

Stem cells and ageing

The potential of stem cells to overcome age-related deteriorations of the body in regenerative medicine

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Ageing is a complex process that involves every cell and organ in the body and that leads to the deterioration of many body functions over the lifespan of an individual (Clark, 1999). With age, for example, the skin loses its elasticity and injuries heal more slowly than in childhood. The same holds true for bones, which turn brittle with age and take much longer to heal when fractured. Lung tissue also loses its elasticity and the muscles of the rib cage shrink. Blood vessels accumulate fatty deposits and become less flexible, which results in arteriosclerosis. The reduced capacity to regenerate injured tissues or organs and an increased propensity to infections and cancers are probably the most prominent hallmarks of senescence (Hayflick, 1994). Although the vulnerability to infectious disease and cancer is caused by a decline of the immune system, the latter is in turn a product of interactions among haematopoietic stem cells and the micro-environments in the bone marrow and the thymus, as well as in the mucous lining of the bronchus and gut systems. Hence, all ageing phenomena—tissue deterioration, cancer and propensity to infections—can be interpreted as signs of ageing at the level of somatic stem cells. As the regenerative prowess of a living organism is determined by the ability and potential of its stem cells to replace damaged tissue or worn-out cells, a living organism is therefore as old as its stem cells.

Mammals, and especially humans, have paid a high price for climbing up the evolutionary ladder: they have lost much of the regenerative power found in lower animals. Whereas humans have only limited potential to rejuvenate their ailing tissues, other organisms show amazing regenerative abilities (Davenport, 2004). On decapitation,

planaria will regenerate a new head within five days. Hydra, a small tubular freshwater animal that spends its life clinging to rocks, is able to produce two new organisms within 7–10 days when its body is halved. After losing a leg or a tail to a predator, salamanders will recover with a new limb or tail within a matter of days. Animals with staggering regenerative potential either have an abundance of stem cells or can de-differentiate specialized tissue cells into stem cells. It has been estimated that about 20% of a flatworm consists of stem cells, and hydra is a “kind of permanent embryo” (Davenport, 2004). Salamanders use a completely different mechanism. When they are in urgent need of a new limb, they convert adult differentiated cells back to an embryonic undifferentiated state. These cells then migrate to the site of injury where they regenerate the missing part.

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To a limited extent, mammals can rejuvenate some types of tissue, such as skin and bone marrow, but they are not nearly as proficient as hydras or salamanders. This regenerative power, as mentioned above, declines with age. Surprisingly little is known about the impact of time and age on the basic units of life, which are the corresponding tissue-specific or adult stem cells. Thus, an understanding of the molecules and processes that enable stem cells to initiate self-renewal and to divide, proliferate and then differentiate to rejuvenate damaged tissue might be the key to regenerative medicine and an eventual cure for many diseases.

Although the concept of stem cells was introduced nearly a century ago by Alexander Maximow (Maximow, 1909), modern stem-cell research began in 1963 when James Till, Ernest McCullough and Lou Siminovitch established assays to detect haematopoietic stem cells (HSCs; Siminovitch *et al*, 1963). The group has since found HSCs in the bone marrow of mice. First, their series of experiments demonstrated that cells from the bone marrow could reconstitute haematopoiesis and hence rescue lethally irradiated animals. Second, using serial transplantations, they established the self-renewal ability of the original bone marrow cells. When cells from splenic colonies in the first recipients of bone marrow transplants were further transplanted into other animals that had received a lethal dose of irradiation, colonies of white and red blood corpuscles were found in the secondary recipients. On the basis of these experiments, the group defined HSCs as cells that have “the abilities of unrestricted self-renewal as well as multilineage differentiation” (Siminovitch *et al*, 1963). This discovery marked the beginning of modern-day stem-cell research. Only in recent years have other somatic stem cells been identified in tissues with more limited regenerative capacity (Bjerknes & Cheng, 1999; Gage, 2000; Weissman, 2000).

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In the context of regenerative medicine and curing age-related diseases, two main categories of stem cells have attracted much attention: so-called adult, somatic or tissue-specific stem cells (Gage, 2000; Weissman, 2000; Ho & Punzel, 2003), and embryonic stem cells (ESCs; Thomson *et al.*, 1998; Amit *et al.*, 2004). The establishment of human ESC lines since 1998 has fuelled the present enthusiasm for stem-cell research (Thomson *et al.*, 1998). But there are differences between these cell types with respect to their therapeutic potential: ESCs have unlimited potential for growth and differentiation, whereas adult stem cells are committed towards specialization. Thus, adult stem cells have the ability to regenerate the tissue from which they are derived over the lifespan of an individual, whereas ESCs have the potential to form most, if not all, cell types of the adult body over an almost unlimited period.

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On the basis of animal models, various studies have claimed that adult stem cells might also have developmental potentials comparable with those of ESCs (Ho & Punzel, 2003). More recent reports, however, have severely challenged the interpretation of initial results that suggested the 'plasticity potential' or 'trans-differentiation' of adult stem cells (Morshead *et al.*, 2002; Terada *et al.*, 2002; Wagers *et al.*, 2002; Ying *et al.*, 2002). Whereas some of the initial experiments that demonstrated the versatility of adult stem cells were not reproducible, other studies showed spontaneous cell and nuclear fusion between adult stem cells and host cells *in vitro* and *in vivo*. Cell fusion might therefore account for some of the phenomena that have been interpreted as evidence for trans-differentiation. Other reports, however, have shown that trans-differentiation occurred without cell fusion, especially under the physiological conditions of the developing fetus, albeit at very low frequencies (Almeida-Porada *et al.*, 2004).

Furthermore, by contrast to ESCs, which can be derived from cell lines established from 4- to 7-day-old embryos, somatic stem cells are elusive. The need for *in vitro* assays to identify human haematopoietic progenitors increased with the advent of haematopoietic tissue transplantation to treat leukaemia. Any assay to measure stem cells must compare the properties of the cells analysed *in vitro* with those of repopulating units tested *in vivo* after a lethal dose of irradiation—an experimental approach that is obviously not possible in humans (Ho & Punzel, 2003). To test human adult stem cells, colony assays, including long-term initiating cell assays (LTC-IC) and myeloid-lymphoid initiating cell assays (ML-IC), have been developed that might serve as surrogate markers for the repopulating potential of the stem cells in a given population (Ho & Punzel, 2003). In addition, surface markers such as CD34, CD133, Thy-1 and HLA-DR have been shown to be associated with the 'stemness' of the cell preparations. Despite all the efforts during the past 40 years, no *in vitro* assay to identify HSCs is considered to be adequate (Weissman, 2000). Thus, there is still no appropriate substitute for the repopulation assay in a mouse transplantation model after a lethal dose of irradiation. An immunocompromised mouse model—such as the SCID mouse model or variations thereof—or *in utero* sheep transplantation models using animals tolerant to human HSCs have been proposed for estimating the repopulating potentials of human HSCs (Zanjani *et al.*, 1992).

Whereas the impact of time and age on the quantity and quality of mouse HSCs has been recently studied, information on senescence in human HSCs is scarce. Studying this is also a challenge because these cells can maintain their function much longer than the average human lifespan. Most of the knowledge we have gained on senescence of stem cells has therefore been gathered from mice. As mice share more than 90% of their genome with humans, but have 30–40-fold shorter lifespans, it is hoped that observations of stem-cell behaviour in mice can be extrapolated to human HSCs. Various studies have indicated that even though similar HSC concentrations could be found in young and old bone marrow, it is the functional ability per cell in the repopulation model that shows a significant reduction with increasing donor

age. HSC senescence is regulated by several genetic elements mapped to specific chromocytos (Chen, 2004). These elements may differ among species, strains and even individuals in the mouse model. In humans, HSC senescence and related pathological effects might not be as obvious as in the mouse model because individual primitive HSC clones can produce progeny that sustain life-long mature blood cell production, which is especially obvious after bone marrow or HSC transplantation.

The huge medical potential of stem-cell-based treatments was first shown when bone marrow transplantation was used to treat patients with hereditary immunodeficiency or acute leukaemia in the late 1960s (Bach *et al.*, 1968; Thomas *et al.*, 1977). Without the benefits of present-day knowledge of immunology and supportive care, morbidity and mortality rates were high (Thomas *et al.*, 1977). Nevertheless, the results were encouraging compared with those obtained from conventional treatment options. Bone marrow transplantation has in the meantime become much more efficient and has been proven to be the only cure for some patients with malignant and hereditary diseases. Although initially identified in the marrow, HSCs were later found in the peripheral blood on stimulation, such as during the recovery phase after myelosuppressive therapy or after administration of cytokines (Korbling *et al.*, 1986). These peripheral blood HSCs have been used successfully in lieu of bone marrow to reconstitute haematopoietic and immune functions in recipients (Ho & Punzel, 2003).

The success of any bone marrow transplantation correlates with the quantity of HSCs in the graft, which are able to reconstitute the blood and immune system after myeloablation. On the basis of our extensive experience in HSC transplantation since 1984, we have found that age represents the main variable and worst prognostic factor for clinical outcome of transplantation. Recent evidence indicates that there is a decline with age in the quantity and quality of the CD34⁺ cells harvested. There is also a change in the ratio of fat to cellular bone marrow with age, which has been well known since the turn of the twentieth century. One way to overcome this problem would be to expand the

human HSC population *ex vivo* before transplantation. There have been numerous such attempts, but progenitors with self-renewal capacity are very demanding. Reports of successful expansion of HSCs derived from human marrow in the laboratory have thus far been controversial. By contrast, CD34⁺ cells derived from umbilical cord blood have been shown to be expandable to a limited extent, which is another indication that the potency of HSCs declines with ontogenic age.

To identify the subpopulation most enriched in HSC candidates, we have studied CD34⁺ subsets derived from various human donors. We determined and compared the relative engraftment potential and functional characteristics of specific CD34⁺ subsets derived from fetal liver, umbilical cord blood, mobilized peripheral blood and adult bone marrow. By means of our single-cell culture system, we have shown that cells with the phenotype CD34⁺/CD38⁻/HLA-DR⁺ from fetal liver have a 2–3-log higher proliferative potential on a cell-to-cell basis than those from adult bone marrow or mobilized peripheral blood.

But the potential of stem cells extends beyond purely clinical use—they could also become a model for studying the genetic and biochemical processes of cellular ageing. For example, early in life, nearly all of the body's cells can divide. After a certain number of divisions, however, they lose their ability to proliferate and DNA synthesis is blocked. Human fibroblasts divide about 50 times and then stop, a phenomenon known as the Hayflick limit (Hayflick & Moorhead, 1961). How this replicative senescence occurs in HSCs and whether this contributes to the gradual loss of the body's capacity to renew tissues remains to be clarified. A multilayer control system involving a host of mechanisms seems to be at work in maintaining the body's cell number and appropriate organ size (McCormick & Campisi, 1991). Genes that upregulate proliferation are countered by anti-proliferative genes. Mutations in these silencing genes have been shown to affect the lifespan of *Caenorhabditis elegans* and might also be involved in the ageing of human HSCs.

The biological clock underlying the limited division potential of somatic cells is the length of telomeres, the repeated DNA sequence at the ends of each chromosome.

Telomeres provide stability to the chromosome and protect them against DNA loss associated with cell division. Their length is maintained by a reverse transcriptase, telomerase, which egg and sperm cells use to restore telomeres to the ends of their chromosomes. Most adult cells, however, lack this capacity and when telomeres reach a critical length, the cells stop proliferating. Whether senescence of HSCs is explained by telomere shortening has been the focus of several rigorous studies (McCormick & Campisi, 1991).

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The role of epigenetics in cellular senescence is most obvious for genes that are subjected to genomic imprinting (Mahlknecht & Hoelzer, 2000). Enzymes that control the status of DNA methylation and histone acetylation are key epigenetic modulators that regulate chromatin stability and gene expression. Chromatin modifications are important for several nuclear processes, including DNA repair, replication, transcription and recombination—life processes that probably change with age. Thus, epigenetic changes could represent critical determinants of cellular senescence and warrant further investigation.

Other than genetic changes, all metabolic activities expose cells to biochemical substances that cause random DNA damage and cellular breakdowns (Finkel & Holbrook, 2000). Among these, oxygen radicals and protein cross-linking have attracted much attention. The mitochondrial process of using oxygen to produce adenosine triphosphate (ATP) creates free oxygen radicals as harmful by-products, which could cause damage to proteins, membranes and DNA as well as to the mitochondria itself. In glycation, glucose molecules attach themselves to proteins, thus starting a chain reaction that leads to cross-linked proteins (Finkel & Holbrook, 2000). Over time, these proteins accumulate and eventually disrupt cellular function (Cerami, 1985). It seems that glycation and oxidation are interdependent processes that accelerate the formation of one another as a cell ages.

Heat-shock proteins (HSPs) are produced when cells are exposed to various stresses, particularly heat (Mahlknecht & Hoelzer, 2000), but they are also triggered by toxic substances such as heavy metals and chemicals, and even by psychological stress. In cultured cells, HSP-70 production declines markedly as the cells approach senescence. The level of HSP production by an animal under stress depends on the animal's age. As they are known to facilitate the disposal of damaged proteins, HSPs might have an important influence on the ageing process but their exact role still needs to be defined.

The regulation of stem-cell activity and the influence of time and age on somatic stem cells are critically important for an organism during development and senescence. Mounting evidence suggests that the genetic and biochemical alterations described above for somatic cells might also have a prominent role in HSC ageing. Using multipotent stem cells derived from bone marrow as a model to study these processes might hold promise for ageing research for several reasons. First, by contrast to most other somatic stem cells, those from bone marrow can be readily isolated without undue risks. Second, two types of stem cell can be extracted from the bone marrow: HSCs and mesenchymal stem cells. Third, much data is available on ageing and senescence of HSCs in mice and some of this knowledge might be extrapolated to human HSCs. Last, human HSCs have been well characterized by parameters that can be used to test the impact of time and age on a specific phenotype of stem cells throughout ontogenic stages as well as throughout lifespan.

Senescence at a cellular level is a complex process. Studies on genetic stability, DNA damage, synthesis and repair, telomere organization, telomerase activity, oxidation and glycation require specific expertise in cell biology, molecular biology, human genomics and proteomics that is often not available in one laboratory. There is a great need for coordinated and integrated efforts to harmonize the definitions, tools and reagents in the characterization of stem cells and in the studies of the aforementioned parameters on the impact of time and age on marrow-derived stem cells. Understanding how stem cells age might have a key role in deciphering the normal ageing process.

Never has there been so much interest from the public, politicians and scientists about stem-cell research. Stem cells hold enormous promise for cell-replacement therapies or tissue repair in many age-related degenerative disorders, such as diabetes, stroke, heart disease and Parkinson's disease. In the context of time and ageing, stem-cell research is important for two reasons. On the one hand, stem cells are a good model to study ageing. Changes associated with the senescence of marrow-derived stem cells might provide pertinent clues to decipher the processes of cellular ageing. On the other hand, finding ways to reactivate stem cells and control their target destination might create unforeseen opportunities for curing degenerative diseases. Whereas many genetic and biochemical changes during senescence of HSCs in mice have been studied in detail, similar examinations in human stem cells still pose a major challenge. A combinatorial and interdisciplinary approach will be essential to exploit stem-cell technology fully for regenerative medicine.

Although ESCs can develop into almost any specialized cell type in the body, their clinical use might be associated with a risk of developing cancers and mixed tumours. It is not clear yet whether adult stem cells can match the ESCs' prowess to differentiate into cells of other organs. Because most studies have focused on the dramatic change in long-term fate, such as the conversion into tissues of another germinal derivation, little is known about the initial steps that lead to a different maturation pathway. Similarly, it is not yet clear which molecular changes are involved in switching to another differentiation programme. Cross-talk with the microenvironment probably determines the long-term fate of stem cells, in terms of both differentiation and the balance between self-renewal and differentiation (Ho & Punzel, 2003). By understanding how the microenvironment communicates with HSCs, we might be able to alter their long-term fate for therapeutic purposes. Given the present status of research, we should keep all options open for studying both embryonic and adult stem cells to appreciate the complexity of their differentiation pathways and their developmental and ageing processes. Only through a thorough understanding of the molecular mechanisms can we acquire the power to manipulate a stem cell's destiny.

ACKNOWLEDGEMENTS

This work was supported by the German Ministry of Education and Research (BMBF) within the National Genome Research Network NGFN-2 (EP-S19T01), the German Research Foundation DFG (HO 914/2-3; Ma 2057/2-3) and the Joachim Siebeneicher-Stiftung, Germany.

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doi:10.1038/sj.embor.7400436