

Stem Cell Research

Paths to Cancer Therapies and Regenerative Medicine

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MANY, IF NOT MOST, TISSUES in complex metazoans contain a rare subset of primitive cells that, at the single-cell level, can self-renew as well as give rise to mature, differentiating daughter cells. It is likely that such cells arose in development to build tissues and organs and are retained in adult life to regenerate these tissues. In the best-studied model, mouse hematopoiesis, long-term self-renewal is reserved for hematopoietic stem cells (HSCs); all of the downstream oligopotent progenitors lack long-term self-renewal and depend on HSC input for their regeneration over time.¹⁻⁴ (Exceptions are the “memory” T and B lymphocytes.) Thus, self-renewal is invested in a rare population of stem cells in tissues, and these cells are the essential and perhaps the only cells important for lifelong tissue regeneration.

But self-renewal is a potentially dangerous property: many cancers, leukemias, and lymphomas contain at least some cells called cancer stem cells (CSCs) that possess self-renewal capacity, albeit in a poorly regulated form.⁵ Therefore tissue development and regeneration and its regulation, as well as cancer development and spread, are properties of different kinds of stem cells, united by the property of self-renewal. This article describes how the thinking and methods that led from the speculation that stem cells exist to their

Most tissues in complex metazoans contain a rare subset of cells that, at the single-cell level, can self-renew and also give rise to mature daughter cells. Such stem cells likely in development build tissues and are retained in adult life to regenerate them. Cancers and leukemias are apparently not an exception: rare leukemia stem cells and cancer stem cells have been isolated that contain all of the tumorigenicity of the whole tumor, and it is their properties that will guide future therapies. None of this was apparent just 20 years ago, yet this kind of stem cell thinking already provides new perspectives in medical science and could usher in new therapies. Today, political, religious, and ethical issues surround embryonic stem cell and patient-specific pluripotent stem cell research and are center stage in the attempts by governments to ban these fields for discovery and potential therapies. These interventions require physicians and physician-scientists to determine for themselves whether patient welfare or personal ethics will dominate in their practices, and whether all aspects of stem cell research can be pursued in a safe and regulated fashion.

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prospective isolation provide new perspectives in virtually all aspects of medical science and could usher in newer and less toxic, curative therapies.

Prospective Isolation of Adult or Tissue Stem Cells

In the 1960s, Till and McCulloch and colleagues provided genetic marking evidence that in mouse bone marrow there are rare cells that can form myeloid colonies in the spleens of irradiated mice, some of which contain cells that can self-renew.^{6,7} It was reasonable to propose the existence of HSCs from these experiments,^{8,9} although later experiments showed that the colony-forming cells were defined myeloid progenitors.¹⁰ The proposal that HSCs probably exist set the stage for prospective isolation of these cells.

The isolation of HSCs required several conceptual and technical advances, including monoclonal antibodies to cell surface proteins,¹¹ high-speed multiparameter cell sorters,¹² and the establishment of clonogenic assays for all blood cell lineages.¹³⁻¹⁷ In a preclinical setting, the cells were shown to regenerate the blood-forming system of lethally irradiated mice.^{11,17} These assays eventually led to the prospective isolation of clonogenic mouse¹ and human¹⁸ HSC populations that self-renew and include in their clonal prog-

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eny all blood cell types.^{19,20} The downstream progenitors in the hematopoietic lineage in mouse and humans have also been isolated.^{3,4,21-23}

The same method was used to isolate a human central nervous system stem cell (hCNS SC) population²⁴ which, at the single-cell level, could give rise to spheres of neural cells that can differentiate to oligodendrocytes, astrocytes, and neurons, as well as self-renewed hCNS SCs.²⁵ Implantation of these cells into the lateral ventricles of newborn immunodeficient mice brains led to site-appropriate seeding of neurogenic zones (subventricular zone of the lateral ventricles and the dentate gyrus of the hippocampus²⁵) by these stem cells, where they self-renew for the life of the mouse. Their daughter cells migrate to sites where they differentiate into the neural cell types and the specific tissue architecture specified by the mouse brain regions they occupy.

These results revealed an unexpected aspect of stem cell biology—the cues for self-renewal, site-specific migration, and site-appropriate differentiation and placement into the microarchitecture, at least for brain stem cells, appear largely to be conserved between mouse and humans. This becomes important in studying preclinical capacities of stem cell therapies, for understanding the behavior of brain cancer stem cells, and for neurobiology. Investigators can now study human neuronal cells in the context of the mouse brain, which should be valuable in the fields of neurodevelopment, function, and perhaps neuropathologies. These neural stem cells can be genetically modified, allowing the opportunity to study particular genes in human neural cells in situ for their role in neurological functions.

Stem cell isolation is now about 17 years old, yet only a few tissue stem cells from mouse or humans have been prospectively isolated to date, and really only HSCs have been transplanted in humans to regenerate any tissue. Yet tissue and organ transplants have shown the need, and the feasibility of regenerative medicine, and so the field has

a long way to go before we understand and exploit their potential.

Preclinical and Clinical Transplantation of Stem Cells and Oligolineage Progenitor Cells

Most clinical hematopoietic cell transplants now use mobilized peripheral blood (MPB), which results in rapid (10- to 13-day) engraftment; this is due to increased numbers of HSCs in MPB vs marrow.^{26,27} Unfortunately, because the clinical community has accepted the term “stem cell transplants” to include a variety of hematopoietic transplants, only the most savvy oncologist will recognize the difference between unpurified mobilized blood cell transplants and HSC transplants. The following proposed terminology may help clarify this problem: all transplants could be called *HCT* for hematopoietic cell transplantation. Mobilized peripheral blood could be called *MPB*; bone marrow, *BMT*; umbilical cord blood, *UCB*; *CD34*⁺-enriched transplants, *CD34 HCTs*; and true stem cell transplants by their identifying characteristics, eg, *CD34+Thy1+HSC*. That way the reader can know what was actually done, rather than requiring credentials in stem cell biology.

For treatment of patients with cancer with their own hematopoietic cells following myeloablative chemotherapy, only purified HSCs were free of cancer cells,²⁸ and these were used in several clinical trials.²⁹⁻³²

In the first human transplantations between HLA-matched siblings, the donor T cells that are present at high levels in bone marrow and MPB (and UCB) recognize the host as foreign and carry out a potentially lethal graft-vs-host disease (GVHD). In mice, rapid and sustained engraftment with pure HSCs could be accomplished without GVHD.³³⁻³⁶ In allogeneic transplants between major histocompatibility complex (MHC)-matched (HLA in humans, H2 in mice) but otherwise genetically distinct pairs, the main requirement for successful engraftment

is temporary lymphoablation of T cells.^{33,35} Even unirradiated immunodeficient mice can achieve 0.1% to 1% donor cells with HSC transplants (D. Bhattacharya, D. Rossi, D. Bryder, I.L. Weissman, unpublished data, 2005). But when the donor expresses unshared MHC alleles, one must also eliminate host natural killer cells,^{35,37} which can kill or reject tissue cells.³⁸ In mice monoclonal antibodies that eliminate host natural killer cells are essential for partial or unmatched HSC or HCT engraftment. Using sublethal irradiation plus anti-T and anti-natural killer antibodies, a “safe” regimen, mice can be transplanted with pure HSCs and be lifelong chimeras without host rejection of the graft or GVHD.³³⁻³⁵

Purified hematopoietic progenitors can also be useful in particular circumstances. For example, mice exposed to murine cytomegalovirus, *Aspergillus fumigata*, or *Pseudomonas aeruginosa* in the immediate post-HSC transplant period die rapidly because their immune defenses are weak. Cotransplantation of common lymphoid progenitors with HSCs blocks murine cytomegalovirus mortality, even if the common lymphoid progenitors are from fully allogeneic donors.³⁹ Similarly, cotransplantation of common myeloid and granulocyte-monocyte progenitors precludes lethality with *Aspergillus* or *Pseudomonas*, again even if the donor is fully allogeneic.⁴⁰

In another example, whole body just-lethal irradiation or exposure to myeloablating chemicals is possible through neglect, intention, and war. The only sure way to be saved from doses of radiation that cause hematopoietic failure, but not irreversible gut damage, is to be transplanted with sufficient numbers of HSCs, requiring some kind of HLA match. However, some progenitors in mice can be radio-protective until the rare surviving host HSCs can regenerate the system.¹⁰

While hematopoietic regeneration is a well-developed field, CNS regeneration is still only experimental. Given that human CNS stem cells engraft and migrate widely in site-appropriate man-

ners in immunodeficient mouse brains (and presumably human brains), several experimental models of neural repair are ongoing. Patients and mice with lysosomal storage diseases, such as Batten disease, undergo both systemic and neural degeneration. Provision of the missing enzymes systemically can result in uptake of the enzymes by diseased cells in the body, but not the brain, and ameliorate systemic disease. Transplantation of hCNSC-derived populations into mice affected with Batten disease results in amelioration of the neurodegeneration in all parts of the brain.⁴¹

Compression injuries of the spinal cord often result in paralysis following local inflammatory events, and among the pathological hallmarks are areas of cord demyelination.⁴² Transplantation of hCNS SCs/neurospheres into immunodeficient mice that had a controlled crush injury at T9 about 9 days after the injury led to cell engraftment-dependent recovery of hindlimb paralysis and coordination.⁴² The engraftment was mainly oligodendrocytic, resulting in effective remyelination, and sustained presence of the graft was required for sustained recovery.⁴²

In these 2 examples neuroprotection was afforded by injected stem cells and depended on their ability to differentiate, migrate appropriately, and function. It is yet to be determined whether regeneration of neural circuits by replacement with cells derived from CNS SCs can occur, and of course, it is yet to be determined which neurodegenerative diseases sustain neuron loss by direct effects on the neurons vs their nurturing environments.

HSC Allografts Induce Lifelong Transplantation Tolerance: The Beginnings of Regenerative Medicine

When HSCs engraft in fully myeloablated/lymphoablated mice, the blood-forming and immune systems are largely, if not completely, derived from the donor. Donor cells that enter the thymus give rise to T cells as well as donor-derived antigen-presenting den-

dritic cells. The dendritic cells, along with the host thymic medullary epithelial cells, delete developing T cells with reactivity to self-proteins that could be expressed in any tissue or organ from donor or host. Thus these HSC chimeras produce T-cell populations that cannot make immune reactions against donor or host but are capable of making other protective immune reactions for the host. As a result these reconstituted mice are permanently transplantation tolerant of grafts of any cell, tissue, or organ from the HSC donor.³⁷

Tolerance induction for tissue and organ grafts should eventually be followed by cotransplantation of HSC- and tissue/organ-specific stem cells from the same donor source.^{34,37} This might be achieved not only from living donors but eventually from classic embryonic stem cell lines or from donor-specific nuclear transfer stem cell lines.⁴³⁻⁴⁵

These possibilities could usher in the era of regenerative medicine, in which curative intent regeneration of diseased organs and tissues can be achieved with stem cells, rather than chronic support with drug therapies. However, thus far the only cell of choice for these regenerative medicine therapies are the self-renewing tissue stem cells rather than more transient progenitors or actual mature, functional cells.

HSC Therapies and Autoimmune Diseases

Several autoimmune diseases have genetic predilections.⁴⁶⁻⁴⁸ In mouse models of these diseases (eg, type 1 diabetes), some of the actual genes involved have been determined, while the rapid advances of genomics will reveal others. In the case of type 1 diabetes, a progressive disease that results in the emergence and activities of anti-islet β cell inflammatory and cytotoxic T cells,⁴⁹ many of the genes are expressed in the progeny of HSCs.^{49,50} It was logical that donors expressing disease-resistance genes might be effective donors for bone marrow⁵⁰ or HSC^{51,52} transplants to hosts with these autoimmune diseases.³² Such allogeneic HSCs can, in fact, block the autoimmune progression of estab-

lished diabetic immune activities in both myeloablative and nonmyeloablative settings, which should lead to clinically acceptable protocols if these findings extrapolate to humans.³⁷

Other autoimmune diseases that have known genetic predilections include multiple sclerosis, rheumatoid arthritis, inflammatory bowel disease, and ankylosing spondylitis.^{52,53} Therefore it is reasonable to propose that when human nonmyeloablative conditioning protocols are developed that allow purified HSC transplants to succeed and to establish lifelong hematolymphoid chimerism, autoimmune disease therapies might be treated by one-time curative intent stem cell regenerative medicine therapies.

Unfortunately, by the time many patients with these destructive autoimmune diseases are treated by HSC therapies they already have irreversible destruction of host tissues, such as β islet cells in well-established type 1 diabetes or oligodendrocyte loss in advanced multiple sclerosis. Thus these patients might require cotransplantation of donor HSCs to block autoimmunity and other organs or tissue stem cells to regenerate damaged tissues.

The extent to which endogenous cells can regenerate extensive lesions is unknown. However, claims that hematopoietic cells participate in conversion to other tissue stem and developed cells, so-called stem cell plasticity,⁵⁴⁻⁵⁸ are not reproducible.⁵⁹⁻⁶⁴ In type 1 diabetes, islets or islet stem cells from HSC donors will be required to regenerate islets to provide insulin, while in multiple sclerosis, CNS stem cells or oligodendrocyte progenitors might be required for remyelination.⁴²

Cancer and Leukemia Stem Cells Provide Unique Targets for Discovery and Therapy

Cancers are derived from an individual's own tissues and organs, usually by a multistep process that gradually changes a normal cell at a defined lineage step to a fully malignant entity. Normal stem cells and cancer/leukemia stem cells (CSCs/LSCs) are

both cell types that possess the property of self-renewal, whereas most other cell types do not. Malignant stem cells self-renew in a poorly regulated fashion, while normal stem cells are strictly regulated in their self-renewal. In the view that malignant stem cells exist and include nonmalignant cells in their progeny, the properties of immortality, invasiveness, tendency to spread, etc, could be properties of the subset of normal cells from which the malignant cells derived, rather than new properties acquired by these cells.⁶⁵ Therefore it became important to have knowledge of both normal stem and progenitor cells and malignant stem cells to determine which properties were new to the malignant cells. For example, HSCs and some progenitors regularly leave their bone marrow niches, enter the bloodstream, then find and engraft marrow niches at a distance.⁶⁶ Leukemia stem cells are derived from HSCs or progenitors; this explains why leukemias are never local to one marrow site.

Although ideas about CSCs/LSCs are at least 30 years old, the hypothesis could not be seriously tested until the steps in normal differentiation from stem cells were defined, and the cell populations in primary tumors prospectively isolated and subjected to a test of malignancy.

The first serious attempt to isolate human acute myelogenous leukemia (AML) stem cells was carried out by John Dick and colleagues, using an assay transplantation of leukemic subsets into immunodeficient mice.^{67,68} The investigators found that most AML samples they studied could only be transplanted with CD34+/38- cells, and therefore concluded that the LSCs were HSCs that had undergone the requisite steps to full malignancy. However, our laboratory had shown that in the CD34+/38- subset, only Thy1+/Lin- cells were HSCs.⁶⁹ To test the hypothesis of Dick et al, we determined the marker profile of aml1-Eto translocation AML: only CD34+/38- Thy1-Lin- cells contained LSCs, while all isolated CD34+/38- Thy1+Lin- HSCs gave rise

to normal multilineage colonies.⁶⁹ Interestingly, up to 40% of these HSCs had the translocation. Patients treated successfully retained about 1% of their HSCs as translocation positive for at least 150 months following treatment, suggesting that LSCs were not classic HSCs but were likely at the multipotent progenitor stage.^{69,70} Furthermore, the aml1-Eto translocation was only one step in the malignant progression and occurred in preleukemic HSCs.

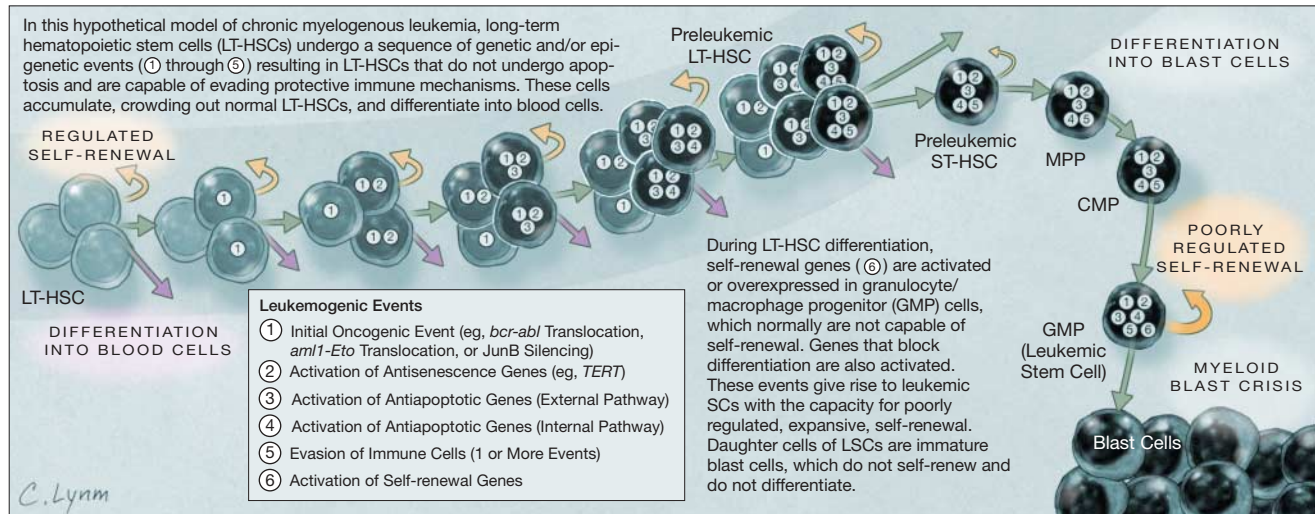
Because several gene products regulate normal HSC self-renewal,^{71,72} we began to test in other leukemias whether the LSC was at the stage of HSC or whether they occurred at a later stage of differentiation and had appropriated hematopoietic self-renewal genes. Along with Clarke, Morrison, and Reya, I proposed a framework of studies to reveal CSCs/LSCs.⁵ Clarke et al had isolated from primary human breast cancers a minor subset of CD44+/24- cells that could transplant the tumor in the mammary gland of immunodeficient mice.⁷³ The resultant tumor had all the histopathological hallmarks of the original tumor, containing 1% to 5% CD44+/24- cells; only these CSCs could retransplant the tumor.⁷³

Mouse models to reconstruct myeloid leukemogenesis showed that the distinct pathways of intrinsic programmed cell death were blocked, the emergent LSCs had evaded both T-cell and macrophage surveillance, these cells overexpressed telomerase reverse transcriptase to evade senescence,⁷⁴ and the AML LSC was at the stage of the granulocyte/macrophage-restricted progenitor (GMP) (C. Jamieson, I. Weissman, unpublished data, 2005).⁷⁵⁻⁷⁷ These LSCs overexpressed β -catenin. In one mouse model, a chronic myelogenous leukemia (CML) syndrome could be induced by blocking the expression of the transcription factor JunB; only HSCs could transplant this CML,⁷⁵ mimicking human *bcr-abl* translocation CML, in its chronic phase. Chronic phase human CML HSCs contain the *bcr-abl* translocation, with normal levels of activated, nuclear β -catenin, a stem cell self-renewal protein^{71,72}; this is not different

from normal HSCs.⁷⁸ At myeloid blast crisis there was a 6-10 fold increase in GMP; these GMPs could replat self-renewing cells in vitro, unlike normal GMPs, and are blast crisis candidate LSCs.⁷⁸ These myeloid blast crisis GMPs expressed high levels of activated nuclear β -catenin, and reported activation of β -catenin target genes. Furthermore, transfected axin, a specific inhibitor of the catenin pathway, blocked leukemic GMP replating.⁷⁸

These findings lead me to propose here a multistep model for myeloid leukemia progression (FIGURE). Important in this formulation is the fact that multiple independent genetic and epigenetic changes must occur to drive a normal cell to an LSC. In the case cited above, the LSC in myeloid blast crisis CML is at the stage of the GMP, normally not a self-renewing population. Most progression events in leukemogenesis would not endow a cell with self-renewal, and if the event occurred in GMP, the short natural life of that altered cell would guarantee that it would not be a sustained event.⁵ Each event to be sustained must be in a self-renewing population. In CML that first event is likely to be the *bcr-abl* translocation in HSC. In AML, it could be *aml1-Eto*. As the single cell that sustained the *bcr-abl* translocation expanded its progeny in the HSC pool, a second rare event, perhaps epigenetic, probably occurred in a daughter cell, and that clone with events 1 and 2 slowly expanded to a size that a third event was possible, and so on. In that model all events except activation of a self-renewal pathway occurs at the stage of long-term HSC in the clone that has sustained the previous events. In this view, only the LSCs have sustained all of the events, especially poorly regulated self-renewal.

Knowing that, isolation of LSC (or other CSCs) could allow for the first time a detailed assessment of these events. This will require a deep genomic analysis of the isolated LSC; since LSCs are less than 5% of the marrow cells in the leukemia, analysis of the entire leukemia would be interesting, but

Figure. Hypothetical Model of Leukemic Progression

CMP indicates common myeloid progenitor; MPP, multipotent progenitor; ST-HSC, short-term hematopoietic stem cell; and TERT, telomerase reverse transcriptase.

not definitive. Patients having these leukemias likely have still existing in marrow HSC clones with n-1, n-2, n-3, n-4, etc, events. In this way the order of events should be knowable, and in analyzing several patients, whether the order is always the same.

By knowing the events that had occurred in LSCs/CSCs, particular molecular targets for drug and immune therapies can be tested. If LSCs/CSCs are isolated and transplanted into immunodeficient mice, such tumor-bearing mice should be useful for pre-clinical testing of diagnostics and drug and immune therapies.

The Potential of Embryonic Stem Cells and Nuclear Transfer-Produced Pluripotent Stem Cells in Human Medicine

About 7 or 8 cell divisions after fertilization of the vertebrate egg, the preimplantation blastocyst is formed and arrives in the uterus. The outer shell of cells (trophoblast) of the blastocyst is adapted to bind to the uterine wall and begins to form the placenta; these cells do not contribute to the embryo. The 15 to 30 cells inside the trophoblast shell will produce all of the tissues and organs of the embryo and subsequent fetus. Many if not all of these inner cell

mass cells are pluripotent, that is, at the single-cell level can contribute to all tissue and organ cells.

Methods to culture these inner cell mass cells to produce embryonic stem cells were accomplished in mouse models in the 1980s^{79,80} and adapted to produce embryonic stem cells from human blastocysts.⁸¹ Mouse and human embryonic stem cell lines are propagated in specialized media to produce very large numbers of embryonic stem cells; changing the conditions in vitro allows them to form disorganized collections of all tissue types in the embryo, called embryoid bodies. The embryonic stem cells are self-renewing in vitro, and single embryonic stem cells contribute to all tissue types; hence, they are pluripotent. Mouse embryonic stem cells have been of inestimable value in studying normal and pathological development, partly by studying their development in vitro, and by injecting them into host mouse blastocysts prior to implantation, wherein they contribute to all tissues and organs. Genetic modification of the embryonic stem cell lines allows the effects of the added or subtracted gene to be tracked in the context of the body and has led to a renaissance of studies of physiology and pathology, as well as

developmental biology. But the human embryonic stem cell lines are defined by the source of blastocysts, assisted reproduction clinics, which neither represent the full genetic diversity of humans nor, with rare exceptions, any definable human genetic disease.

A new technology called nuclear transfer allows the production of embryonic stem cell lines from predefined donors, including patients with genetically determined or influenced diseases. This involves removing the chromosomes of an unfertilized oocyte, replacing them with a nucleus from a somatic cell (eg, skin) to form a pseudozygote. The inner cell mass cells from these embryoid blastocysts were used to produce nuclear transfer pluripotent stem cell lines (nuclear transfer stem cells). In mice it has been shown that one can produce nuclear transfer stem cell lines from mature cells, eg, lymphocytes, odorant receptor neurons, and skin cells.^{43,82,83} In all of these cases the chromosomal makeup of the cell lines is derived from the donor nucleus, while the mitochondrial DNA is largely from the oocyte; because they are mixed chromosomal/mitochondrial composites, they are not clones.

Production of nuclear transfer stem cells from mice with a genetic severe combined immunodeficiency results in stem cells that can make every cell in the body except lymphocytes. Correction of the defect with the correct gene at the nuclear transfer stem cell level results in repaired stem cells whose blood-forming system can be manipulated and transplanted into the original immunodeficient line to ameliorate or cure the disease. Production of nuclear transfer stem cell lines for the production of patient-specific transplantable cells is popularly known as therapeutic cloning, but that is neither a scientific nor an accurate term. The term accepted by most responsible groups, including the International Society for Stem Cell Research⁸⁴ and the National Academies of Science, Medicine, and Engineering,^{85,86} is nuclear transfer stem cells. Clearly, production of patient-specific nuclear transfer stem cell lines offers an unprecedented opportunity to study these diseases.⁸⁷

Translating Embryonic Stem Cell and Nuclear Transfer Discoveries to Regenerative Medicine: Politics and Religion Collide With Medical Science

A recent report from the National Academies⁸⁵ has developed guidelines for the production and use of human nuclear transfer stem cells, including the production of human/mouse chimeras with nuclear transfer stem cell–derived adult stem cells.⁸⁵ There is ample precedent for transplanting human cells and tissues into immunodeficient mice: the first human HSC was discovered and validated by preclinical trials in such mice,¹⁸ as well as the first human CNS stem cell.²⁴

I have previously proposed⁸⁸ that human genetic disease pluripotent stem cell lines could be created using somatic nuclei from patients with these diseases in the nuclear transfer production of stem cells. Presumably, the tissue and organ stem cells derived from such patient-specific stem cell lines could be isolated and studied *in vitro*

and also transplanted into the cognate organs in immunodeficient mice using these techniques. If the transplanted tissue stem cells develop the same pathologies as the patients, a new era of studying disease pathogenesis will have arrived. This has precedence in mouse studies, as described above.

A huge investment has been made in the Human Genome Project, and through the application of those technologies to patient samples, many genes associated with diseases have been and will be identified and cloned. These represent target genes in the pathogenesis of these diseases. To validate which genes play a role in the particular disease vs those that are simply associated, gene correction of the disease genes can be accomplished in the pluripotent stem cell line, and the tissue stem cells derived from them transplanted to cognate organs in the mice to test whether such gene correction precluded disease development. The answers to these questions are of intense interest to those that wish to work with validated targets, from pharmaceutical companies testing small molecules on the proteins encoded by pathogenic genes to gene therapy companies hoping to correct the genes *in vivo*, to the stem cell therapy companies that need to know which cell types need replacement.

Patient-specific nuclear transfer stem cell lines are portable and should be available to the best and brightest of the biomedical community committed to study and treat these human diseases, which in aggregate are quite common. But to some individuals, working with authentic embryonic stem cells, taken from (and thus sacrificing) authentic embryo blastocysts with high potential to implant and develop in assisted reproductive technologies, is anathema. The religious and ethical background to such beliefs equates this ball of cells with born humans. Presumably, these individuals must oppose current assisted reproductive technologies, which are responsible for the legal disposal today of large numbers of unused blastocysts. Surprisingly, there is

even more objection to production of nuclear transfer stem cells, taken from embryoid blastocysts that have little or no chance to implant and produce a newborn in any species tested to date.⁸⁹ The arguments against these experiments are first that they enable human reproductive cloning, and second that scientists would be creating embryos solely for experiments, again embryos with the same rights as born humans.

The National Academy of Sciences panel I headed in 2002 voted unanimously to advise responsible entities to enact a legally enforceable ban against human reproductive cloning,⁸⁹ relying on the force of law to prevent a conspiracy of scientists, gynecologists, nurses, anesthesiologists, and hospitals from attempting this. The panel voted unanimously to advise responsible bodies to allow (and regulate) nuclear transfer stem cell research. In 2001, by executive order President Bush allowed human embryonic stem cell research on lines created before August 9, 2001, but not to fund any subsequent embryonic stem cell production or nuclear transfer stem cell production. So the major barrier to the derivation and the distribution of new embryonic stem cell lines or human nuclear transfer patient-specific pluripotent stem cell lines for widespread study into the pathogenesis and therapy of these human diseases is at this time national politics.

Currently, the few human embryonic stem cell lines derived from blastocysts that could be used for study using US government funds are likely not useful for therapies as they are potentially contaminated with pathogens from mouse cells.⁹⁰ Bills pending in Congress would create criminal penalties with \$1 million fines and 10-year jail sentences for those convicted of conducting human nuclear transfer research,⁹¹ or for patients treated with products derived from nuclear transfer research, or for physicians prescribing therapies derived from nuclear transfer research. While such research has been effectively halted in the

United States, the recent successes by Hwang et al to develop a method for efficiently producing human nuclear transfer patient-specific stem cell lines ensures that such cell lines will be available, if licensed from the Republic of Korea.^{44,45} California has taken a state's rights approach to the issue, passing a bill that approves and regulates new embryonic stem cell and nuclear transfer research via the legislature and signed by the governor. To ensure that such research would be carried forward, Californians passed by a 59%/41% majority a state initiative directly voted by its citizens, Proposition 71.⁹² Lawsuits by entities that objected to the passage of this proposition have prevented its enactment.

Governments have intervened previously on the type of biomedical research that can be done, such as famously occurred when Stalin took the advice of Trofim Lysenko and banned Darwinian genetics in favor of Lamarckian views. Russia suffered greatly for this ideological ban, and until recently could not participate in the biomedical revolution of modern genetics. In the United States, recombinant DNA research was opposed for safety, ideological, and religious reasons, but in the end it was regulated, not banned, ushering in the era of biotechnology that now cures and treats hundreds of thousands of Americans each year.

Physicians must wonder which precedents to choose and who is morally responsible for lives lost because of political actions. While some are relieved with a ban that places normal and embryoid preimplantation blastocysts beyond experimentation, they should understand that such a choice will certainly delay or prevent, at least in the United States, timely therapies derived from research for those patients who have a narrow window during which those therapies could help or save them. This is rightfully a debate as old as the Galileo case and the Scopes trial, and members of the biomedical community should learn and explain the alternatives to patients and to politicians.

Summary

The prospective isolation of stem cells and the studies of their biology have led to important new insights into biology, development, tissue regeneration, pathological conditions, and cancer. All of this comes from applying "stem cell thinking" to already-known processes. Even more applications to medicine are expected in the future, especially if all aspects of stem cell research can be pursued in a safe and regulated fashion.

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