0031-3998/06/5904-0001R
PEDIATRIC RESEARCH
Vol. 59, No. 4, Pt 2, 2006
*Printed in U.S.A.*


# Mammalian Stem Cells

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**ABSTRACT:** Stem cells are quickly coming into focus of much biomedical research eventually aiming at the therapeutic applications for various disorders and trauma. It is important, however, to keep in mind the difference between the embryonic stem cells, somatic stem cells and somatic precursor cells when considering potential clinical applications. Here we provide the review of the current status of stem cell field and discuss the potential of therapeutic applications for blood and Immune system disorders, multiple sclerosis, hypoxic-ischemic brain injury and brain tumors. For the complimentary information about various stem cells and their properties we recommend consulting the National Institutes of Health stem cell resources (http://stemcells.nih.gov/info/basics). **(*Pediatr Res* 59: 1R–8R, 2006)**

## EMBRYONIC STEM CELLS (ESCS)

***Isolation.*** In the mammalian embryo, following fertilization of the egg by a sperm, several cell divisions take place without any growth in total volume, so the cells (now called blastomeres) get progressively smaller. They also rearrange to form a hollow sphere of cells (blastocyst) surrounding a fluid-filled cavity called the blastocoel. The cells of the blastocyst then segregate into an outer layer called the trophectoderm, and an inner cell mass (ICM). The cells of the trophectoderm (trophoblasts) become the fetal contribution to the placenta, while the cells of the ICM give rise to the embryo proper. Around days 5–6 after fertilization in mouse and days 8–9 in humans the cells of the inner mass can be isolated and put in culture. The trophectoderm is removed and the ICM is plated on to a feeder layer of mouse or human embryonic fibroblasts (1), which is essential for the survival of the ICM (2). The ICM then flattens into a compact colony of ESCs, which is than mechanically dissociated and re-plated several times to give rise to stable cell line. It is important to stress that ESCs are an artifact of culturing and differ from the original ICM cells, notably in their pattern of epigenetic modifications. Remarkably, cultured ESCs retain pluripotency and are capable of generating all tissues (including germline) of chimeric mice after the injection into the blastocyst. When the adult chimeric mice have gametes generated from cultured ESCs, breeding of such chimeras will produce an animal composed entirely of the progeny of cultured ESCs. This event is called the germline transmission. It is critical to note that ESCs themselves cannot generate the entire embryo.

***Properties.*** Under certain conditions hESC populations can divide almost indefinitely while undifferentiated, but under other conditions they can differentiate into many cell types in the body (2–6). When undifferentiated hESCs are transplanted into an animal, they often form a tumor (teratoma) containing the cells from all three germ layers (7,8). Indeed, the ability of hESCs to form a teratoma after injection is the accepted criterion for identifying hESC lines as such. This is a very crude but necessary test of pluripotency in the absence of the techniques available in mice (blastocyst injection, chimeric mice and germline transmission). As a result, these limitations preclude testing if cultured ESCs are actually capable of generating the entire neuronal diversity of the human brain, for example, as this would require injection of undifferentiated hESCs either into human blastocysts or into the brains of human volunteers, followed by detailed neuroanatomical examination. AQ: 1

When cultured in the laboratory, hESCs grow as compact colonies and usually require the presence of feeder cells for their survival. hESCs can be cloned, albeit at very low efficiency (3). The feeder cells are typically mouse fibroblasts that have been treated with mitotic inhibitors to prevent their proliferation. Human feeder cells can be used as well as feeder-cell conditioned medium, which presumably contains appropriate growth factors (9–11). Recently, bFGF was identified as the factor secreted by fibroblasts to repress serum-derived BMP2/4 signaling (100 ng/mL bFGF is required) and sustain the undifferentiated proliferation of hESCs in the absence of fibroblasts or conditioned medium (12).

ESCs in culture have a specific morphology, and they express characteristic surface antigens and nuclear transcription factors. The surface antigens include the stage-specific embryonic antigen SSEA-4 and the teratocarcinoma recognition antigens TRA-1-60 and TRA-1-81 (9). The transcription factors include the POU (pit-oct-unc)-domain transcription factor Octamer-4 (Oct-4), associated with the expression of particular elements of the embryonic genome (6). Recently, a new functional marker of the ES cells, NANOG, was discovered simultaneously by two independent teams of investigators (13,14). Interestingly, NANOG alone can confer the self-renewal and pluripotency to mouse ES cells in the absence of conventional LIF signaling. Moreover, in human ES

Received November 16, 2005; accepted December 21, 2005.
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**DOI: 10.1203/01.pdr.0000205154.86517.2a**

**Abbreviations: BM,** bone marrow; **ESC,** embryonic stem cell; **HLA,** human leukocyte antigen; **HSC,** hematopoietic stem cell; **ICM,** inner cell mass; **NSC,** neural stem cell; **SVZ,** sub-ventricular zone

cells identification of OCT4, SOX2, and NANOG target genes using genome-scale location analysis revealed co-occupancy for a substantial portion of their target genes (15). The above mentioned gene triad together with other modulator genes represent the core transcriptional regulatory circuitry in hES cells.

***Differentiation.*** When undifferentiated hESC colonies are detached from the feeder layer and transferred into serum-containing medium, they form multi-cellular aggregates called embryoid bodies (EB) which can contain cell types representing all three germ layers of the body: endoderm, mesoderm, and ectoderm. However, the exact composition of each EB is stochastic and unpredictable; therefore, many labs have been trying to develop protocols for directly controlling the differentiation of hESCs.

Exogenous differentiating factors have been useful in favoring differentiation into specific derivatives: retinoic acid and nerve growth factor for neuronal differentiation (16); basic fibroblast growth factor and platelet-derived growth factor for glial precursors (17); 5-aza-2′-deoxycytidine for cardiomyocytes (18); bone morphogenetic protein-4 and transforming growth factor-beta for trophoblasT-cells (19); sodium butyrate for hepatocytes (20); and various cytokines for hematopoietic cells (21). Differentiation into the primitive neural stem cell stage during neural lineage commitment has been proposed to be a default pathway for mouse ES cells (22). In the case of hESCs, culturing of small clusters of pristine pluripotent hESCs in a neurobasal medium supplemented with bFGF and EGF generates a uniform population of human neural precursors (AT, unpublished observation). AQ: 2

Several groups (17,23–25) have produced neuronal precursors from either mouse or human ESCs and tested them by injection into the developing brain of a mouse or rat. The transplanted cells were incorporated into the host brain, migrated along appropriate tracks, differentiated into neurons in a region-specific manner and made synaptic contacts with host neurons. In some cases the transplanted cells also gave rise to glia and astrocytes.

## SOMATIC STEM CELLS IN THE ADULT ORGANISM

Classical embryologists developed the concept that as mammals developed, their cells became progressively more determined for a certain tissue fate and the tissues progressively lost the potential for repair or regeneration. However, recent work has shown that many mammalian tissues including bone marrow, skin, gut lining, blood vessels, endocrine glands, mammary gland, prostate, lung, retina, and parts of the nervous system contain stem cell populations that might self-renew and generate somatic cells normally as well as mobilize, proliferate and differentiate in response to wounding or disease. The level of self-renewal and degree of differentiation varies drastically between somatic tissues. Almost a quarter of century ago, the realization that custom microenvironments might control hematopoietic stem cells led Schofield (26) to call such regions "niches." A stem cell niche can be defined as a specialized subset of tissue cells and extracellular matrix that harbor one or more somatic stem cells and control their self-renewal and differentiation throughout the life of an organism. Somatic stem/precursor cells from some tissues (*e.g.* germinal zones in CNS) can be isolated and grown in culture. During *in vitro* propagation, CNS precursors are said to retain multi-potency, *i.e.* the ability to differentiate into major cell types appropriate to their original site. It is important to stress that the rigorous definition of pluripotency would require the demonstration that cultured stem/precursor cells are capable of generating the entire variety of cell types of not only the tissue of origin, but other tissues as well.

***Hematopoietic stem cells.*** Since the pioneering experiments of Till and McCulloch (27,28), hematopoietic stem cells (HSCs) are perhaps the most rigorously studied somatic stem cells. The current most-accepted phenotype for the prospective isolation of mouse HSCs (1:5,000 cells) include the c-kit$^+$, *Sca*I$^+$, Flk2$^+$/Thy1.1$^{low}$, Lin$^-$ cell population from bone marrow (BM) (29,30). It has been proposed that very primitive pluripotent HSCs are quiescent (31,32). However, while long-term HSCs are normally maintained at constant numbers, these cells do proliferate and increase in number *in vivo*, for example after reconstitution of lethally-irradiated recipients with a single HSC (33,34). Thus proliferating long-term HSCs must be present in the BM under some circumstances. There is some evidence that c-kit ligand could be crucial for the maintenance of HSCs in the quiescent state within the BM (35). AQ: 3

Another clear example of actively proliferating long-term HSCs is in the fetal liver. In fact, fetal liver HSCs are even more potent in long-term reconstitution assays than adult HSCs (36). Mobilization of HSCs by cyclophosphamide and granulocyte colony-stimulating factor also result in proliferation of HSCs in BM and spleen (37). However, mobilized multi-potent progenitors isolated from the spleen were less efficient than normal BM multi-potent progenitors in engrafting irradiated mice (37).

Several gene products have been proposed to play a role in HSC self-renewal *in vitro* and *in vivo*, including activated Notch1 (38), HoxB4 (39,40), thrombopoietin (18,41,42), activated $\beta$-catenin (43,44), FGF-1(45) and Bmi-1 (46,47). However, several issues remain unresolved. While both HoxB4 and FGF-1 were found to act on whole BM, they did not promote the amplification of prospectively isolated HSCs (45). On the other hand, retroviral transformation of HSCs with constitutively active forms of Notch1 and $\beta$-catenin generated cells that were not able to radio-protect recipients and resulted in significant skewing in the reconstituted mice in favor of T-cells and B-cells, respectively (38,44). Currently, no methods can maintain/amplify multi-potent human HSCs *in vitro*.

***Mesenchymal stem cells.*** In addition to the hematopoietic system, bone marrow also contains a supporting tissue called stroma. It was originally thought to simply provide a structural framework for the hematopoietic system, but it has now been found to contain several cell types with other functions and potentials. Most importantly, it contains a population of mes-

enchymal stem cells (MSCs) (48), which are strongly adherent and can therefore be isolated by culturing marrow on an appropriate substrate and washing other cells off. Mesenchymal stem cells can give rise to many kinds of connective tissue cells including those responsible for remodeling of cartilage, bone, fat, and vascular tissue (49). MSCs are likely to participate in maintenance of the essential microenvironment necessary to support the hematopoietic stem cells in the bone marrow (48).

The results of bone-marrow transplantation studies have led to the conclusion that this remarkable tissue can also produce cells that can circulate to various other sites in the body and contribute to even more tissues including endothelium, muscle, liver, pancreatic islets, heart, brain, lung, kidney, and retina (50,51). A number of studies have claimed an amazing plasticity or trans-differentiation of these bone marrow-derived cells (52–58). However, much of this plasticity is clearly the result of cell fusion (59–61). Future rigorous analysis will clarify the extent and the usefulness of trans-differentiation.

***Neural stem cells.*** Cultured neural cells, defined by their clone-forming ability (neurospheres), self-renewal capability and multi-potency, were first isolated from embryonic and adult mice (62). The *in vivo* origin during development (63) and distribution in the adult (64–67) have since been analyzed in detail. Although still a matter of debate (68), the prevalent model of adult neurogenesis in rodents is based on the results from the laboratory of Alvarez-Buylla (67). The pluripotenT-cells in the sub-ventricular zone (SVZ) of the adult rodent brain are thought to be GFAP+ cells (called type B-cells) with morphologic characteristic of astrocytes. These cells divide quite rarely and give rise to mitotically active transiently amplifying cells (type C cells), also located in the sub-ventricular area. In turn, type C cells give rise to the post-mitotic highly migratory neuroblasts (type A cells), which migrate along the rostral migratory stream (RMS) toward the olfactory bulb to generate granule cells and periglomerular interneurons (69). Similar cells have been found in fetal, neonatal, and adult human brain (70,71). However, despite the identification of the unique astrocytic ribbon in human SVZ (72), the phenomenon of massive migration along RMS in adulthood has not been established in humans. Moreover, neurosphere-forming cells from fetal human brain can be prospectively isolated using the marker AC133 (70). Single neural progenitor cells divide and, in the absence of a substrate, gradually grow into balls of 10–10,000 cells called neurospheres. Neural precursor cells migrate out from neurospheres and can give rise to neurons, astrocytes, and oligodendrocytes (70,71,73).

The presence of neural stem cells in the adult brain accounts for the finding of adult neurogenesis, well established in rodents and humans, in many regions of the brain including the SVZ of the anterior lateral ventricles and the dentate gyrus of the hippocampus {Eriksson, 1998 #29; Gage, 1998 #34; Uchida, 2000 #112; Gage, 2002 #35; Roy, 2000 #92; Lu, 2000 #62; Gould, 1999 #37; Gould, 1999 #37}.

## STEM CELL-ASSOCIATED GENES

Based on expression analysis of neural and hematopoietic stem cells, we have formulated the hypothesis of stem cell-associated genes (74). Transcripts that are enriched in several types of somatic and embryonic stem cells, but down-regulated in differentiated cells, may participate in defining the stem cell phenotype and might be involved in basic stem cell functions, such as self-renewal and the ability to regenerate the cellular complexity of a given tissue.

Several following publications put forward the "stemness" hypothesis by forcing the identification of common genes in embryonic, neural and hematopoietic stem cells (75–77). However, very limited overlap between the three independent studies (1 common gene) has been raising doubts about the usefulness of such extreme generalization. Indeed, the original suggestion was specifically limited to somatic stem cells and merely suggested to "to test whether these candidates molecules are involved in stem cell self-renewal, differentiation or plasticity" (74). In practice, the "shared" gene pool is enriched in functionally "interesting" genes (*e.g.*, stem cell phenotypes in knock-out animals), thus we consider the stem cell-associated expression as a particular filter to gauge the overwhelming complexity of the microarray analysis datasets.

## STEM CELLS, CANCER, CANCER STEM CELLS

The heterogeneity of human tumors was recognized a long time ago and lead to the idea that only some cells, named cancer stem cells, are capable of initiating the tumor (78). Indeed, such cells have now been identified in several types of tumors such as brain (79), breast (80) and blood (81). Common molecular pathways were suggested to be operational in somatic and cancer stem cells (82). For example, the Bmi1 gene was implicated in self-renewal of somatic stem cells as well as propagation of tumors (46,83,84). Tumor stem cells may also have an epistatic relationship with somatic stem/precursor cells. Indeed, because neoplastic transformation often requires several consecutive mutations to occur, this is unlikely to happen in the differentiated post-mitotic compartment. On the other hand, the mitotically active stem/precursor cell pool is likely a suitable milieu allowing the accumulation of mutations eventually leading to the transformation.

## STEM CELL THERAPIES

***Blood and immune system disorders.*** Transplantation of bone marrow, containing HSCs or purified cell fractions from bone marrow, has been used for over three decades in the treatment of disorders of the blood-cell production system. Children with Severe Combined Immune Deficiency have been successfully treated by bone marrow transplantation since 1968 (85). Leukemia patients have been successfully treated with chemotherapy to destroy the bone marrow followed by rescue with bone-marrow transplants from HLA-identical twins or siblings or HLA-matched donors (85). For lymphoma and leukemia, the patient's own bone marrow may be harvested in advance when the disease is in remission, cleared of undesirable cells (residual malignant cells, and cells

that could mediate a graft-*versus*-host immune reaction), and frozen so that it can subsequently be used to restore the patient's hematopoietic system after chemotherapy. More recently, peripheral blood (after treatment of the donor with cytokines to mobilize stem cells) has been found to be a better source of stem cells to restore hematopoiesis (85). Umbilical cord blood (UCB) can also be used and has several advantages over bone marrow as a source of HSCs, especially in pediatric patients (86). In principle, the HSCs are the only cells required to restore the ablated hematopoietic system. As a proof of principle, the transplantation of extensively purified mobilized peripheral blood $CD34^+$, Thy-$1^+$ HSCs, used as the sole source of the hematopoietic graft from 22 patients with recurrent or metastatic breast cancer, resulted in rapid and sustained hematopoietic engraftment (87).

***Metabolic diseases.*** Another group of diseases being treated with HSC transplantation is the lysosomal storage disorders, including the mucopolysaccharidoses such as Hurler's Syndrome. These diseases involve the harmful accumulation of specific cell components, due to the absence or inactivity of a specific enzyme normally involved in their degradation. In these cases, stem cell therapy would function primarily for enzyme replacement, by providing cells containing an active form of the missing or defective enzyme. Since disease progression may lead to extensive damage, early diagnosis and treatment is essential (88). At present, HSC transplantation is effective primarily for soft-tissue, non-central nervous system organs such as spleen and liver, with only limited effectiveness for bone and cartilage, and little to no effectiveness for the CNS (88).

## EXPERIMENTAL THERAPEUTIC APPLICATIONS

***Autoimmune diseases/multiple sclerosis (MS).*** Multiple Sclerosis (MS) is a debilitating neurologic disease in which chronic inflammation of the CNS leads to multiple impairments of motor, sensory, and cognitive functions. It affects about a million people worldwide, about 200,000 of them in the U.S. The fundamental feature of the disorder is that the individual's own immune system attacks and destroys the myelin sheath that normally surrounds nerve fibers. Some of the destroyed myelin regenerates spontaneously, although it is not clear exactly which cell type is responsible for producing this myelin. Current treatments mainly use the immunomodulator $\beta$-interferon to slow the progression of the disease.

Two different manipulations of stem-cell populations are being tested as potential treatments for MS. First, since the disease results mainly from the development of a population of immune cells that attack the myelin sheaths of neurons, the replacement of the stem cell population generating these immune cells is a logical goal. In fact, the possibility of this kind of treatment was first recognized when some MS patients being treated by transplants of blood stem cells for other diseases showed remission of their MS symptoms. In a clinical trial of hematopoietic stem-cell (HSC) transplantation for MS, 20 out of 26 patients appeared to stabilize (84). These trials included a combination of total body irradiation and chemotherapy followed by peripheral blood stem cell transplantation. About 250 patients are currently in Phase I and Phase II open clinical trials (89).

Soon after their discovery, neural stem cells (NSCs) were recognized as having tremendous potential for the cell-based repair of neurological damage (90). However, it seems likely that the first applications will be for therapy of neurological diseases that do not require the establishment of new neuronal circuitry, notably MS (91–93), Parkinson's disease (94), and metabolic diseases. Thus, NSC therapy could be used to repair the de-myelination caused by the immune attack. NSCs support the production of neurons and glia in parts of the normal brain throughout adulthood. They can be isolated from either fetal or adult brains, expanded extensively and maintained safely in a chemically-defined medium; they can be directed into a neuronal fate or an astrocyte fate by treatment with different growth factors; and they can be safely frozen and thawed, thus eliminating the need for continuous maintenance (95,96). Their ability to migrate over long distances in the body and to apparently home in on diseased areas also makes them uniquely suitable for cell therapy of diseases, including MS, that are multi-focal (affecting many locations in the body) (97).

In a mouse model of MS, NSCs implanted into the brain survived well, could home in on the de-myelinated region, differentiate into oligodendrocytes, stimulate the increased production of host oligodendrocytes and re-myelinate the damaged fibers. They also reduced astrogliosis, further demyelination and axon loss (91). These cells were equally effective if administered *via* the circulation, after which they passed through the blood-brain barrier and entered the brain. Many of the treated mice were completely cured of the disease, and the cells did not produce tumors.

Embryonic stem cells (ESCs) are also an attractive option for treatment of demyelinating diseases. When transplanted into rodents suffering from demyelinating disease, they can differentiate into glial cells and remyelinate affected axons (17,98,99). However, these cells also produce teratomas (100) so the controls over their differentiation will have to be analyzed more thoroughly before they can be seriously considered for use in stem-cell therapy. It has already been shown that ESCs can be induced to differentiate into oligodendrocyte precursors, and that these can be used to promote myelination of axons in the shiverer mouse (101), which suffers from defective myelination.

The remarkable ability of stem cells to home in on diseased areas appears to be a response to the inflammation at the disease site. Inflammation involves the production of a well-characterized set of molecules including proteins that function in adhesion between cells (*e.g.* integrins), proteins that act as signals between cells to attract them to each other and to activate them in various ways (chemokines and cytokines), and specific receptors for these proteins (102,103). An intriguing discovery regarding the mechanism was that two proteins (CD44 and Very Late Antigen-4), shown to be required for NSCs to home in on inflamed regions, are the same proteins that attracted the inflammatory lymphocytes to the site in the first place (93).

## POTENTIAL THERAPEUTIC APPLICATIONS

***Hypoxic-ischemic brain injury.*** Neonatal hypoxia-ischemia is a major contributor to chronic neurologic dysfunction (104–106). A clinically relevant rat model was established in neonatal rats by Vannucci and colleagues (107) and then adapted for mice (108–111). Neonatal hypoxic-ischemic injury has been shown to increase cell proliferation and neurogenesis in the SVZ and peri-infarct striatum, and to directly correlate with the degree of damage (112). BrdU, doublecortin and Map2 labeling identified extensive migration of newborn neuroblasts and terminally differentiated NeuN-positive neurons. Mature newborn astrocytes and oligodendrocytes were also detected 2 wk after injury. However, few newly generated cells were found 3 wk after injury, implying that the endogenous precursors failed to survive and differentiate (112). The neonatal hypoxia-ischemia model has neuronal destruction in the same brain regions as in adult animals, but also causes necrosis of white matter, which is linked to oligodendrocyte precursor death and disrupted myelin production (110). Thus, this model can be used to assay both neurogenic and oligodendrogenic/myelination potential of the transplanted neural precursors.

***Metabolic diseases affecting the brain.*** As mentioned above, HSC therapy for metabolic diseases has had some, but limited success (88). This limit is particularly true for the CNS. In animal models of some of the lysosomal storage disorders, however, implantation of NSCs into the brain significantly ameliorates the detrimental effects of the disease on the brain (113,114). These data suggest that combination stem cell therapy, using HSCs for the periphery and NSCs for the brain, might be a much more effective treatment strategy for these patients. In addition, transplantation of another stem cell component of bone marrow, the mesenchymal stem cells, may be beneficial for the bone and cartilage defects found in these diseases (115).

***Brain tumors.*** NSCs have a remarkable ability to migrate through the body and through normal tissues to accumulate in various types of tumors, both neural and non-neural (116). This provides a potential avenue to developing radically new types of cancer treatment, especially for tumors that infiltrate the brain so extensively that they cannot be effectively removed by surgery or chemotherapy. In such cases, it may be possible to use the homing ability of NSCs to deliver chemotherapeutic agents accurately and exclusively to the tumor cells.

In studies on an experimentally-induced glioma in mice, NSCs were implanted either into the tumor or at sites within the brain but distant from the tumor. When implanted directly into the tumor, they spread through the tumor, and when they were implanted into other sites in the brain they migrated to the tumor and spread through it (117). Even when they are delivered simply by injection into the circulation, NSCs can also target both brain tumors and tumors at other sites including prostate cancer and malignant melanoma, without significant accumulation in normal tissues (117). In addition, NSCs have been genetically engineered to produce various products that could be delivered directly to the tumor (118). They can be designed to release cytolytic viruses that destroy adjacent cells, to produce anti-tumor proteins, or to secrete enzymes that will locally convert inactive pro-drugs into active chemotherapeutic compounds (119). NSCs may also contribute to recovery of tissues damaged by cancer. They may differentiate directly into neurons and other damaged cell types, but they may also promote the ability of host cells to replace diseased tissue, especially if they are genetically engineered to produce appropriate neurotrophic factors. NSCs that are engineered as therapeutic agents can also be specially tagged so that they can be monitored *in vivo* after injection (120).

## AVOIDING OR OVERCOMING IMMUNE REJECTION IN STEM CELL TRANSPLANTATION

Even though HLA matching remains the single most effective method for minimizing immune rejection, it is anticipated that the use of stem cells from donors will require the same kind of immunosuppression that is being used with other organ transplant therapies. This is clearly true for HSCs and presumably will also be true for other adult stem cells. However, ESCs may not elicit the same kind of immune response that is associated with transplantation of more mature cells or tissues. Injection of ESCs into immune-competent mice failed to induce an immune response (121), suggesting that any ESC line may be immunologically compatible with any recipient, obviating the need for either therapeutic cloning or a large ESC bank. These studies, however, must be replicated and expanded to include specific differentiated cell types.

The ideal solution to the potential problems of immune rejection of transplanted stem cells would be to generate stem cells that are genetically identical to the patient. In principle, this can be done by replacing the nucleus of a donor stem cell with a nucleus from a somatic (body) cell of the patient, and growing up the resulting cell into a new stem cell line, which hopefully would retain its stem-cell properties and could be used in stem cell therapy. This process of somatic cell nuclear transfer has been proven in animal models (122) and tested with human cells by researchers (123) who showed that the resulting ESCs retained stem-cell characteristics, including expression of several stem-cell specific markers, differentiation *in vitro* and *in vivo* into all three germ layers, and continuous proliferation.

## SCIENTIFIC ISSUES ARISING IN STEM CELL THERAPY

***Use of animal cells or products.*** Feeder cells from other mammals have often been used for the culture of ESCs, and this may limit the use of the stem cells in therapy according to current FDA regulations (124). However, this regulatory hurdle can be cleared by demonstrating that the ESCs grown on non-human feeder cells do not carry non-human (or human) pathogens (viruses, bacteria). In addition, it has recently been shown that ESCs cultured in the absence of animal cells but in the presence of animal-cell-derived products may show the presence of animal-specific molecules (125). Although it has been suggested that this may obviate the use of the cells for therapeutic applications, the clinical use of similarly-treated

cells or other implantable products has been underway for many years (FDA Xenotransplantation Action Plan). In addition, it is possible to significantly reduce the animal components simply by culture of the cells in non-animal based systems (125).

***Tumor-forming potential of ESCs.*** One of the basic properties of undifferentiated ESCs is their ability to form a teratoma. Implantation of this type of cell, therefore, carries substantial risk (126). Undifferentiated ESCs are thus unlikely to be used directly in therapeutic applications. Differentiation of an ESC eliminates its capacity to form a tumor. Thus, ESCs will be used to generate lines of cells that are at least partially differentiated, and these derived cell lines will be used therapeutically (127).

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# AUTHOR QUERIES

**AUTHOR PLEASE ANSWER ALL QUERIES** **1**

AQ1: AUTHOR— Please define hESC at first use

AQ2: AUTHOR— Please define EGF at first use

AQ3: AUTHOR—Changed "neg" to "−" in superscript marking for "Lin" OK?

AQ4: AUTHOR—PLEASE PROVIDE REFERENCE 124 OR DELETE FROM THE TEXT.