Published in final edited form as:

Stem Cell Rev. 2008 September ; 4(3): 137–147. doi:10.1007/s12015-008-9036-y.

The Paradoxical Dynamism of Marrow Stem Cells: Considerations of Stem Cells, Niches, and Microvesicles

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Abstract

Marrow stem cell regulation represents a complex and flexible system. It has been assumed that the system was intrinsically hierarchical in nature, but recent data has indicated that at the progenitor/stem cell level the system may represent a continuum with reversible alterations in phenotype occurring as the stem cells transit cell cycle. Short and long-term engraftment, in vivo and in vitro differentiation, gene expression, and progenitor numbers have all been found to vary reversibly with cell cycle. In essence, the stem cells appear to show variable potential, probably based on transcription factor access, as they proceed through cell cycle. Another critical component of the stem cell regulation is the microenvironment, so-called niches. We propose that there are not just several unique niche cells, but a wide variety of niche cells which continually change phenotype to appropriately interact with the continuum of stem cell phenotypes. A third component of the regulatory system is microvesicle transfer of genetic information between cells. We have shown that marrow cells can express the genetic phenotype of pulmonary epithelial cells after microvesicle transfer from lung to marrow cells. Similar transfers of tissue specific mRNA occur between liver, brain, and heart to marrow cells. Thus, there would appear to be a continuous genetic modulation of cells through microvesicle transfer between cells. We propose that there is an interactive triangulated Venn diagram with continuously changing stem cells interacting with continuously changing areas of influence, both being modulated by transfer of genetic information by microvesicles.

Keywords

Stem cell; Cell cycle; Continuum; Areas of influence; Differentiation; Microvesicle; Cell communication; Lung injury; Co-culture; Gene expression

Introduction

We present here a deconstruction of the current idiom, proposing that a stem cell is not a stem cell (as presently recognized) and that there are no specific cellular niches but rather areas of influence (AOI). Observations of the lability of stem cell phenotype associated with cell cycle passage, the complexity of stromal cell–stem cell interactions and the existence of

microvesicle based mechanisms for genetic information transfer from tissue cells to marrow stem cells allow for the construction of a triangulated Venn diagram to model the stem cell system and its regulation.

The past as a harbinger of the future

The infusion of marrow cells into syngeneic irradiated mice results in bumps on the spleen, which turned out to be clonal. The spleen-colony-forming unit (CFU-S) represented the first definition of a marrow stem cell [1]. This assay has weathered hard times; it was not clear which was the appropriate day for counting and correlates with long-term engraftable stem cells were poor. However, it may well be that this is as good a candidate for a long-term repopulation stem cell as any currently on the market. Consider that the colonies contained erythroid, megakaryocyte, and granulocyte elements plus showing variable self-renewal. They evidenced great proliferative and differentiative potential and, importantly, heterogeneity. In a brilliant foreshadowing of where we are now, Till, McCulloch and Siminovitch [2] suggested that their studies of the CFU-S indicated that “relevant control mechanisms were operative at the level of populations rather than single cells”. They further proposed that the behavior of individual stem cells was analogous to that of individual radioactive nuclei. Populations of nuclei give decay with a highly predictable half-life, but it was impossible to predict exactly when an individual nucleus will undergo radioactive decay.

The hierarchical model

When morphologically recognizable differentiated granulocytes were studied, there was a clear hierarchy with a progressive loss of proliferative potential, coupled with a gain of differentiated characteristics [3–8]. Thus, the discovery of granulocyte-macrophage progenitors assayed in in vitro clonal culture [9, 10], immediately indicated the existence of an orderly hierarchy with CFU-S differentiating into colony-forming unit-granulocyte-macrophage (CFU-GM) and the latter then differentiating into morphologically recognizable granulocyte species. There followed the description of erythroid and megakaryocyte progenitors, both of low and high-proliferative potential, the latter termed burst-forming units [11–19]. Subsequently, hematopoietic colonies were described consisting of all hematopoietic lineages including lymphocytes and in virtually all combinations. This expanded the model significantly, but still enabled an orderly and sensible model in which different stem/progenitor cells gave rise to others with less proliferative potential and more specific characteristics. Primitive multi-cytokine responsive stem/progenitors were also described including CFU-blast, CFU-GEMM, and the high-proliferative potential colony forming cell [20–31]. These cells and others grown on stromal layers were felt to be close to the most primitive long-term repopulating hematopoietic stem cell [32–34]. While the system was more complex, it still allowed for a satisfying hierarchy.

Most recently, in elegant studies using antibodies to cell surface epitopes and FACS separations, the hematopoietic system has been ordered in a very attractive hierarchy. This work, largely by the Weissman group [35–55], has defined the most primitive cell as lineage negative Thy1^{low}, c-kit⁺, sca-1⁺, and FLK2⁻, terming it the long-term hematopoietic stem cell (LT-HSC) along with a series of stem/progenitor cells arising from this cell. These cells

are distinguished by differences in their surface markers which can then be used to physically separate them by FACS. This system, which is now widely accepted, proposed that the LT-HSC gives rise to a short-term-repopulating cell (ST-HSC), which in turn gives rise to a multipotent progenitor. This cell then differentiates into a common myeloid progenitor (CMP) and a common lymphoid progenitor (CLP), and these latter two further differentiate into granulocyte-macrophage or megakaryocyte-erythroid progenitor (MEP) and pro T and B cell progenitors. A simplified version of this model is presented in Fig. 1.

Gene expression profiles have been developed for these progenitor/stem cells classes, which are consistent with their lineage fates [35, 50, 54]. In further work with single cell RT-PCR, these profiles generally appeared to hold, although there was some heterogeneity [51]. These and other studies have shown that the different classes of progenitor/stem cells may be further classified by expression of a wide variety of genes, many of which had previously been assigned to non-hematopoietic lineages or to differentiated hematopoietic cells [50–52, 54, 56]. Transcription factor profiling in individual hematopoietic progenitors by digital RT-PCR has revealed additional heterogeneity for gene expression [51]. We had previously utilized a gene display approach evaluating lineage negative rhodamine^{low} Hoechst^{low} (LRH) stem cells stimulated to transit cell cycle by IL-3, IL-6, IL-11, and steel factor [56]. We defined 637 transcripts expressed in stem cells and not in lineage positive cells and showed that gene expression at a quiescent versus a cycling time point showed a major shift. In this study, we categorized “quiescent” genes and “cycling” genes. More recently, we have published work showing that there are different gene expression profiles with stem cell cycle passage and a good deal of intrinsic heterogeneity in gene expression of the lineage negative Sca-1+ progenitor stem cell class [57].

There is some flexibility within the model, i.e., the phenotypes are not necessarily “hard-wired”. Further studies have shown that the ST-HSC may be further subdivided based on Flt3 [58] and MPP by VCAM-1 [59] and that MEP may originate from a multipotent progenitor or from the CMP. Studies have also shown that endoglin [60] and differential dye efflux (such as rhodamine and Hoechst) [61–63] can be markers for the LT-HSC, as can a number of other determinants.

The sum total of characterization of the hematopoietic stem cell classes reviewed above has resulted in a hierarchy which is very satisfying to the orderly scientific mind and dismissive of anything smelling of chaos or undue complexity. The wonderful evolution of our knowledge of the coagulation system should inform this debate. The present hierarchical system is much too orderly and neat, without the necessary redundancy, nonlinearity, and complexity required by critical biologic systems.

Questions about the validity of a non-stochastic hierarchical model

There were, however, early indications that the marrow stem/progenitor cell system might not be a highly ordered hierarchy. As noted above, Till, McCulloch and Siminovitch [1, 2] emphasized the heterogeneity of CFU-S and the stochastic nature of its renewal. This immediately suggested that the system might not be strictly hierarchical. Subsequent work by Suda, Suda and Ogawa [30] also provided evidence against a hierarchical model. They followed the fate of cells resulting from a stem cell division, when the stem cell population

was cultured under permissive conditions. In approximately 20% of the cases, the daughter cells from one division demonstrated various differentiation outcomes; one cell might give rise to megakaryocytes and erythrocytes, while the other sister clone gave rise to granulocytes and macrophages. This certainly did not fit with a hierarchical scheme of differentiation; in fact, it was strong evidence against such a scheme. Recently, Takano et al [64] studying asymmetric division and lineage commitment at the level of the hematopoietic stem cell, also presented data against a purely hierarchical system and against the existence of the CLP and CMP as definitive precursors of all lymphoid and myeloid cells. Most recently, Sieberg et al [65, 66], using mathematical modeling and murine transplants, have proposed that their data indicates the existence of 18 different primitive stem cells out of a possible 58 stem cells. They showed also that these stem cells bred true to specific lineages after transplantation. We have reinterpreted their elegant work [67] (not agreed to by the authors) as being consistent with a stem cell population with continuing phenotypic change over time. This interpretation included the assumption that once a stem cell in the appropriate responsive state is interrogated with a differentiation stimulus, it is then fixed in a differentiation hierarchy. Since the number of potential stem cells would be increased when studies are extended to mice in different biologic states, such as old versus young mice, it is clear that the actual number of possible stem cells could rapidly increase exponentially. This would, in essence, constitute a continuum and appears to be supportive of the continuum concept developed below. A continuum of features and function also fits with the continued observations that the most purified hematopoietic stem cells are universally heterogeneous when virtually any parameter (except for the parameters used for separation) are evaluated.

The continuum model

In studies of marrow cell CFU-S and in vitro myeloid colony formation, we were struck with the day-to-day variability of results. This was generally attributed to “biologic variability” and led to carrying our relatively large numbers of experiments and then meaning the results, a process which seems intrinsically flawed. Given the known heterogeneity of stem cells and the shifts in phenotype over time (vide infra), each experiment needs to be individually interpreted. In any case, further work has strengthened the importance of individual experiment interpretation given the nature of the stem cell system.

Our initial insights derived from work on the impact of cytokine stimulation and cell cycle progress showing that as murine hematopoietic stem cells progressed through one cell cycle from dormancy, long-term engraftment was largely lost, but then recovered [68]. This dramatic phenotype shift suggested that we might need to reinterpret our hierarchical classification of stem/progenitor cells. Subsequent work over the past 10 years has shown that many phenotypic characteristics of marrow stem cells show reversible changes tied to cell cycle passage. In these studies, either whole marrow or highly purified LRH or lineage negative (Lin-) Sca-1+ cells were stimulated by cytokines to progress through cell cycle and different aspects of their function tested, including long and short-term engraftment [56, 68], in vivo homing [69], expression of a variety of genes [56, 57, 70], progenitor numbers [71, 72] and in vitro differentiation [70]. In the studies with LRH, there is a highly synchronized

march through cell cycle such that up to 98% of the cells are in S phase at one point in culture [70], and the synchrony persists for out to 5 cycle transits [73]. Employing LRH stem cells, stimulated by thrombopoietin, FLT3L, and steel factor or by steel factor, IL-3, IL-6, and IL-11, we have shown that engraftment is largely lost in late S-phase and that at the G1/S interface, there is a wave of megakaryocyte differentiation. These changes occur during one cycle transit and are reversible. Thus, the fate of a stem cell as to engraftment and differentiation shifts at different points in cell cycle and shows reversible changes. *In toto*, these studies indicate that the potential of the marrow stem cell continually changes with cell cycle passage and that these changes are reversible, i.e., not unidirectional. We view these as windows of transcriptional opportunity which will continually change as histone/chromatin coverage alters with cell cycle passage. When the stem cell is interrogated with a differentiation signal appropriate to its functional state, it will then proceed to commit to a specific lineage pathway. We have shown that GM-progenitors and LT-HSC show inverse shifts with cycle passage before cell division [72], suggesting that this progenitor and stem cell may be the same cell in different functional states. We further suggest that the different classes of progenitor/stem cells described in Fig. 1 exist on a continuum rather than in a hierarchy and that their regulation is not a unidirectional one, as is usually proposed. In Table 1, we show the different characteristics of stem cells which show reversible functional changes tied to cell cycle.

Further work using separated cycling Lin-Sca-1+ cells separated on the basis of DNA content by Hoechst staining and FACS have shown that megakaryocyte differentiation is tied to cycle in the absence of cytokine exposure. Of course, if the marrow stem cell is a non-cycling cell, this work is of less significance. However, a number of investigators using in vivo BRDU labeling of LRH or Lin- Sca-1+ c-kit+ cells have shown that the stem cell is a cycling cell which progressively labels over time [44, 74, 75]. Other studies on freshly isolated cells have shown up to 20% of HSC are in S-phase at isolation [76]. Thus, cycle shifts are continually occurring at the HSC level and would mandate that reversible changes in phenotype potential would be taking place continually.

Thus, the stem cell model is that of a continuum of potential as illustrated in Fig. 2. This continuum could include all of the described stem/progenitor phenotypes, or as an alternative “partial continuum” model, could include the more primitive types with discrete differentiation steps accounting for the more mature progenitors. As we will develop further below, this has to be interfaced with a probabilistic matrix, including soluble cytokines and cellular based modulators.

A probabilistic matrix or areas of influence—niches reinterpreted

There has been a great deal of attention directed to the stem cell niche as a determining factor for stem cell fate. The niche is usually viewed as one or several specific cell types. Recently, the osteoblast [77, 78] and the endothelial cell [79] have been most prominent. The microenvironmental influences on a stem cell are what we have termed a probabilistic matrix, probably influenced in large part by soluble or membrane anchored growth factors along with various other impacting factors such as oxygenation and physical position. We feel these microenvironmental influences are critical, but have strong reservations about the

idea that there are specific niche cells for specific stem cells. Given the continuum concept of continuous phenotypic change, there would have to be innumerable different niche cells to accommodate the great heterogeneity of stem cells. This may in fact be the case, but we would then prefer to consider such a cellular network as an area of influence, not a specific niche.

It is of interest that virtually every possible marrow cell has been interpreted as a niche cell at one time or another. The adventitial reticular cell [80] is still an interesting candidate niche cell which had far ranging projections in the marrow, touching a wide range of marrow cells. Macrophages were a component of Dexter stromal cultures and had long been recognized a nurse cell for erythropoiesis [81, 82]. The preadipocytic fibroblast, the other component of murine Dexter stroma, is another putative niche cell [83]. Granulocytes and megakaryocytes seemed to locate near sinusoidal endothelial cells, and the bone cortex has been identified as a region of relative stem cell concentration by many investigators [77, 78, 84–86]. The osteoclast is another candidate niche cell [87]. Other work has indicated that regional hypoxia may be important and that stem cells with low or high reactive oxygen species may preferentially reside in different “niche” areas [88, 89]. It has been further suggested that the osteoblastic niche may have very low oxygen reactive non-proliferating stem cells and the endothelial niche high oxygen reactive species proliferating stem cells. Further complexity is added by intriguing observations that matrix elasticity may determine the functional nature of different marrow support cells [90]. In visualizations of Lin- Sca-1+/- Dexter stromal cell interactions, we have seen that multiple stem cells may roll along lamellopodial extensions which we have termed proteopods. Other studies suggest that marrow cells may enter and exit stromal cells, and some visualizations suggest that stromal cells may have polarity, nurturing on one side and inhibiting on the other (Fig. 3).

Much of what we assume about the niche cell relates to studies on homing and subsequent engraftment. In the paper which coined the term “Niche”, Scofield [91] laid out the following characteristics defining a “niche”: (1) It is a defined anatomic location, (2) It is required for stem cell expansion, (3) It preserves stem cells by limiting differentiation, (4) It is limited in size and limits stem cell numbers, and (5) It is capable of reverting daughter cells to a stem cell fate.

Homing is the arrival of marrow stem cells to marrow spaces. Studies of whole marrow populations are uninformative, and studies after the first stem cell divisions will be clouded. Therefore, direct homing needs to be considered with purified cell types and within the first 12 h of cell infusion. Employing these criteria and using Lin- Sca-1+ cells labeled with the cytoplasmic fluorescent label, CFSE, and measuring cells in the marrow at 3 h (plateau had been determined to occur by 1 h), we determined that approximately 7–9% of cells homed to marrow in a non-irradiated mouse [69]. We determined total marrow cellularity in BALB/c mice and, using the non-irradiated mouse model with male-to-female transplants and following Y chromosome + cells, we showed that final engraftment (short or long-term) was determined simply by the ratio of donor-to-host cells. There was no limitation on engraftment and, therefore, there were no limitations imposed by niche availability [92]. If 40,000,000 cells were infused into a BALB/c mouse with a total cellularity of 530,000,000, the final % of donor cells was 6.9 (72 transplanted mice). If one simply did a mathematical

calculation of donor-to-host cell ratio, one arrived at essentially the same percentage. Other work showing that irradiation actually impaired homing to marrow, supporting the concept that there are no limiting niches [93]. This did not indicate that niches or areas of influence were not important, simply that they were not limiting.

A large number of molecules and genetic regulators have now been implicated in niche stem cell regulation, and this is out of the scope of this review, but considering our rethinking of the nature of the niche, the role of most of these would also have to be reconsidered.

Overall, the marrow microenvironment is a suitably complex mix of different cell types, matrices, and soluble molecules. We hypothesize that its phenotype continually changes in interacting with the continually changing phenotypes of marrow progenitor/stem cells. We are doubtful that the so-called niche is represented a single or only several cell types and prefer to designate the marrow microenvironment as consisting of areas of influence, which while showing gross stability, are continually adjusting to individual circumstances.

Cellular communication and phenotype change via microvesicles

The capacity of marrow cells to convert to cells of non-hematopoietic lineages, so called stem cell plasticity, has been repeatedly confirmed by a relatively large number of investigators, although the mechanisms involved here remain unclear. This area has given rise to a controversy relating to whether transdifferentiation really occurs and has focused, in part, on the phenomena of cell fusion. We have addressed the controversy in a previous communication, referring to most of the points as Ignoratio Elenchi, essentially irrelevant conclusions or red herrings [94]. A number of animal studies have established that adult marrow-derived cells are capable of differentiating into an expanding repertoire of non-hematopoietic cells, including the lung [95–113]. We have focused our “plasticity” work on the capacity of marrow cells to convert to epithelial lung cells. In these studies, donor marrow cells marked by either green fluorescent protein expression or the Y chromosome were engrafted into lethally irradiated mice, and cells carrying the donor marker which were negative for CD45 and positive for cytokeratin or surfactant were analyzed [95]. There were significant numbers of donor derived cells with epithelial markers and no CD45 ranging between 3–7% depending upon the specifics of the experimental models. In these studies, we were also able to show that the capacity to convert to epithelial cells changed reversibly as the marrow cells transited cell cycle.

The mechanisms underlying the development of lung characteristics in transplanted marrow cells remain unclear. Information transfer from injured cells to marrow cells would seem to be involved. Cell to cell fusion, as noted above, provides a clear mechanism, which appears operative in some models, but more subtle variants of fusion may be responsible for the cellular changes observed in other settings. It has been found that membrane-derived vesicles released from the surface of activated eukaryotic cells can exert pleiotropic effects on adjacent cells. Microvesicles may be secreted by activated normal cells and play a role in cellular communication [114–116]. They have been found to transfer CD41, integrin, or CXCR4 [117–120] as well as HIV and Prions [121–124] between cells. Embryonic stem cell microvesicles have been reported to reprogram hematopoietic stem/progenitor cells via the horizontal transfer of mRNA and protein [125]. Similarly, tumor-derived microvesicles have

been shown to carry several surface determinants and mRNA and to transfer some of these determinants to monocytes [126]. Apoptotic bodies from irradiated EBV-carrying cell lines have been seen to transfer DNA to a variety of co-cultured cells and integrated (not episomal) copies of EBV resulted in expression of the EBV-encoded genes EBER and EBNA1 in recipient cells at high copy number [127]. Extracts from T lymphocytes containing transcription factor complexes could induce fibroblasts to express lymphoid genes [128]. Investigators have evaluated the protein and mRNA content of microvesicles in a limited number of studies. In a recent study by Deregibus et al [129], RNA was extracted from endothelial progenitor microvesicles and microarray and data analysis carried out. They found a total of 298 transcripts, 183 of which were associated to RefSeq identifiers and the remaining Unigene, suggesting that the particles did not contain a random sample of cellular mRNA, but rather a specific subset.

There have also been a limited number of proteomic analyses on microvesicles of different origins. Jin et al determined the whole proteomic profile of plasma microvesicles from healthy donors [130] after isolation by ultracentrifugation at 250,000×g and flow cytometry analysis, proteins from microvesicles were separated by 2D-electrophoresis and identified by MALDI TOF/TOF. They identified 83 proteins out of 169 spots. Banfi et al [131] analyzed the proteome of endothelial cell-derived procoagulant microvesicles. They identified approximately 80 cellular proteins, including cytoskeletal proteins, chaperonins, nucleosomal proteins, enzymes, annexins, and proteins involved in folding and signaling. Annexins had previously been described as a category of proteins associated with microvesicles [132]. The microvesicle proteome also reflects the mechanism of particle formation, which explains the presence of cytoplasmic metabolic enzymes. Garcia et al [133] analyzed the platelet microvesicle proteome. They identified 578 proteins, 10% of them belonging to plasma membrane proteins. Again, the majority of proteins (48%) are normally located in the cytoplasm. Miguet et al [132] reported on a thorough proteomic analysis of malignant lymphocyte membrane microparticles. They identified 390 proteins, 131 (34%) belonging to the plasma membrane proteins, and 216 (54%) being cytoplasmic proteins. The composition of microvesicles is dependent on the cell from which they originate, and proteomic studies may allow the determination of different protein expression in different cell types. In the case of tumor or other malignancies, they may carry their proteomic “signature” from the affected site into the blood stream or other body fluids [134, 135]. In a recent study, Cho et al [136] demonstrated that –MS/MS combined with isotope-coded affinity tag (ICAT) labeling technique enables quantitative analysis of paired microvesicle samples. This method was applied to investigate differences in proteome between microvesicles generated from platelets and those isolated from plasma.

One possible mechanism for marrow to lung cell plasticity was that lung-derived microvesicles transferred to marrow cells and altered their genetic phenotype. Work by Jang et al [137] who showed that marrow cells cultured across from liver cells but separated by a cell impermeable membrane, began to express mRNA for albumin suggested such a mechanism.

In order to address this possible mechanism, we utilized co-culture of lung cells, irradiated or not, across from marrow cells, but separated by a cell impermeable membrane (0.4

micron) and analyzed the genetic characteristics of the marrow cells [138]. The model is illustrated in Fig. 4.

In our studies, we found that marrow cells expressed high levels of mRNA for clara cell specific protein, aquaporin-5, and surfactants B, C, and D, all lung specific mRNA. Higher levels were seen at 7 days of co-culture and if the lungs were derived from mice which had been exposed to 500 or 1200 cGy of whole body irradiation 5 days before sacrifice. Conditioned media from irradiated or non-irradiated lungs also could induce expression of lung-specific mRNA in marrow cells. When the lung conditioned media was ultracentrifuged “converting” activity pelleted and the pellet was found to contain high levels of lung-specific mRNA. Electron microscopy of the pellet showed that it contained membrane bound microvesicles. Microvesicles derived from green-fluorescent protein expressing transgenic mice glowed green, and the membranes could be further labeled with the red fluorescent dye, PKH26. These double-labeled microvesicles could be purified by fluorescent-activated cell sorting, and the purified microvesicles were also found to contain high levels of lung-specific mRNA. When purified microvesicles were incubated with different marrow cell populations, they were found to enter a minority of the marrow cells, around 1–2%. These included granulocytes, non-descript mononuclear cells and, in separate experiments, Lin- Sca-1+ murine stem/progenitor cells. Marrow cells co-cultured across from lung also showed an increased capacity to convert to pulmonary epithelial cells after transplantation into lethally irradiated mice. In more recent experiments, the universality of the phenomena of tissue transfer of genetic phenotype was addressed. Co-culture experiments with murine brain, liver, or heart across from marrow cells were established. In each case, tissue specific mRNA was found in co-cultured marrow cells. Of additional significant interest were observations that cell cycle status of marrow stem cells affected their capacity to take up microvesicles. These studies clearly illustrate the potential of tissue-derived microvesicles to alter marrow cell phenotype, although the precise mechanisms at present remain to be elucidated.

Summary

Altogether, the above considerations suggest that stem/progenitor regulation is under exquisitely flexible control with the capacity to respond to a myriad of conditions. We propose that marrow stem cells continually alter their cell phenotype in a reversible fashion, related to cell cycle status, and that microenvironment is also continually in flux being able to match the appropriate stem cell phenotype. These would be your areas of influence or “niches”. Finally, these two interactive systems would then be further modulated by a continuous stream of genetic information via microvesicle transfer from different non-hematopoietic tissues, the latter being strongly influenced by tissue injury. This concept is presented in Fig. 5.

Acknowledgements

Grant funding has been provided by NCRR, NIDDK, and NHLBI, #P20RR018757, R01DK061858, R01HL073749 and #K08HL086868.

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Fig. 1.

The hierarchical model. LT-HSC=long-term hematopoietic stem cell, ST-HSC=short-term hematopoietic stem cell, MP=multipotent stem cell, CLP=common lymphoid progenitor, CMP=common myeloid progenitor, GMP=granulocyte-macrophage progenitor, MEP=megakaryocyte-erythroid progenitor, Pro-DC=dendritic cell progenitor, Pro-NK=natural killer cell, Pro-B=B cell progenitor, Pro-T=T cell progenitor

Fig. 2.

The stem cell continuum model: a model of potential. LT-HSC=long-term hematopoietic stem cell, ST-HSC=short-term hematopoietic stem cell, MP=multipotent stem cell, CLP=common lymphoid progenitor, CMP=common myeloid progenitor, GMP=granulocyte-macrophage progenitor, MEP=megakaryocyte-erythroid progenitor

Fig. 3.
Schematic design of stem cell–stromal cell interactions

Fig. 4.

Lung, bone marrow cell co-culture. Bone marrow cells are co-cultured with lung fragments, separated by a cell-impermeable membrane. Co-cultured marrow cells are analyzed for the presence of lung cell-specific mRNA by Real Time RT-PCR

Fig. 5.

A complex flexible regulatory cell system. A triangulated Venn diagram presenting a model of stem cell regulation. The stem cell continually changes phenotype related to cell cycle state (the continuum) and interacts with soluble cytokines and cell surface mediators or changing areas of influence (AOI, a probability matrix) to determine fate outcomes. This is interfaced with genetic intercellular communication and phenotype change mediated by

mRNA laden microvesicles and this later interacts differentially with stem cells at different points in cell cycle. The butterfly and storm represent elements of chaos

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Table 1

Stem cell shape shifting

Short and long term engraftment [56, 68, 139, 140]
Adhesion protein expression [141, 142]
Homing to marrow [69]
Cytokine receptor repertoire and responsiveness [143]
Global gene expression [56, 57]
Progenitor levels [71, 72]
Differentiation into megakaryocytes, granulocytes, and epithelial lung cells [70]

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