 tory genes that appear to regulate oligodendrocyte differenti-
673 ation also appear to regulate pancreatic islet development
674 [100–103]. A combination of FGF, Shh, Wnt, and BMP
675 proteins appear important in organizing neural development,
676 cardiac differentiation, and limb formation. It is therefore
677 reasonable to assume that a successful strategy of prolifera-
678 tion, self-renewal, and differentiation may be reiterated in
679 multiple stem cell populations and these genes could be
680 candidate “stemness genes.” Given the paucity of data, this
681 is necessarily speculative and we welcome a response
682 from readers.

683 It is intriguing to note that in most cell populations de-
684 scribed, FGF and insulin/IGFs appear important. ES cells,
685 MSCs, HSCs, and NSCs all require FGF to maintain their
686 self-renewal. Likewise, some members of the Hox gene
687 family and the Sox (SRY-related genes containing a HMG
688 box) family of genes are expressed in stem cell populations
689 and are important for their self-renewal. HoxB4, for exam-
690 ple, is important for HSC proliferation and overexpression

640 **Table 7.** Pathways regulating senescence

641 Pathways	Comments
642 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.
643 DNA repair and apoptosis pathways	Stem cells are radiation sensitive but often resistant to other apoptotic signals.
644 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.
645 Mitochondrial and oxidative stress pathways	Prolonged lifespan requires special mechanisms to regulate mitochondrial stability and response to oxidative stress.
646 Immortality genes	Genes that prolong lifespan or bypass senescence have been identified. These include Mortalins, and MORF's.
647 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.

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

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

Table 8. Common growth factors and transcription factors

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

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 NSC. 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

(Q4) 798 **Table 9.** ES cells vs MAPCs

799	Property	ES cells	MAPCs
800	Growth potential	indefinite	indefinite
801	Contributes to germ cells?	yes	?
802	Common growth factors	yes (mouse)	yes (mouse)
803	Differentiates to most cell types	yes	yes (lower efficiency)
804	Expression of telomerase	yes	?
805	Expression of Oct-4	yes	low levels
806	Expression of Rex-1	yes	yes
807	Expression of SSEA-1	yes	yes (subset)
808	MAPCs lineage markers	absent	low levels Flk-1, Flt-1, AC133, CD44, CDw90, KDR, B2-microglobulin, higher levels CD13, CD49b
809			
810			
811	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, coesodermin, TRA-1-81/TRA-1-60	absent

816 Modified from Orkin and Morrison, 2002.

817 are likely to be more similar to each other than to differenti- 851
 818 ated cells and this similarity likely identifies common app- 852
 819 proaches to responding to the environment. We suggest that 853
 820 comparing two populations of stem cells is likely to provide 854
 821 insights into overall theme or broad mechanisms, but this 855
 822 type of analysis breaks down when comparisons are general- 856
 823 ized across all types of stem cells (as was indicated by 857
 824 [15,16]). 858

825 We end by offering one further caution in comparing stem 859
 826 cell populations. Our comparisons of ES cells across species 860
 827 (mouse D3 and human H1 ES cells) suggest that homolo- 861
 828 gous stem cells isolated from very similar stages of develop- 862
 829 ment use different strategies to achieve the same output (see 863
 830 Table 10). Thus mouse ES cells use LIF to self-renew 864
 831 while human ES cells require FGF. HSCs in humans do not 865
 832 express Sca while rodent cells do. Occasionally the same 866
 833 gene may be expressed in the same pattern but different 867

834 **Table 10.** Distinguishing features of mouse and human ES cells: Major 870
 835 differences between mouse and human ES cells based on work from 871
 836 our laboratory (Ginis et al., Brandenberger et al. are summarized) 872

837	Marker	Mouse	Human
838	Morphology	More diverse	Rounded with
839			sharp boundaries
840	SSEA1	Present	Absent
841	SSEA4	Absent	Present
842	Vimentin	Absent	Present
843	Trophoectoderm markers	Absent	Present
844	β-III tubulin	Present	β-5 tubulin present
845	LIFR	High	Low/variable
846	Gp130	High	Low/variable
847	FGF4	High	Absent/undetectable
848	HRASP	Required	pseudogene
849	E-hox	Required	No orthologue present
850	Fox-D3	Present/required	Low/absent

859 promoter/regulatory events orchestrate its pattern of ex- 851
 860 pression. The promoter regions of some ES cell-specific 852
 861 genes, for example, show little sequence homology though 853
 862 they share similar patterns of expression. Thus, comparison 854
 863 across species should be done with caution as the compari- 855
 864 sons may be confounded and suggest that comparisons of 856
 865 different stem cell populations within a single species will 857
 866 be a better strategy to identify common “stemness” genes. 858

867 Overall, we conclude that the idea of “stemness” is ap- 859
 868 pealing but that care needs to be utilized in using this term. 860
 869 Stemness genes are genes that are common to one particular 861
 870 type of stem cell in a particular species which make this 862
 871 population unique and distinguishable from other cells. 863
 872 Some of these genes appear to be shared with other stem 864
 873 cell populations and will be a subset of those common to one 865
 874 particular population of stem cells. This subset of “stemness” 866
 875 genes are those that reflect common strategies utilized by 867
 876 particular subsets of stem cells. It is likely that there will 868
 877 be exceptions to their universal nature, i.e., some stem 869
 878 cell populations will not express one or more of these mark- 870
 879 ers or when more subsets of stem cells compared, fewer 871
 880 stemness genes will be unique. So far, few universal markers 872
 881 have been identified by large-scale gene array analysis, and 873
 882 the ones we have discussed using an empirical approach 874
 883 likely represent a large cross-section of potential common 875
 884 stem cell markers. In particular, absence of markers of differ- 876
 885 entiation will likely be important. Identifying both common 877
 886 and distinct gene patterns in stem cell populations will 878
 887 serve in defining stem cell populations and assessing the 879
 888 differentiation and transdifferentiation ability. 880

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