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STEM CELLS FROM BIRTH TO DEATH: THE HISTORY AND THE FUTURE

Gerald de Haan[1] and Gary Van Zant[2,3]
[1]Department of Stem Cell Biology,
University of Groningen, A. Deusinglaan 1,
9713 AV The Netherlands
[2]Departments of Internal Medicine and Physiology,
University of Kentucky Medical Center, 800 Rose St.,
Lexington, KY 40536-0093 USA

ABSTRACT

The concept that adult stem cells, despite their impressive proliferative potential, are immortal has been challenged by experimental studies of hematopoietic stem cells. In this review, we discuss the properties that characterize a stem cell, the growing list of tissues in which stem cells are found, how they can be identified and isolated, how stem cells may transdifferentiate, and the findings that illustrate how age affects the hematopoietic stem cell population. We propose that an aging stem cell population affects tissue and organ homeostasis, particularly in response to environmental stresses, and we hypothesize that through this mechanism the functional status of stem cells affects the longevity of the organism.

STEM CELLS: AN INTRODUCTION

Stem cells can be ascribed three functional attributes that distinguish them from other cells in the body (1). They have the capacity to replicate to form daughter cells with similarly extensive developmental potential; that is, to self-replicate. They have the capacity to differentiate into progenitor cells which through amplification and differentiation give rise to large numbers of mature, functionally replete cells necessary for carrying out specific tissue requirements. Lastly, stem cells are able to respond to external cues to meet the needs for more or less differentiated cells by exercising flexibility in the choice of the first two options, or to assume a state of quiescence . Stem cells are most abundantly found in tissues in which the mature, differentiated cells, responsible for conferring tissue and organ function, have short lifespans and require continuous replacement. Historically, the skin, liver, the gut lining, and the bone marrow have been the most studied examples of such self-renewing tissues, but recently it has become evident that other organs undergo continuous cellular renewal. For example, the latter are now known to include cardiac myocytes and parts of the brain, to name just two examples of a growing list (2-6).

Stem cells of the bone marrow are the cellular source of at least eight lineages of blood and lymphoid cells with disparate functions ranging from carrying oxygen to identifying and killing tumor cells (7). Because clinical transplantation of bone marrow stem cells has been used for almost fifty years to successfully treat hematologic malignancies and congenital hematologic defects (8), it is the most well-studied stem cell population. Despite the scientific and clinical interest in stem cells, they have been difficult to study for several reasons. First, they are a rare cell population in the tissues in which they occur. For example, only one in about 100,000 cells in the bone marrow has stem cell potential (9,10). Until recently, it has been difficult, if not impossible, to identify stem cells prospectively. Once it was widely accepted that the variety of blood cell types was derived from a common stem cell (the monophyletic theory) around the turn of the last century (11,12), scientists have used the best tools available at the time for the study of these cells. Early in these studies, the microscope was the instrument of choice and hence a great deal of effort went into finding and describing stem cells in the bone marrow. A consensus was more or less reached that stem cells resembled small to medium-sized lymphocytes whose cytoplasm had blast cell characteristics (13-16). In the 1940s and 1950s it was discovered that hematopoietic stem cells were very radiosensitive (17,18), and that transplantation of (unirradiated) bone marrow stem cells into irradiated recipients could effectively rebuild the ablated lympho-hematopoietic system (19,20). A way was thus opened for a functional assay of stem cells, even if they could not be identified easily by morphology. This advance also made possible the clinical stem cell transplantation described above (21,22). The functional assay owed to the capacity of stem cells to generate progeny that could be identified both morphologically and functionally. However, in so doing, the original stem cells under study are lost and can be identified only retrospectively through their progeny. Nonetheless, functional demonstration of a stem cell's unique capabilities remains the 'gold standard' in stem cell biology (23).

Prospective identification of stem cells was made possible by techniques to identify cell parameters that, through empirical study, were found to characterize stem cells (24,25,15). In particular, monoclonal antibodies raised to a wide diversity of cell surface proteins coupled with the development of fluorescence-activated flow cytometry enabled the identification and sorting of individual cells with a prescribed set of identifying cell surface markers (26-30). Antibodies could be directly tagged with fluorochromes or indirectly labeled using biotin-streptavidin intermediates, and these coupled with the discovery of ways to measure parameters such as DNA content to determine cell cycle kinetics (31,32), and to assess a cell's antiporter function by pumping out cell permeable dyes opened the way for a multi-parameter approach to stem cell analy-

[3]To whom correspondence should be addressed at:
Division of Hematology/Oncology,
Markey Cancer Center, Rm. CC408
University of Kentucky
800 Rose St.
Lexington, KY 40536-0093
Telephone, (859) 323-5719; Fax, (859) 257-7715
e-mail, gvzant1@uky.edu

sis and sorting (33-37). Preparation of relatively large populations of highly enriched, and viable, hematopoietic stem cells for research and even clinical purposes is now not only possible but routine in many labs.

STEM CELLS AND AGING

In most if not all tissues, post-mitotic, functionally mature cells have a limited lifespan, which can vary from several days to multiple years. For example, most neurons of the central nervous system are post-mitotic and are not replaced during an individual's lifespan. In the hematopoietic system, cell lifespans vary from only a few hours for neutrophilic granulocytes to several months for erythrocytes and even years for some lymphocyte subsets. Remarkably, very little is known of mechanisms that regulate the longevity of these post-mitotic end stage cells. It is likely that at least part of this dramatic variation in cellular lifespan depends on cellular damage that is encountered by these cells as they carry out their normal functions; such damage may affect the RNA- and DNA-based transcription machinery, and protein protection mechanisms (38-43). Obviously, cells such as erythrocytes which lack a nucleus, must accumulate damage in cytoplasmic components, most likely the plasma membrane, that are in turn detected by the appropriate organ (spleen in this example) which removes them from the circulation. In most nucleated cells, accumulated cellular damage typically triggers apoptosis, which provides the mechanism for their removal and prevents the accumulation of damaged, non-functional cells in tissues. Although any relationship between lifespan of an organism's cells and the lifespan of the organism itself is controversial, Rohme has nonetheless pointed out that the natural lifespan of erythrocytes amongst 11 mammalian species is directly correlated with their natural organismal longevity (44).

The limited lifespan of many adult cells implies that in order for any tissue to maintain its optimal physiological function, continuous replenishing of aged cells must occur. Rare, tissue-replenishing stem cells thus constitute a crucial population of cells from which massive numbers of newly formed post-mitotic cells are derived. One may postulate therefore that although most of the deleterious consequences of aging are directly targeted at widely abundant, post-mitotic, and fully matured cells, the burden of the aging process in the long term is carried by tissue-specific stem cells. The molecular mechanism of stem cell aging remains as elusive as that of post-mitotic cells. Telomere erosion has been speculated to play a role, and evidence from transplantation studies exists to substantiate this claim (45-50). However, given the exceptionally long length of telomeres in the cells of laboratory mice and the modest shortening that occus following stem cell transplant, it appears as if there must be alternative mechanisms that function to serve as a cumulative replication counter in cells (51). The accumulation of several types of cellular damage by stem cells may provide a limit to its replicative potential. Such limitations must be grounded in physiologically relevant mechanisms, since unlimited replicative potential may easily transform into tumorigenesis (52). In this vein, a recently generated mouse with a mutated allele of the tumor suppressor gene, *p53*, displays pleiotropic effects that point out the physiological balance that is usually maintained between tumor suppression and aging (53). Mice heterozygous for the mutant allele (*p53m*) show enhanced resistance to naturally occurring tumors, but apparently at the price of premature aging. They have shortened lifespans accompanied by generalized organ atrophy, osteoporosis, and a particularly acute intolerance to stress. Interestingly, the bone marrow, and presumably the hematopoietic stem cell population therein, responds to myeloablative doses of 5-fluorouracil with a very slow and incomplete recovery, suggesting that hematopoietic stem cells may be particularly affected by the mutant allele. There is now increasing evidence to suggest that cellular aging, which manifests itself as senescence, in mammals prevents uncontrolled (i.e. malignant) proliferation (54,53). It may be relevant in this context to realize that a tumor cell meets a major and crucial qualification of stem cells, namely its ability to self-renew.

A challenging contradiction thus emerges: if stem cells were truly self renewing and inexhaustible, as they should be by definition, they would indefinitely sustain the delivery of new cells to the various tissues, and aging may not occur. However, as deterioration of function can readily be demonstrated in almost any somatic tissue during aging, one conclusion that may be drawn is that stem cells in fact are not able to self renew forever, and may be functionally, if not numerically, depleted in old age (55). At a single cell level, aging and self-renewal are mutually exclusive. It is exactly because of this paradox that the concept of stem cell aging has been studied in considerable detail over the last three decades. Because of the unprecedented experimental model systems that have been, and still are, available for the exploration of hematopoietic stem cells, it is stem cell aging research in the field of hematology that has in particular advanced most dramatically.

If stem cells play an essential role in the aging process, this would intuitively suggest that aging is differentially regulated and possibly substantially more complicated in stem cell-containing mammals compared to lower organisms. It is noteworthy that in flies and worms, organisms that are essentially entirely post-mitotic and thus devoid of stem cell populations, a number of genes have been identified that affect organismal lifespan (56-60). Elucidation of longevity affecting genes has proven to be far more challenging in stem cell-containing mammals such as mice and human, although two recent examples of genes causing dwarfism in mice clearly have significant effects on lifespan (61,62).

HEMATOPOIETIC STEM CELLS AND AGING

Does stem cell renewal prevent hematopoietic aging? The ability of the hematopoietic stem cell population to maintain itself through self-renewal should prevent depletion during aging and suggests that the stem cell population may be protected from aging. The fact that hematopoietic function is maintained at essentially normal levels in aged mice and humans supports this concept. Moreover, in the commonly used C57BL/6 strain of laboratory mice, the stem cell population expands by several-fold from young adulthood to old age (63-67). Thus, when bone marrow from old C57BL/6 animals was competed

with young marrow in the repopulation of hematopoietic tissues of irradiated transplant recipients, old stem cells had a significant competitive advantage (68). However, other mouse strains show distinctly different effects of age on stem cell populations and the results argue that the C57BL/6 strain may be the exception rather than the rule. For example, comparisons of old and young marrow from CBA/CaH-T6, DBA/2 and BALB/c mice revealed a significant disadvantage of old stem cells in the repopulation of irradiated transplant hosts (69,68,70-72). Perhaps not surprisingly, when stem cells from old C57BL/6 mice were serially transplanted through a succession of irradiated transplant recipients, age-related dysfunction in the stem cell population became apparent, illustrating that the difference among mouse strains is quantitative rather than qualitative (73). Numerous studies have shown that age-related changes in the function of stem cells begin well before old age and can even be detected between hematopoietic tissues before and soon after birth. Fetal liver stem cells have a competitive repopulation advantage over bone marrow cells of young adult animals (70,71). Human stem cells show a similar pattern. Primitive hematopoietic stem and progenitor cells sorted by flow cytometry from human fetal liver, umbilical cord blood, and adult bone marrow were cultured in vitro to measure the numbers of differentiated progeny they ultimately generated (74). Sorted stem and progenitor cells from human fetal liver generated the largest number of progeny, followed in order by cells from cord blood and adult bone marrow. These results show that the developmental and proliferative potential of primitive hematopoietic cells, which is highest in fetal life, diminishes through development, young adulthood and finally in old age.

STEM CELL PLASTICITY

Three levels of aging can be recognized: aging at the level of the individual cell, aging at the level of tissues/organs, and finally, aging at the organismal level. The accumulation of damage at the level of an individual cell will ultimately lead to its demise and removal usually by apoptosis and phagocytosis. As a result of both genetic and environmental influences, cells of certain tissues will accumulate more damage than those of others. Consequently, the proliferative pressure on tissue-specific stem cells will be higher in some organs than in others. If tissue-specific stem cells were functionally limited in the type of cells they are able to produce, and could only provide end-stage cells functioning in the same tissue as the one in which the stem cells resided, those organs that cease to function first will ultimately limit the physiological integrity of an aging organism. The regenerating capacity of tissue-restricted stem cells would thus determine the integrity of that specific tissue/organ. However, it has become evident that at least some stem cell populations, most notably those derived from the bone marrow, may not be as restricted in their proliferative potential as has been the dogma in the stem cell field for many years.

Until very recently, it was thought that although stem cells within a given organ system had broad developmental potential for producing a spectrum of organotypic cells, separate stem cells existed for each organ. To revert to terms used in the hematopoietic stem cell debate of a century ago, organ-specific stem cells were thought to be part of a polyphyletic developmental scheme where each stem cell type contributed to the renewal of cells only within its respective tissue or organ. It is now becoming clear that under at least some experimental conditions, a stem cell's developmental potential may be greatly expanded by transplantation into the microenvironment of another organ. For extensive reviews of this burgeoning field, the reader is referred to (23,75,76). A switch in the type of progeny a stem cell can produce, is apparently not even restricted by the embryonic cell layer from which an organ arose. Thus, hematopoietic stem cells purified from the bone marrow, a mesodermally derived organ, may give rise to hepatocytes when transplanted into regenerating liver, whose embryonic origin was the endoderm (77). Ectodermally-derived stem cells from the brain are apparently able to reconstitute the mesodermally-derived bone marrow of irradiated transplant recipients (78) and, conversely, transplanted bone marrow stem cells are able to give rise to glia and neurons in the central nervous system (79-81). To further illustrate the unexpected extent of stem cell developmental switching, Krause et al. purified murine stem cells from the bone marrow using antibodies to cell surface markers and cell elutriation, which separates cells on the basis of size (82). The enriched stem cell population was subsequently labeled with a fluorescent dye (PKH26) that is incorporated to cell membranes and subsequently injected into lethally irradiated female recipients. Two days later, male, dye-labeled cells that had homed to and lodged in the bone marrow were purified from the hosts by flow cytometry and injected at limiting dilutions into lethally irradiated secondary hosts. Extensive examination of cytokeratin-positive epithelial cells in the major organs many months after transplant revealed significant chimerism for Y-chromosome-containing cells. Thus, donor-derived epithelial cells in the secondary recipients were detected in the lung, liver, gastrointestinal tract and skin. In a clinical study by Quaini et al., examination of the hearts of a group of male patients who up to a year and half earlier had undergone a heart transplant in which the donor was female, revealed that as many as ten percent of cardiomyocytes were Y chromosome-containing recipient cells (4). Moreover, undifferentiated male cells bearing cell surface antigens characteristic of stem cells (c-kit, MDR1, Sca-1) were found in the transplanted (female) hearts. These surprising outcomes indicate that stem cells from unknown, but possibly a variety of self-renewing tissues may normally circulate and seed other organs. It is known that small, but measurable, numbers of hematopoetic stem cells normally circulate in the blood (83,84), and the results of the study by Krause et al. (82) discussed above demonstrate that stem cells purified on the basis of their ability to home to the bone marrow have extensive potential to transdifferentiate into epithelial cells in a wide variety of other organs. A number of chemotherapeutic drugs and hematopoietic cytokines has been found to cause a dramatic efflux of hematopoietic stem cells from the bone marrow into the circulation (85-90), raising the numbers of circulating cells capable of at least immuno-hematopoietic engraftment to a level that they can be harvested by leukocytapheresis and used clinically for stem cell transplantation.

Plasticity in stem cells' developmental potency may be explained by two general possibilities. The first is that a common population of totipotent stem cells is present in all organs, perhaps as a vestige of embryonic development, and that under normal physiological conditions, they are generally quiescent. The organotypic stem cells with restricted developmental potential, perhaps derived early in development from the totipotent population, account for the organ-specific cell renewal, if any, that normally occurs. Under conditions of physiological stress such as following total body irradiation and/or chemotherapy and transplantation, totipotent stem cells, as well as organotypic stem cells, may be activated and participate in the rebuilding of a given tissue.

A second possibility is that organotypic stem cells are all totipotent, but normally contribute only to the developmental lineages of the organ in which they reside. The tissue cells of the local microenvironment provide instructive cues for the relevant developmental program. In this scenario, transplantation of a stem cell to a new tissue environment would alter the external cues and thus alter the development program to be consistent with the organ of residence. Circulating stem cells from any organ would thus be uncommitted to any developmental program and ready to respond to new envrionments in which they may lodge and be integrated into the architecture of the tissue. Small numbers of stem cells normally circulate in the blood and these would account for the presence of donor-derived cardiac myocytes following bone marrow transplantion in the example above.

At the present time there is insufficient evidence to prove which of the hypotheses is correct, or if there are additional ones that need to be considered. The necessary data to resolve the issue may be provided by tracking the cell fates of genetically marked stem cells. For example, if bone marrow stem cells were to be retrovirally marked in vitro and subsequently transplanted, the progeny of individual stem cells could be identified by the unique proviral insertion pattern in the stem cell genome. The presence of hematopoietic cells and cardiac myocytes, for example, with the same genomic integration pattern would demonstrate that the same stem cell, or its progeny, is capable of giving rise to these disparate cell types. This result would not distinguish between the possibility that a totipotent stem cell in the first scenario was labeled and its progeny, produced through self-renewal seeded both organs or if hematopoietic stem cells were genetically marked, underwent self-renewal in the marrow and subsequently seeded the myocardium. On the other hand, if there was no overlap between marked clones in the heart and bone marrow, it would show that the first scenario is more likely correct in that marked totipotent cells produced progeny restricted to the developmental lineages in the organs in which they resided.

STEM CELL PLASTICITY AND AGING

Although the mechanism of stem cell plasticity is thus far from clear, it is apparent that tissue-specific stem cells have not lost all developmental properties once possessed by their ancestors, and that adult somatic stem cells may prove to be far more plastic than previously imagined. What possible consequences may stem cell plasticity have for the process of aging? Let us start out by cautiously stressing that it is still very early days in the field of stem cell plasticity, and some of the most dramatic examples of stem cell plasticity (e.g. muscle into blood) may need to be readdressed as proper control studies are carried out (91,92). If it turns out that in unperturbed organisms, the developmental potential of somatic stem cells indeed exceeds by far the confinement of the tissue in which they reside, one may speculate that such cells play a crucial role in the organismal aging process. As a result of both genetic and environmental influences, certain tissues will, with age, be more impaired in functioning than others. Consequently, the proliferative pressure on tissue-specific stem cells varies from organ to organ, possibly resulting in exhaustion of a local stem cell pool. Organs with extensive stem cell proliferation will cease to function first, and will ultimately limit the physiological integrity of the entire aging organism. Here we propose that stem cells are continuously redistributed throughout the body and seed the "neediest" organs. The neediest organs would be those in which the replicative exhaustion of tissue-specific stem cells has (almost) been met, i.e., those organs that have aged most dramatically. We hypothesize that stem cells, possibly predominantly derived from the bone marrow, thus feed progenitors in other organs, and consequently may play a part in organismal aging.

One of the most essential aims of future research in this field should be to assess to what extent stem cell plasticity occurs during normal physiology. In other words, do stem cells only show plastic features when they are ectopically, and highly artificially, placed in "inappropriate" environments? Or, alternatively, is stem cell plasticity a process that takes place continuously? It is a challenging speculation to propose that whereas DNA-repair and protein-protection mechanisms may extend the lifespan of an individual cell, stem cells may extend the lifespan of an organism. The extent of stem cell plasticity, by whatever mechanism it may be regulated, might thus interfere with the rate at which organismal aging occurs.

INTRINSIC REGULATION OF STEM CELL AGING

The scenario described above places extensive, and one might think complete, control of stem cell function in the extrinsic signalling provided by the local tissue microenvironment. However, there is extensive evidence that intrinsic controls are important in stem cell function as well. We and others have employed genetic mapping to identify loci that determine stem cell numbers, proliferative activity, response to cytokines, and response to aging (93-95,67,96-98,73,99). Building on natural inter-strain variations in stem and progenitor cell parameters, loci have been genetically mapped using recombinant inbred sets of mouse strains, and by using backcrosses and F2 intercrosses. This is a relatively recent approach to the understanding of stem cell regulation and this forward genetic strategy, proceeding from phenotype to gene, has the advantage of starting with a physiological stem phenotype that is naturally polymorphic between mouse strains. As such, the approach is likely to lead to a discovery of genes that play a role in homeostatic stem cell regulation in vivo, and thus may be broadly applicable to a number of

species, including humans. Although this genetic approach is still in its infancy, as more stem cell polymorphisms are mapped between mouse strains, and genetic coordinates are obtained for more loci, a consensus may be reached on the locations of critically important stem cell loci. Identification of the genes may proceed by at least three main strategies. One is positional cloning by methodically closing the gap between the expression of the stem cell phenotype and the exact DNA sequence responsible. One route to this goal is to generate congenic mice in which the genomic interval surrounding a mapped locus is backcrossed onto the genetic background of the partner strain. If the interval contains the gene affecting stem cell function, as indicated by the genetic mapping, it might be expected to confer the phenotype when introgressed into the background strain genome. Confirmation of mapping has been obtained in such a way for several of the mapped loci regulating hematopoietic stem and progenitor cell numbers in the bone marrow (67).

Another approach may take advantage of the consensus map locations and by carefully examing the list of candidate genes within each of the consensus map intervals, look for genes whose products are members of common regulatory pathways that may provide the mechanisms for the stem cell phenotype. It might be expected that stem cell polymorphisms between different pairs of mouse strains would lead to the mapping of different loci, but when examined on a larger scale, a common family of genes might be uncovered each of which plays a different role in a common mechanistic pathway.

A third approach to identify the biological molecules that influence functional stem cell properties is to search for genes that are differentially expressed in cells isolated from various strains of mice, or indeed from mice of different ages. Such quests can now be undertaken on a genome-wide scale by employing DNA-expression chips. Predictably, these comparisons of expression profiles will result in the obligate list of differentially expressed transcripts, and it may not be trivial to interpret the biological relevance of each individual gene for the trait at hand. However, in conjunction with confirmed genetic information on the chromosomal position to which specific traits map, the list of possible candidate genes may shrink in length quite dramatically. Only those differentially expressed transcripts that map to the critical interval remain, and those that do not map to these regions may be differentially expressed as a consequence of the biological variation induced by the gene at the primary locus.

A fourth strategy that is yielding important progress in the elucidation of genes causing human diseases, direct association between simple nucleotide polymorphisms (SNPs) and candidate genes, is significantly complicated by practical methods to measure the numbers and functions of stem cells in large human populations.

Positional cloning, candidate gene searching, and expression profiling all will benefit from increasing refinement of DNA sequencing of the human and mouse genomes. In particular, sequencing of the genomes of additional strains of mice in addition to the four (C57BL/6, A, 129 and DBA/2) now available will significantly advance this line of investigation.

CONCLUDING COMMENTS AND FUTURE DIRECTIONS

We predict that a molecular study of naturally occurring variation in stem cell phenotypes as they are encountered in regular inbred strains of mice will be of crucial relevance for our understanding of similar variation in normal, and indeed diseased humans. Alternative approaches, such as the introduction of germ-line gene deletions by specific gene targeting (100), mutagenesis (101,102), or gene-trap insertions (103) all have proven to be valuable as well. These approaches by definition induce Mendelian traits in affected individuals, which obviously simplifies the detection of the underlying gene, but it is not clear how relevant these models are for the study of complex traits, such as the study of stem cell aging.

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