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Canadian Stem Cell Scientists Take the Prize

Essay

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This year, the recipients of the Lasker Award for Basic Medical Research are Ernest A. McCulloch and James E. Till. The landmark studies of McCulloch and Till in the 1960s defined the hallmark properties of stem cells: the ability to self-renew and differentiate.

Not a week goes by that the scientific community or the media fails to report on stem cells. The topic is being discussed in the Congress of the United States as well as in governmental bodies around the globe. The funding of stem cell institutes by state governments and private benefactors is considered daily. How did we get there? The revolution began almost 50 years ago with the fundamental studies of two Canadian scientists, Dr. Ernest A. McCulloch and Dr. James E. Till. The pioneering work of McCulloch and Till now has been recognized by the Lasker Foundation, with their receipt of the 2005 Lasker Award for Basic Medical Research. The landmark studies of McCulloch and Till in the 1960s defined the hallmark properties of stem cells: the ability to self-renew and differentiate. Working with mouse bone marrow cells, McCulloch and Till developed an assay to quantitate a class of early blood-forming progenitor cells and to define the potential of these cells both to self-renew and to undergo extensive differentiation into many different blood cell types. Stem cells are very difficult to study due to their rarity (that is, 1 in 10,000 bone marrow cells in the mouse). In addition, their quiescent nature diminishes the ability to obtain a complete stem cell phenotype or "footprint" to aid in their identification and purification.

In the Beginning

From 1960 to 1963, McCulloch and Till demonstrated that cells residing in the mouse bone marrow, upon transplantation to a heavily irradiated mouse recipient, migrate to the spleen and form discrete colonies (nodules) (McCulloch and Till, 1960; Becker et al., 1963). Within the nodules, they identified donor cells belonging to several hematopoietic lineages. They used abnormal chromosome marker studies to establish the clonal nature of the nodules. The authors concluded that these nodules represented the progeny of single transplanted bone marrow cells based upon the direct linear relationship between the number of cells transplanted and the number of colonies observed. They demonstrated that the cells within the colony could give rise to additional colonies after serial transplantation into secondary recipient mice, the now classic assay for self-renewal potential. They also realized that the nodules on the surface of the spleen—which they called colony-forming units-spleen (CFU-S)—had variable capacity for self-renewal, suggesting a functional heterogeneity within the population. This heterogeneity has led to the concept that not all stem cells are created equal, even though stem cells as a class are the most primitive cells within a tissue and provide the organ with a renewable source of differentiated progeny. During the next decade, assays for more mature clonogenic progenitor cells of bone marrow grown in vitro were developed. A hierarchy was established and as these cells differentiated they were shown to lose the self-renewal potential characteristic of the earliest cells in the stem cell compartment. The observed heterogeneity also implied that regulation of clonal expansion may be stochastic. These basic tenets of stem cell biology, namely, that the stem cell must demonstrate both self-renewal and differentiation potential, have remained both conceptually and practically the same even given the decades of additional research to understand how stem cells maintain homeostasis of the organism.

Controversies in the Field

It was nearly 30 years after the discovery of CFU-S by McCulloch and Till that bone marrow hematopoietic stem cells (HSCs) were isolated successfully, enabling HSCs to be studied directly rather than through colony-forming assays. Yet this delay did not deter McCulloch and Till, their colleagues, and their students from continuing to establish the fundamental physiological roles of HSCs and hematopoietic progenitor cells. They realized that the clonogenic assays, although quantitative, did not directly measure the engraftment potential of the stem cell. The colony assay for CFU-S, although providing an observable window on the potential of mouse HSCs in the short term (that is, 1 to 2 weeks post-transplant), did not establish the potential of these cells for long-term engraftment. Other groups set out to identify the external factors that influenced the self-renewal and differentiation of HSCs. Identification of new growth factors suggested extrinsic regulation rather than intrinsic control of stem cell properties. As scientists discovered these growth factors, they began to argue against the stochastic hypothesis of clonal expansion of stem cells. They developed an alternative deterministic hypothesis: that HSC renewal and differentiation were regulated by the external microenvironment. These two opposing arguments, heated at times, have been at least partially reconciled by recognition that the clonal expansion and regulated differentiation of HSCs both contribute to long-term engraftment of donor HSCs in recipient bone marrow. However, each hypothesis will be challenged over and over again, as they have been most recently with studies showing the conversion of HSCs into cells of other tissues.

Finally, a long-term reconstituting cell population containing CFU-S was isolated in 1988, described in the classical paper by Irving Weissman's group (Span-

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grude et al., 1988). Now the long-term reconstituting cell could be studied directly. Through proof-of-principle experiments, scientists could examine the self-renewal and long-term reconstituting capacity of HSCs that heretofore were measurable only indirectly by the colony assay developed by McCulloch and Till decades earlier. Several different isolation technologies were developed for HSCs based on function and phenotype. Our group showed (Jones et al., 1990) that CFU-S, although multipotent, did not copurify with the long-term reconstituting cell. Then the hierarchy of HSCs was expanded yet again: into early and later stem cells, committed early and later progenitors, and fully differentiated cells.

One can reason that the earliest stem cell in development is the zygote. Clearly the fertilized egg has a potential for differentiation (pluripotency) that far exceeds that of any adult tissue. Embryonic stem cell lines, both mouse and human, exhibit both self-renewal and extensive differentiation capacity in vitro. However, one of the questions under great debate, both by scientists and by political groups worldwide, is the question of whether stem cells in the adult retain their capacity for self-renewal and pluripotency? These studies have led to the concept of stem cell plasticity. Once again the biological principles set forth by the studies of McCulloch and Till in the early 1960s prove relevant. These early studies required transplantation of clonogenic HSCs to obtain successful engraftment and rescue of the irradiated mouse recipients. Studies in the 1960s and 1970s demonstrated functional repair of injured bone marrow by the stem cells. Parallel studies of pluripotency today also need to demonstrate functional repair of injured tissues (heart, lung, liver, etc.). Do the injury signals (that is, the microenvironmental factors produced by the injury) directly regulate HSC differentiation or is repair by HSCs a random event? This question remains the subject of ongoing experiments by many different groups.

Therapy: Then and Now

In the 1960s and 1970s, in the middle of a cold war and during a heightened nuclear threat, it was prudent for the military to consider the effects of ionizing radiation on soldiers. Research on treating lethal irradiation by bone marrow transplantation was militarily justifiable and sponsored. These sponsored basic research studies of McCulloch and Till were complemented by the initiation of therapeutic intervention protocols at several medical institutions. Transplantation of bone marrow cells to treat bone marrow failure caused by, for example, aplastic anemia or hematological malignancy became the treatment of choice for otherwise fatal diseases. Additional sources of enriched HSCs were sought because within the bone marrow these stem cells are rare. Subsequently, both umbilical cord blood and mobilized peripheral blood were found to be enriched sources of HSCs. These sources were tested for their ability to provide rapid and durable engraftment of bone marrow, once again adhering to the criteria established by the studies in animals of McCulloch and Till and their colleagues in the 1960s. Umbilical cord blood contains more long-term engrafting HSCs than bone marrow, but the number of cord blood cells available for transplant is limited.

Let us fast forward to the late 1990s to look at additional sources of stem cells. Human embryonic stem cell lines have been established in vitro. The ability to grow pluripotent human embryonic stem cells in vitro might provide an inexhaustible source of stem cells for therapeutic and research purposes. Early experiments have shown that cell lines derived from human embryos may have both unlimited self-renewal capacity and differentiation potential. The implications and promise for therapy have become enormous. We are at the beginning of developing research approaches to use this source of stem cells to benefit humanity, but the challenges are formidable. We should apply the same stringent biological principles to characterize human and mouse embryonic stem cells that McCulloch and Till used to successfully characterize the HSCs of mouse bone marrow 45 years ago: Do they self-renew and differentiate extensively as measured by serial transplantation and long-term reconstitution?

Many researchers continue to observe in animals that bone marrow cells in general and in some cases a marrow population enriched for stem cells can repair many types of injured tissues. Thus, bone marrow cells appear to be remarkably plastic, transforming into cells of the liver, lung, and brain upon transplantation into injured recipients. Of particular note, in an animal model of myocardial infarction (Orlic et al., 2001), the direct injection into heart muscle of a stem cell enriched population from bone marrow or mobilized peripheral blood HSCs resulted in an improvement in cardiac muscle function. This has led to clinical trials in patients with heart disease with early results showing some improvement. Whether this is really plasticity remains a matter of debate, but importantly, these studies have led to the identification of cardiac stem cells that may be useful for therapy in the future. Scientists are now searching for stem cell populations in many different tissues hoping that their isolation will lead to a therapeutic approach for regenerative medicine.

Cancer Stem Cells

After establishing the basic tenets of stem cell biology, McCulloch and Till turned their attention to leukemia. From the late 1970s until today, the Toronto group has been instrumental in comparing the properties of normal stem cells with those of malignant cells. They predicted that in acute myelogenous leukemia the culprit was abnormal gene expression rather than a block in differentiation. They found that in myeloid cell lines self-renewal could be equated with immortality and suggested that determination of differentiation in leukemia was promiscuous. This infidelity could be a result of genetic reprogramming similar to the plasticity that has most recently been observed with normal stem cells. As a result of these studies, the concept evolved that within a tumor there is a small population of initiating cells that are now termed cancer stem cells. Currently, this concept is under intense investigation. Solid tumors of tissues such as breast and lung are being examined to identify this rare cell type. It is hoped that removal of this initiating cell by selective destruction

will lead to more efficacious therapies. Once again, characterization of the proliferative and differentiation capacities of these abnormal stem cells follows the biological properties outlined by those earlier pioneering studies of McCulloch and Till.

Concluding Remarks

The importance of stem cell biology should not be underestimated given the potential for these cells to repair injured tissues, to become the target for destruction following malignant transformation, and to allow us to understand genetic and epigenetic changes during cellular proliferation and differentiation. Forty-five years ago, two basic scientists found nodules on the spleen of irradiated rodents transplanted with donor bone marrow. These nodules represented for the first time the clonal expansion of a stem cell. The cell itself would be difficult to capture, but McCulloch and Till developed an assay to study its behavior. When new students join my lab, I insist that they set up a spleen colony assay. The assay teaches techniques such as intravenous injection and sterile handling of bone marrow cells. It also shows reproducibly the clonal nature of stem cells as the students carry out the same cell dose-response curve for injected bone marrow cells that McCulloch and Till used to so elegantly show that the number of spleen colonies is directly proportional to the number of bone marrow cells injected.

I can think of no more deserving a team for the Lasker award than that of Earnest McCulloch and James Till, whose revolutionary studies ignited the field of stem cell biology so many years ago.

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