

Stem Cell–Based Medicine¹

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THE CONCEPT OF A STEM CELL is a singularly optimistic idea: a cell imbued with two extraordinarily generative properties. First, the capacity for ongoing self-replenishment, and, second, the ability to produce daughter cells that can make all mature cell types. It is a concept that logically follows the cell theory of life first proposed by Schleiden and Schwann in 1839: everything is made up of organized units called cells. Virchow extended that theory to state that all cells derive from other cells, and, therefore, logically there must be progenitor or stem cells.

However, the stem cell as a stated entity of mammalian biology was not formally articulated until a lecture given by Maximow to the Hematologic Society of Berlin in 1909 (Maximow 1924). In his speech, he proposed that blood contains a cell type that can self-renew and give rise to all mature blood cells. The concept made intuitive sense and led to efforts to define the genealogy of blood cells. What mature blood cells were related to immature cells that might reside in the bone marrow or spleen? Lineage tracks were proposed, but the root of the tissue lineage, a stem cell, remained elusive.

When Till and McCulloch presented their near-perfect experiment in 1963, the concept of the stem cell was finally made real (Becker et al. 1963). The experiment they performed took advantage of the complementary expertise of radiation biology and stem cell biology that the two brought to the task. Till and McCulloch took bone marrow from one animal and transplanted it by intravenous injection into another animal that had been irradiated with lethal doses of gamma irradiation. The recipient animals were rescued by the donor marrow, generating blood from cells produced in the recipients' marrow and spleen. The

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spleen ended up being the critical issue; marrow either has cells or doesn't have them, but when cells are there they do not retain a cohesive architecture. Rather, they become admixed.

But in the spleen, cells remain closely packed when one cell divides to form many others. Therefore, the cells that engraft in the spleen form a cluster or "colony" of cells. Those could be shown to contain an array of blood cells, suggesting the broad potential of the cell that initiated the colony. However, a means of definitively demonstrating that the cells were truly clonally derived, that is, derived from a single stem cell, was needed. Till and McCulloch ingeniously accomplished this by taking advantage of the known ability of irradiation to cause DNA damage detectable by crude examination of chromosomes. By exposing animals to sub-lethal irradiation immediately after transplantation, they were able to induce traceable chromosomal abnormalities. These abnormalities were randomly generated, and so, if a cell survived, it had a signature abnormality in its chromosomal structure that could be detected in descendant cells. By examining many cells within a colony and finding the same chromosomal change (karyotype), they confirmed that each colony came from a single cell. By then also transplanting some of those cells into a second animal and showing that colonies again formed, they confirmed the self-renewing capability of the source cell and thereby experimentally validated the existence of the stem cell. These were the seminal experiments in stem cell biology, establishing the existence of stem cells in adult tissues.

With adult stem cells defined, and the impact of radiation exposure on people recognized through experience with nuclear weapons, bone marrow was tested as a source of stem cells in humans. The dawn of stem cell medicine was thereby begun. What was not clear at the time was that the presence of stem cells was more than just a curiosity of the blood. Many adult tissues now appear to have primitive cells contained within them that can serve to replenish mature cell types. Now, the challenge is to better define these cells and use that information to achieve regeneration of other tissue types. Much of the detailed information we have about adult or somatic stem cells has been learned from studying the blood. It is still not clear just how representative that tissue is and how generalizable principles learned from it may apply to stem cells in other tissues. In particular, it is still to be defined whether the practice of stem cell medicine for blood diseases will be useful in other types of conditions. At present, stem cell therapy for hematologic disease and cancer is limited to cell replacement—an appealing, but excessively simplistic, constraint on the possibilities for stem cells in medicine.

Stem cell biology will almost surely have a much broader array of uses in medicine than currently conceived. The popular conception of

stem cell approaches to disease resembles automobile repair: create an exchangeable part, lop out the old, and insert the new. This model is an easy extension of the success of bone marrow transplantation for conditions like aplastic anemia and leukemia. However, achieving this kind of tissue replacement is uniquely suited to the blood system. Blood stem cells, by nature, circulate in the bloodstream and find their way to the niche that supports their function, producing mature blood cells. So a bone marrow transplant need only inject blood stem cells into the bloodstream, and they will engraft in the bone marrow and become fully integrated as the blood-producing tissue of the body. It will clearly be a task of a different magnitude to deal with complex, fixed tissues of complex order, such as the nervous system or heart.

However, in addition to replacing injured tissues by transplantation, other opportunities have emerged. These include using the stem cells as a target for drug-based therapies and using stem cells in models of disease.

The idea of stem cell-targeting drugs is dependent upon the presence of stem cells in adult tissues. Quietly, this science has developed dramatically over the last ten years. It has become clear that mammalian tissues such as the brain, heart, intestine, bone, muscle, and eye maintain a population of primitive cells that serve as a stem or progenitor (a population with less self-renewal capacity than a stem cell) pool to maintain tissue integrity. Virtually all tissues have some ongoing loss and have evidence of some ongoing production of mature cells under specific circumstances. This is true even for tissues previously thought to be relatively inert in cell production beyond a particular stage of development, such as the heart. For some of these tissues, cell production is at a very low level in the body, yet the stem cells themselves have considerable potential to divide and produce new cells. A particularly striking example is the brain. When some brain stem cells are isolated and cultured in a Petri dish, the stem/progenitor population robustly grows. We know how poorly these cells respond to *in vivo* tissue injury; stroke victims do not have the prospect of substantial brain regeneration. This being the case, the context in which the stem cells reside clearly has a weighty impact on the way they function. Context should be considered on both a macro level, in terms of the state of the organism (young, old, dealing with systemic illness), and a micro level (the immediate neighboring cell types and their products). The micro level is also known as the "stem cell niche," and is the critical determinant of whether stem cells persist, whether they expand, and whether they generate daughter cells that can mature and replenish the mature cells of the tissue.

The stem cells in residence in tissues represent potential opportunities for therapeutic intervention. Understanding the components of their regulatory niche and how those components govern the stem cell

represents a presently underexploited opportunity in regenerative medicine. For example, my laboratory has been interested in discerning what bone does for bone marrow. Blood cell production occurs in the bone marrow, yet it has been entirely unclear what bone might be providing for the blood-forming cells in the bone marrow. The bone must be more than a site of convenience for blood-forming stem cells to reside in. To explore this, we and others undertook model systems whereby we could genetically modify bone components, either the bone-forming cells called osteoblasts, extracellular matrix glycoproteins that are abundant in bone, or even the unique mineral composition of bone (Adams et al. 2005; Calvi et al. 2003; Nilsson et al. 2005; Stier et al. 2005; Zhang et al. 2003). In each of these contexts, we were able to document a role in regulating the number or function of hematopoietic stem cells. These studies demonstrated the critical role of bone in blood stem cell function in the body.

Activating the osteoblast with either a constitutively active parathyroid receptor hormone (a hormone long recognized as critical for new bone formation), eliminating osteopontin (a matrix protein of bone) by genetic deletion, or altering the ability of stem cells to respond to the ionic calcium of the bone, changed blood stem cells. Osteoblast activation changed the number of stem cells in our model and an independent model using a different receptor modification (Calvi et al. 2003; Zhang et al. 2003). The deletion of the osteopontin gene led to an increase in the number of stem cells, and the absence of the calcium-sensing receptor on stem cells led to their inability to localize or engraft in the bone marrow. Interestingly, each of these interactions has a potential drug therapy opportunity, as each is the target of drugs that are already on the market for other purposes.

One such application is the simple re-purposing of parathyroid analogues currently used for the treatment of osteoporosis. These drugs are known to activate and increase in number the very cells we demonstrated are important in governing how the blood stem cell functions. Testing whether analogues of this hormone would affect blood stem cells in mice, we observed three highly clinically relevant effects. First, the hormone increased the number of stem cells that could be generated and potentially used in a donation of stem cells for subsequent use in transplantation. Second, it protected stem cells from the adverse effects of sequential chemotherapy. Third, it enabled a more potent regeneration of the bone marrow of transplanted animals, leading to a substantial improvement in mortality when limiting numbers of stem cells were used (Adams et al. 2007). These are all settings encountered regularly in the care of patients with hematologic disease, including a wide range of malignancies. This has led to active clinical trials testing

whether the compounds used for treating osteoporosis can have an impact on the outcome for patients undergoing stem cell transplantation for leukemia, lymphoma, or multiple myeloma.

In a somewhat different scenario, it may be possible to target the stem cells directly, rather than through intermediate niche components. We recently demonstrated one such example targeting the mesenchymal stem cells that are known to be present in bone and are capable of generating bone, cartilage, muscle, and fat (Mukherjee et al. 2008). We used a currently marketed drug, a proteasome inhibitor, that had been developed for the treatment of patients with multiple myeloma. Myeloma is a lethal malignancy of antibody-producing lymphocytes that is associated with profound erosions of bone. The drug had been observed to reduce the number of malignant cells and to improve the bone lesions.

Interestingly, some patients did not experience an improvement in the tumor, yet still had some benefit in terms of bone disease. We found that the drug acts on mesenchymal stem cells, driving their differentiation toward bone-forming cells even when used at very low doses. This effect resulted in an improvement in bone parameters when the drug was used in a mouse experiencing the osteoporosis of estrogen loss after menopause. Therefore, drugs might also be developed that can directly affect stem cell behavior to encourage more robust regeneration, forcing the cells to respond more vigorously. Such strategies would be of great potential benefit if they could be applied to stem cell populations that exist in other tissues where repair is often poor after injury. The brain and heart are organs of obvious interest, but considerable basic research is required to determine whether the principles of the bone and bone marrow can be applied.

One unanticipated outcome of increased attention to stem cell biology is a changing paradigm for cancer and cancer therapy. Normal tissues are organized so that a very tiny fraction of the cells represent self-renewing stem cells. All of the more specialized cells that make up the tissue are derived from the stem cells, but, unlike stem cells, they do not have the capacity to self-renew and are generally destined for death. A hypothesis has been put forward that cancer tissue may be similarly organized. This hypothesis has been tested in the blood due to the availability of tools for the identification of subsets of cells enriched for stem cells in the normal bone marrow. Using similar tools, investigators assessed human leukemias and also found such a population of self-renewing cancer stem cells (Lapidot et al. 1994). These cells were a fraction of the cancerous cells, but were the cells responsible for the persistence and spread of the cancer. If other fractions of the cancer cells were used, they did not produce or maintain cancer. For example,

methods used to isolate normal stem cells could be used to isolate a fraction of leukemic cells that could reconstitute leukemia, whereas other subgroups within the leukemia did not. These studies demonstrate that there are indeed malignant cells that meet the criteria of stem cells in cancer. This has been shown for leukemia and now in a wide range of other human tumors as well. Since the relevant population that enables cancer persistence is small, the current practices used for drug development may not detect an effect on cancer stem cells. This concept has led to a re-thinking of the way cancer drug therapies are developed. If the cancer stem cells can be identified and followed, these cell types may indeed be the most important to monitor in our efforts to eradicate disease. If we simply look at what happens with the bulk of the tumor (the current means by which cancer drug therapies are developed), we may find only drugs that improve, but do not root out, disease. The cancer stem cell has been likened to the queen bee in a beehive. If you eliminate the worker bee population in a tumor without eradicating the queen, the hive will come back. If, however, you eliminate the queen, no matter how many workers remain, the hive will eventually die out. The cancer stem cell hypothesis and experimental support for it have provided a very different way of thinking about developing oncology drug products.

An extension of the cancer stem cell hypothesis is the potential dependence on a stem cell niche. We know that cancers do not metastasize just anywhere; there are favored sites that reflect at least some degree of dependence on the microenvironment. The nature of the niche and the sensitivity of the cancer stem cell to it may be quite different from those of normal stem cells of the same tissue. This raises intriguing possibilities. Might we be able to identify niche-acting drugs that can change the microenvironment of the cancer stem cell, making it more hostile for that cell type? Might compounds be found that foster support of normal stem cells over that of malignant stem cells and thereby change the kinetics of cancer? Might inhibiting the cancer stem cell interaction with its niche render the cancer stem cell more sensitive to drug-induced apoptosis? All of these issues are under active investigation and represent unique dimensions of the stem cell field not often appreciated in the popular press.

The final area where emerging stem cell biology is likely to have an impact is in modeling chronic human disease. We currently have very poor models for many complex and devastating human ailments. Recent work demonstrating that a mature cell, such as a skin cell, can be re-programmed to become a pluripotent stem cell (iPS) has opened enormous opportunities for understanding and developing drugs for these conditions (Park et al. 2008; Takahashi et al. 2007; Yu et al. 2007).

Perhaps the best example is Huntington's disease, a notoriously malicious X-linked neurodegenerative disease that has virtually 100% penetrance and fatality, but induces symptoms only as men approach middle age, often after reproduction. We currently cannot study the neural cells that are affected by this disease. If we can generate pluripotent cells from the skin cells of people known to carry the genotype of the disease, we can then use these iPS cells to generate the types of neurons known to be affected by the disease. When we have abundant numbers of such cells in hand, they can then become the reagent against which pharmacologic agents can be tested. In addition, they can become the reagent for identifying other genes that may alter the state of disease. The advent of iPS, therefore, has become an enormous opportunity to begin to better understand and treat some of mankind's worst threats.

Stem cell biology is therefore providing us with specific tools that may enable better drug and cell therapies for disease. It is changing our thinking about tissue organization under normal and malignant conditions, affecting what we can expect from other therapies of the future. A nation's investment in this field has been mired in political and ethical controversy that has limited advances and discouraged the young from becoming involved. It is time for the science to take center stage and for the minds of the young to engage this promising field so full of optimism and opportunity.

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