Perspectives on the properties of stem cells

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Much about stem cells is controversial. For example, even the question 'what is a stem cell?' arouses controversy. One definition of stem cells is that they are "primal undifferentiated cells which retain the ability to differentiate into other cell types…[which] allows them to act as a repair system for the body, replenishing other cells as long as the organism is alive"[1]. This definition is controversial for at least two reasons. First, it could be interpreted to imply that a stem cell is simply an undifferentiated cell that is able to give rise to differentiated descendants (*i.e.*, that 'stem cell' is equivalent to 'progenitor cell'). Second, this definition neglects a crucial property of stem cells—that some of their descendants must retain stem cell properties.

We suggested in1963 that stem cells have two defining properties that are evident at cell division[2]. First, progeny arising from division may retain stem properties; that is, the stem cells have made new stem cells, the property called self-renewal. Alternatively, the progeny of stem cell division may have lost the capacity for self-renewal; instead, they may either differentiate or enter into a series of terminal divisions, finally yielding an organized tissue such as an organ or a population of functional blood cells. Beyond these two central properties, stem cells are identified and named according to the function of their descendants (for example, marrow stem cells) or their place in development (for example, adult stem cells or embryonic stem cells). Stem cells also vary in the diversity of their differentiated descendants. Those with unlimited potential are called totipotent stem cells. Equivalent designations are used for cells whose potential is limited (pluripotent stem cells, multipotent stem cells, bipolar stem cells, etc.). Much research forms the basis of these various designations, and questions about the self-renewal potential and the differentiation potential of various kinds of stem cells continue[3].

The introduction of the concept of plasticity is a good example of the general impact of stem cells throughout biology and medicine. Here we provide a few other examples, such as the impact of stem cells on the development of diversity and cancer. Furthermore, recent work in many laboratories is showing the possibility of functional uses of stem cells, especially in regenerative medicine. These experiments are controversial not only in the scientific sense, but also in their ethical consequences. These possibilities, and their accompanying debates, account in large part for the intensity of the current interest in stem cells. These controversies will be mentioned briefly here, but not considered in detail.

An early example of stem cell diversity

We first detected 'spleen colony-forming units' (CFU-S) in mouse bone marrow in experiments reported in 1961 (ref. 4). These experiments had been carried out as part of our collaborative research on the radiation sensitivity of normal mouse bone marrow cells[5], and the context within which this work took place has been described[6]. On the basis of our early results, we wondered whether these CFU might be some kind of stem cells, but we were cautious about jumping too quickly to this interpretation. Until we had obtained more convincing evidence, we preferred to refer to these 'units' only in functional terms. We named them initially on the basis of the function that we had actually observed: their ability to form macroscopic colonies in the spleens of irradiated mice.

One of our first problems in seeking such evidence was how to define stem cells in functional terms. Previous attempts to identify stem cells had been based mainly on speculations about their probable morphology.

We soon realized that, as noted above, one crucial defining property of stem cells must be their capacity for self-renewal. The self-renewal of cells able to form colonies in the spleens of irradiated mice was first shown in conceptually important experiments published in 1963 (ref. 2). When individual spleen colonies were assayed for their content of new colony-forming cells, almost all of the individual colonies were found to contain at least a few new colony-forming cells. When coupled with our equally important earlier finding that individual colonies were clones derived from single cells[7], this result showed that the cells able to give rise to spleen colonies indeed had a capacity for self-renewal.

The second point of interest in the results obtained by Siminovitch *et al.*[2] in 1963 was the observation that the distribution of numbers of new colony-forming cells per colony was very heterogeneous. A few colonies contained large numbers of new colony-forming cells, but the majority of colonies contained much smaller numbers. We wondered why.

Three possible explanations were considered. The available evidence did not support the view that the heterogeneous distribution was simply an artifact of the experimental procedures used. For example, the colonies tested were significantly more homogeneous with respect to their total cell content than in respect to their content of CFU. Furthermore, no gross feature of the colonies, such as colony size or color, could be correlated with their content of new CFU. Indeed, no criterion emerged for the detection of colonies with high or low content of colony-forming cells, except the criterion of test by injection into irradiated recipients.

A second possibility was that individual colony-forming cells had inherited different capacities for self-renewal. To test this possibility, colony-forming cells derived from individual colonies were themselves tested for their capacity to give rise to new CFU. No evidence of inheritability of high levels of self-renewal of CFU was obtained, but these experiments were complicated by the occurrence of a systematic decrease in the proliferative capacity of colony-forming cells with repeated passage[8].

A third possibility was that the heterogeneity developed during the formation of a colony. A model of the processes involved in the formation of a colony was needed. We

Figure 1 Ernest McCulloch.

developed such a model[9], on the basis of two of the defining properties of stem cells that are evident at cell division. The content of an individual colony after a period of growth may be considered to be the result of a balance between production of new stem cells by self-renewal and the loss of stem cells (e.g., by differentiation, entry into a series of terminal divisions, or cell death).

It might be expected that, in the stimulatory environment of the heavily irradiated mouse, the loss of stem cells might be a quite orderly process that should yield relatively uniform colonies. But the observed variation in new CFU per colony was so large that the control of the process involved in colony formation seemed to be quite lax[2]. Perhaps the relevant control mechanisms operated at the level of populations, rather than single cells? Indeed, perhaps a stochastic process was involved, such that the choice to self-renew (or not) at each division of a stem cell was uncertain. From this perspective, the behavior of individual stem cells might be regarded as analogous to the behavior of individual radioactive nuclei. Populations of such nuclei decay with a highly predictable half-life, but it is impossible to predict exactly when an individual nucleus will undergo radioactive decay.

The stochastic model that we proposed[9] was elaborated upon by others[10]. An important prediction of such stochastic 'birth-and-death' models of stem cell proliferation and differentiation is that it should be impossible to show conclusively that a purified population of stem cells is indeed 100% pure. This is because in any bioassay for stem cells in which they are required to proliferate, the first cell division that they undergo is a pivotal one. If, by chance, this first division does not result in self-renewal, that particular stem cell cannot be distinguished from a progenitor cell that was already, before division, committed to a terminal differentiation pathway. Stochastic models of stem cell development continue to be controversial. Highly purified populations of hematopoietic stem cells are now available[11,12]. But, because of the experimental difficulties and uncertainties involved in stem cell purification, this particular prediction of stochastic models will be very difficult to either prove or disprove.

Some current issues in stem cell biology

Plasticity

The nature of stem cells has been questioned further with the description of stem cell plasticity. Until recently, scientists thought that stem cells functioned only in systems that depended on proliferation and differentiation; these obligatory renewal systems include hematopoiesis, epithelial mucosa and skin. Other systems respond to injury by divisions in cells that have already differentiated. For example, after partial hepatectomy, mature liver cells divide and replace the parenchyma that was removed; in a similar fashion, renal tubule cells can resume proliferation in the face of injury. These long-held ideas have now been challenged by experiments that suggest that hematopoietic stem cells can give rise to liver, muscle and brain cells. Furthermore, subsets of cells from these organs can repopulate blood and marrow. Some, such as oval cells of liver and satellite cells of muscle, have surface markers, such as Sca and c-Kit, that usually are associated with hematopoietic stem cells. Studies of this kind have been recently reviewed[13,14].

The plasticity of stem cells remains very controversial[15]. If adult stem cells that have the potential to differentiate and produce the functional cells of several organs do exist, then such cells might well be targets for carcinogenic events. It follows that may exist malignancies that arise as transformations in plastic stem cells. The phenotypes of such tumors might continue to reflect the very extensive differentiation potential of plastic stem cells. One candidate phenotype is the well-documented clinical presentation 'metastases with unknown primary site'[16,17].

Cancer

Cancer is a population of malignant cells, either clustered as tumors or dispersed, as in leukemias. All cancers are derived from single cells—cancer stem cells—and the descendants of these cells are clones. These clones contain newly formed cancer stem cells and malignant-appearing cells that lack the capacity for continuous proliferation. Thus the development and continuous growth of cancers depend on the activity of stem cells. These are then the targets for effective therapy.

Cancers vary greatly in their cellular composition; their behavior, growth and response to treatment reflect this variation. It is a goal of research to develop methods that measure this variation and relate the measurement to outcome. Many techniques have been developed; the most valuable are those methods that measure either stem cell number or a crucial property of the stem cells of a particular tumor.

Malignant transformation of a normal stem cell is almost always genetic. The altered expression of the malignant genome is the phenotype of the cancer. Sometimes, the genetic change can be identified in chromosome preparations. A dramatic example came from work on chronic myeloblastic leukemia (CML). References relevant to the research summarized below are available in a recent review[17].

An abnormality, soon named the Philadelphia chromosome, is now known to be a reciprocal translocation between chromosomes 9 and 22. It is found in more than 90% of cases of CML and is diagnostic of the disease. A small limited region on chromosome 22 is termed the breakpoint cluster or Bcr. An oncogene, *ABL*, originally isolated from Abelson murine leukemia virus, fuses with Bcr, to make the fusion protein Bcr-Abl. This protein is a tyrosine kinase, with enhanced activity. Transfer of the Bcr-Abl gene either *in vivo* or *in vitro* causes abnormal stem-cell proliferation. These findings make it highly probable that Bcr-Abl is the immediate cause of CML.

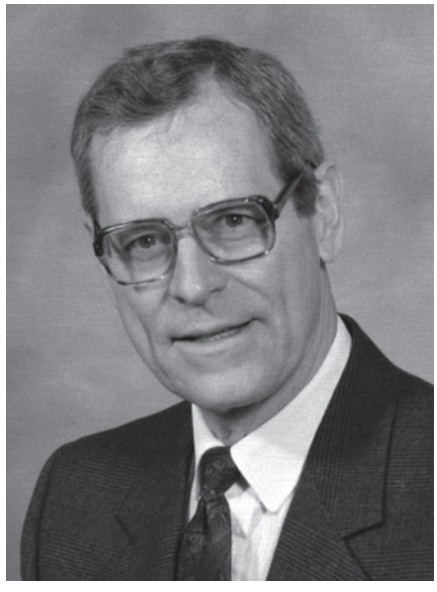

Figure 2 James Till.

The molecular pathology of CML was the basis for a large program with the aim of finding a drug that binds to an important component of the fusion protein Bcr-Abl. The tactic was to seek tyrosine kinase inhibitors; one compound, 2-phenylaminopyrimidine, with some capacity to inhibit protein kinase C, was chosen for further work. Another compound, STI571, was found to be an excellent tyrosine kinase inhibitor; culture experiments showed that STI571 was active in decreasing colony formation by cells with the 9:22 translocation, whereas normal cells were not affected. Its mechanism seemed to be binding to the ATP-binding site on the Bcr-Abl protein. This mechanism was confirmed by three-dimensional structural studies that showed the position of the ATP-binding cleft and the localization of STI571 to it.

The preclinical development of STI571 quickly led to phase 1/2 clinical trials, based on individuals who had failed while on the standard treatment with interferon. In this early work, many of these individuals with end-stage disease entered remission and, in some, the Philadelphia chromosome–positive cell population was eliminated. Quickly, large trials were organized; these showed the value of treatment with the drug in the chronic phase, accelerated phase and blast phase of CML. The drug, now known as imatinib mesylate (Gleevec), provides evidence that successful treatment of cancer requires not only the identification of the causative stem cell, but also a direct attack on the molecular lesion in the malignant stem cell.

Why now? Possibilities and controversies

Our basic research that led to a prestigious Lasker Award was initiated over 45 years ago. One might reasonably ask (as we have asked ourselves): why now?

A detailed response to this question is beyond the scope of this commentary. However, at least four reasons can easily be identified. One is the vast amount of innovative research that has led to the identification of various categories of stem cells. A discussion of these categories, intended for a wide readership, was published recently[18]. These categories range from the totipotent fertilized egg (zygote), through pluripotent embryonic stem cells, to multipotent or bipotent adult stem cells.

Another reason is the exciting possibility that embryonic stem cells might provide a means to regenerate damaged organs, such as heart, brain, pancreas, etc.

A third reason is the enormous publicity that stem cells have received, as a consequence of major controversies over how best to regulate (or not) research on such stem cells. An example was the publicity given to the birth of Dolly the sheep, which showed that it was feasible to clone a mammal (and so, perhaps, a human?).

A fourth reason is the ongoing controversy about whether any (or how many) adult bone marrow cells may have the potential to transform into other cell types (plasticity) that is analogous to that of embryonic stem cells. Those who are opposed to the use of embryonic stem cells in research or practice have emphasized the great importance, from an ethical perspective, of the controversy over plasticity. Some of the ethical controversies related to research on stem cells, from an Australian perspective, have recently been reviewed[19].

Because our basic research, initiated in the 1960s, provided the first functional identification of a stem cell of the blood-forming system, it does (with the benefit of hindsight) seem that we set the stage for subsequent research on adult and embryonic stem cells. We weren't deliberately seeking such cells, but, thanks to a felicitous observation originally made by McCulloch, we did stumble upon them. Our experience provides yet another case study of both the value of fundamental research and the importance of serendipity in scientific research.

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