STEM CELLS – THE ULTIMATE BODY REPAIR KIT

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ABSTRACT:

The new millennium promises to guide in the age of human genome. So far, a different area of biology, the stem cell biology – has captured both scientific and international news headlines. Stem cells are generally very early stage cells that have the ability to differentiate into other specialized types of cells. Stem cells hoist the prospect of regenerating failed body parts and curing diseases that have so far defined drug – based therapy. This review attempts to give an overview of stem cell biology and scientific factors surrounding it.

Keywords: *Embryonic stem cells; Adult stem cells; Stem cell markers; Hematopoietic stem cells*

INTRODUCTION:

Stem cells bridge the cleft between the fertilized egg that is our origin and architecture that we become. The stem cells supply the cells that construct the adult bodies and as the age, replenish worn out, damaged and diseased tissues. And depending on the source, they have potential to form one, many or all cell types of an organism. Stem cells are isolated from two sources generally, the adult stem cells and embryonic stem cells. Recently, cord blood stem cells are isolated for use in regenerative medicine. Depending on the cell source, stem cell may be totipotent, pluripotent, multipotent or unipotent [Table 1].

Table 1: Definition of Terms

Totipotent cell: - able to give rise to all cell types. In mammals, only the fertilized egg and early cleavage stage blastomers are truly totipotent cells of inner cell mass and ES cells are unable to differentiate into cells of the tropectoderm lineage.

Pluripotent cell:- able to give rise to all cell types found in embryo and adult animal

Multipotent cell:- able to give rise to more than one differentiated cell type.

Unipotent cell:- able to give rise to a single cell type.

Lineage:- The natural progression from an immature cell type to one or more differentiated cell types.

Lineage restriction:- The inability of one lineage to give cell type of another, that is, to cross lineage boundaries.

The common characteristics of stem cells include extensive proliferative potential and ability to give rise to one or more differentiated cell types in early mammalian embryos. But embryonic cells lose these properties as differentiation follows and growth promoting signals decline subsequently becoming adult progenitor cell or a differentiated cell (1). The adult progenitor cells can operate at 'steady state', i.e., they can generate in an average of one replacement stem cell and one tissue cell at each division with no apparent limit. Adult stem cells are hence controlled by particular microenvironments known as 'niches' (2). Unlike embryonic stem cells that are isolated from 'blastocyst stage' of embryo for culturing, adult stem cells are isolated from 'niches'. Cord blood contains hematopoietic stem cells, progenitor cells that can form red blood cells, white blood cells and platelets. However Cord blood stem cells are not embryonic stem cells.

In most tissues, stem cells are rare. As a result, stem cells must be identified prospectively and purified carefully in order to study their properties. Fluorescence Activated Cell Sorter (FACS) and Visual Assessment can aid in separation of stem cells from normal cells. Stem cells are often identified by the presence of markers. The markers may be ligands, cell surface protein receptors, cytoplasmic proteins, transcriptional factors or genes (3). The chance of stem cells becoming cancerous in regenerative medicine has led researchers to focus on three aspects between stem cells and tumor cells. First, the similarities in the mechanisms that regulate self-renewal of normal stem cells and cancer cells; second, the possibility that tumor cells might arise from normal stem cells; and third, the notion that tumors might contain 'cancer stem cells' – rare cells with indefinite proliferative potential that derive the formation and growth of tumors (4).

Embryonic stem cells

Human embryonic stem cell (hESC) are non – transformed cells that are self-renewing and pluripotent or multipotent for a tissue type, and highly proliferative with the following characteristics: (i) can be isolated from the inner cell mass (ICM) of the blastocyst, (ii) proliferate extensively *in vitro*, (iii) maintain a normal euploid karyotype over extended culture, (iv) differentiate into derivatives of all three germ layers, (v) express high levels of transcriptional factor, Oct4 and (vi) show telomerase activity (5 - 7). The percentage of hESC lines successfully

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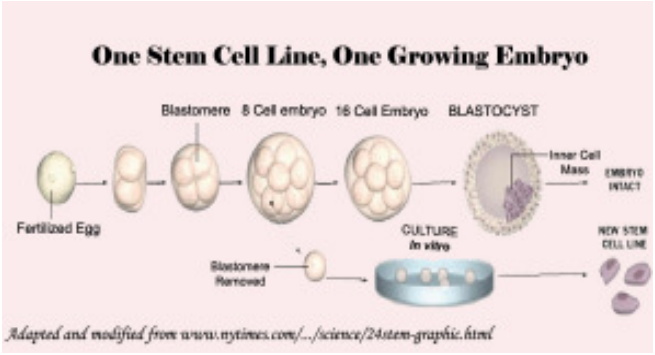
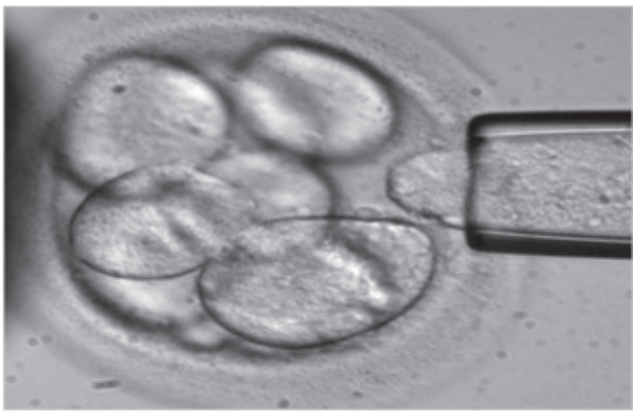


Fig. 1: Stem cells developed without destroying the embryo



Adapted from www.healthcare.zdnet.com/images/stem-cell-harvest.jpg

Fig. 2 : Blastomere removal using pipette

derived from ICMs ranges from 5%-100%; this wide range reflect differences in number of embryos used for derivation, embryo quality or derivation techniques (8). Recently, cell lines have also been isolated from morula stage embryo or from later stage embryos (7 - 8 days) (9) (Fig. 1& 2). Therefore, the current hESC lines have been derived from embryos with different characteristics and this was used earlier for conducting diagnostic tests in the normal course of *in vitro* fertilization (10).

The cell line should reliably differentiate into an appropriate cell population and remain stable during expansion and differentiation as well as after transplantation. Generating and maintaining the cells in defined culture conditions will allow the establishment of more reproducible cultures that can be maintained in multiplex laboratories. Use of cells exposed to these components for cell replacement therapies may thus carry the risk of infection by nonhuman pathogens. Some progress has been made toward the elimination of xenogeneic components in hESC derivation and culture. For instance, one hESC line has now been derived using lysed Mouse Embryonic Fibroblast (MEFs) (11).

Culturing hESC requires an appropriate substratum. The use of a single matrix such as laminin or fibronectin has been successful in the maintenance of hESC. The adherence of hESC to each other, although critical in maintaining cell-cell interactions, has presented several challenges. Regardless of the passaging technique either mechanical or enzymatic, it is important that the hESC remain in clusters to preserve the integrity of the culture. This situation makes it difficult to generate cultures with consistent cell density from passage to passage. However, there are currently no clinically applicable protocols for achieving this goal.

Markers currently used to characterize hESC include glycolipids, glycoproteins and transcriptional factors. These surface markers were identified on human embryonal carcinoma cells or in human preimplantation embryos, such as SSEA - 4, TRA - 1 - 60, and TRA – 1 - 81 (12). hESC also express surface antigens such as AC133, c-kit (CD117), flt3 (CD135) and CD9 (13-15). The transcriptional factors that serve as markers have a critical role in maintaining self-renewal e.g. Oct 3/4 (16 - 17).

Adult stem cells

The adult stem cells (ASC's) are unspecialized or undifferentiated cells that are found in differentiated tissues maintained in a stable micro environmental niche and most of the cells are lineage restricted. The niche safeguards against excessive stem cell production that could lead to cancer. Stem cells must periodically activate to produce progenitor or transit amplifying (TA) cells that are committed to produce mature cell lineages (18). Maintaining a balance of stem cell quiescence and activity is a hallmark of a functional niche. ASC's can be found in the bone marrow, blood stream, cornea and retina of the eye, dental pulp of the tooth, liver, skin, gastrointestinal tract and the pancreas. However, there is not much evidence that ASC's unlike the ESC's are pluripotent (19, 20). Although differentiation potential is slightly decreasing in long-term cultures, it is possible to keep cell lines up to passage 140. Adult stem cells reside in various organs in a specific cellular environment called the niche, in which they are kept in an undifferentiated state (21 - 23). These stem cells possess an extensive self-renewal capability bearing an indefinite proliferative potential. Until recently; adult stem cells are believed to be lineage-restricted with limited differentiation potency, compared with embryonic stem cells. Pluripotent embryonic stem cells are blastocyst-derived cells and proliferate unlimited in an undifferentiated state, being capable of giving rise to cells found in all three germ layers (24, 25) However, this stem cell plasticity was recently shown also for adult stem cells by various groups (26). Different examples of the versatility of adult stem cells have been demonstrated from bone marrow (27) umbilical cord blood (28), testes (29) and pancreas (30). They differentiated into various cell types cutting across lineage boundaries.

Pancreatic stem cell niche

Adult pancreatic stem cells are able to differentiate spontaneously *in vitro* into various somatic cell types. Stem cells isolated from rat pancreas show extensive self-renewal

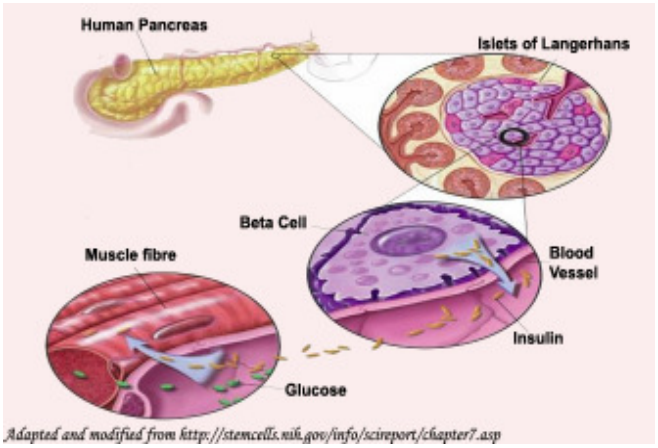


Fig. 3 : Pancreatic stem cell niche

ability and grow in highly viable long-term cultures. Several approaches are being used for isolating and culturing stem cells or islet precursor cells from fetal and pancreatic tissue (Fig.3). These cells were formerly termed pancreatic stellate-like cells because of their morphologic and immunohistochemical similarities to pancreatic stellate cells, which are located within the interlobular septa and interacinar areas of the pancreas (31). The cells can be engineered to avoid immune rejection. Recent studies in mice show that embryonic stem cells can be coaxed into differentiating insulin-producing beta cells, and new reports indicate that this strategy may be possible using human embryonic cells as well (32). Additionally, these cells express typical stem cell markers such as Oct - 4, nestin and SSEA - 1. We have previously reported a simple but effective method for isolation of stem cells from the exocrine pancreas. Before transplantation, they could be placed into nonimmunogenic material so that they would not be rejected and the patient would avoid the devastating effects of immunosuppressant drugs. Since their discovery three years ago, several teams of researchers have been investigating the possibility that human embryonic stem cells could be developed as a therapy for treating diabetes.

Intestinal stem cells niche

Epithelial villus and its surrounding pericryptal

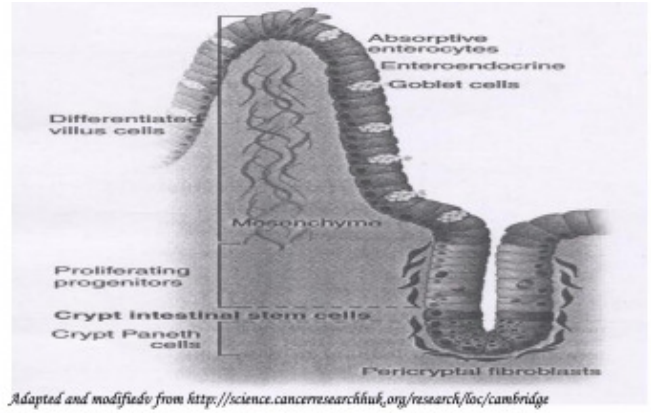


Fig. 4 : Intestinal villi showing crypt cells

fibroblast and mesenchyme in the small intestine make up an anatomical unit that generates four cell lineages: absorptive enterocytes and the goblet, enteroendocrine and paneth cells of secretory lineage. Intestinal stem cells and transit amplifying (TA) cells within the crypt regenerate the entire villus every 3 to 5 days (33). Progeny of activated ISCs migrate upwards to become TA cells. When they reach the top of the crypt, TA cells stop proliferating, differentiate, and assume their appropriate positions within the villus structure (34). Although asymmetric cell division along the vertical crypt axis is an attractive mechanism, this process has yet to be rigorously demonstrated in the ISC system (Fig.4).

Hair follicle stem cell niche

Skin epidermis and its associated structures arise from two stem cell populations within the hair follicle and inter follicular regions (35). One, in the basal layer of skin, normally gives rise to stratified skin layers. A second, the hair follicle stem cell, resides in a region of the outer root

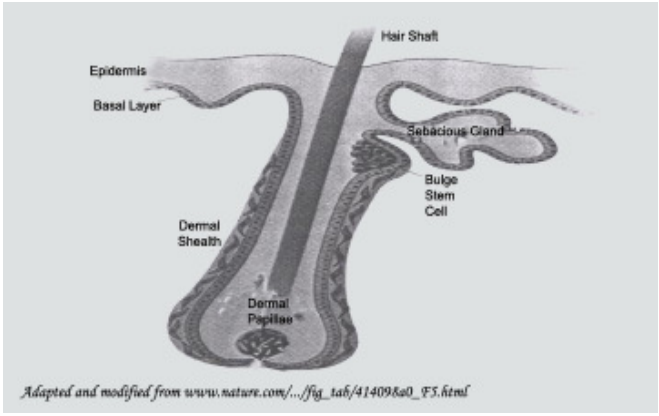


Fig. 5: Stem cells with their niche in hair follicle

sheath called the bulge, and it is responsible for the regeneration of hair and sebaceous glands (36 - 39). It had been suggested that bulge stem cells are also responsible for the long-term replenishment of the interfollicular epidermis (Fig.5). It is now clear that bulge stem cells are not required for normal epidermal homeostasis, although they can contribute transiently to this tissue in wound healing (40 - 44).

Neural stem cell niche

Neural stem cells are a subtype of progenitor cells in the nervous system that can self-renew and generate both neurons and glia (Fig. 6). Adult neural stem cells have now been found in the two principal adult neurogenic regions, the hippocampus and the sub ventricular zone, and in some non – neurogenic regions, including spinal cord (45, 46). Recent findings in stem cell research indicate the presence of stem cells in the hippocampus-a region in brain, which is important in memory. Curious observations in regeneration of neural cells in foetal and adult brain resemble the undifferentiated cells in a developing embryo that give

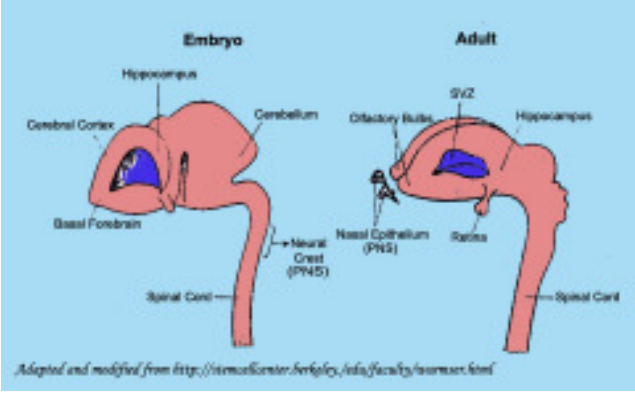


Fig. 6 : Neural stem cell niche

rise to nervous tissue (47). Stem cells are under active consideration as a source of donor tissues for neuronal cell therapy for Parkinson's disease (48), Huntington's disease (49), spinal cord injury (45), stroke (50) and multiple sclerosis (51).

Hematopoietic stem cells

The stem cells that form blood and immune cells are known as hematopoietic stem cells (HSCs). They are ultimately responsible for the constant renewal of blood, the production of billions of new blood cells each day.

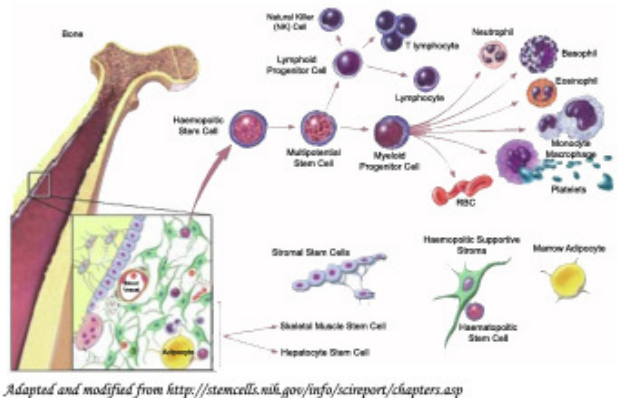


Fig. 7: Hematopoietic stem cell

HSCs are found in the bone marrow, peripheral blood, umbilical cord blood, fetal hematopoietic system, ESC and EGC. Umbilical cord blood and placenta are a rich source of HSCs (Fig.7). The main focus of study of HSCs lies in mainly finding ways to safely and efficiently expanding the numbers of transplantable human HSCs *in vitro* or *in vivo* (52). Cell-surface markers of undifferentiated hematopoietic stem cell found in human are CD 34,CD59,CD38 (low) and c-kit (53, 54). HSCs demonstrate sufficient plasticity that they can differentiate into bone, cartilage, neural cells, pneumocytes, muscle, skin, endothelial, epithelial cells and hepatocytes (55).

Sources of Hematopoietic stem cells

Bone Marrow

Bone marrow transplants by anesthetizing the stem cell donor, puncturing a bone, typically a hipbone and drawing out the bone marrow cells with a syringe. About 1 in every 100,000 cells in the marrow is a long-term, blood-forming stem cell; other cells present include stromal cells, stromal stem cells, blood progenitor cells, and mature and maturing white and red blood cells (56 -59).

Umbilical Cord Blood

In the late 1980s and early 1990s, physicians began to recognize that blood from the human umbilical cord and placenta was a rich source of HSCs. This tissue supports the developing fetus during pregnancy, is delivered along with the baby, and, is usually discarded. Since the first successful umbilical cord blood transplants in children with Fanconi anemia, the collection and therapeutic use of these cells has grown quickly (60, 61)

Peripheral Blood

It has been known for decades that a small number of stem and progenitor cells circulate in the bloodstream, but in the past 10 years the researchers have found that they can coax the cells to migrate from marrow to blood in greater numbers by injecting the donor with a cytokine, such as granulocyte-colony stimulating factor (GCSF). The donor is injected with GCSF a few days before the cell harvest. To collect the cells, doctors insert an intravenous tube into the donor's vein and pass his blood through a filtering system that pulls out CD34+ white blood cells and returns the red blood cells to the donor, from which just 5 to 20 percent will be true HSCs (54, 62).

Heart and Cardiac Muscle

Heart tissue has a limited regenerative capacity: thus; the use of HSCs for cardiac repair has been found to be of clinical relevance (Fig. 8). Researchers are now exploring ways to save additional lives by using replacement cells for

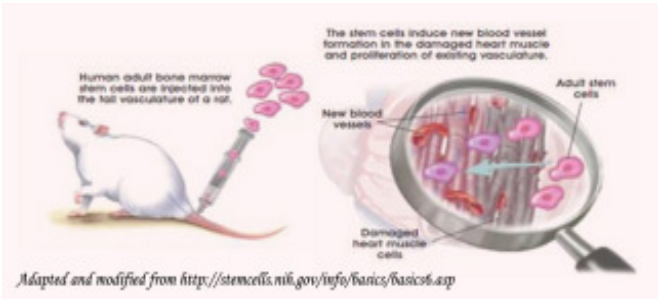


Fig. 8 : Heart muscle repair with adult stem cells

dead or impaired cells so that the weakened heart muscle can regain its pumping power. Like the mouse stem cells, the human hematopoietic stem cells can be induced under the appropriate culture conditions to differentiate into numerous tissue types, including cardiac muscle. In 1998, Kajstura and colleagues (63) discovered the presence of myocytes undergoing mitosis in the failing human heart. Such cells were found to be scarce, explaining the lack of significant myocardial regeneration after myocardial

infarction. These cells have subsequently been demonstrated to be self – renewing, clonogenic and able to give rise to myocytes, smooth muscle and endothelium. These cells have subsequently been defined as Lin^{-} $c\text{-}kit^{P08}$ cells within human hearts (64). A series of animal studies and clinical trials have been used to attempt to determine whether stem cells could be useful in treating myocardial infarction (65, 66). Stem cells injected into the bloodstream leading to the damaged rat heart, these cells prevented the death of viable myocardial cells and reduced progressive formation of collagen fibers and scars (67).

Cancer Stem cells

The cancer stem cell (CSC) model of tumor development and progression states that tumors, like normal adult tissues, contain a subset of cells that both self renew and give rise to differentiated progeny. As in other tissues, the stem cells are the minority of the whole organ, and are the only cells that can maintain tumor growth indefinitely. The self renewal properties of the CSCs are thus real driving force behind tumor growth. The identification of markers that allow the prospective isolation of CSCs from whole tumor tissues will allow us to develop an understanding of several important biological properties of CSCs. Cancer stem cells can only be defined experimentally by their ability to recapitulate the generation of a continuously growing tumor. Cancer stem cells share many characteristics with normal stem cells (68-70). A few cancer stem cells could evade treatment and later give rise to a tumor, referred to as cancer relapse. The tumors formed are really the progenies of the cancer stem cells. Like all progenies of stem cells, they multiply rapidly. However, the progenies of cancer stem cells are not like normal progenies, whose growths are tightly controlled.

A well known property of normal stem cells is their dependence upon their microenvironment, or 'niche' to maintain their quiescent and undifferentiated state, while maintaining their proliferation and differentiation potentials (71, 72). The discovery of signaling pathways that play a functional role in CSC self renewal is extremely important from a therapeutic perspective, as some of these pathways have known chemical inhibitors (e.g. the Hh pathway can be inhibited by cyclopamine treatment), or function as inducers of differentiation (e.g. BMPs). The development of methods for the prospective isolation of CSCs is thus the first step, which then opens the door to a variety of approaches that could ultimately lead to CSC – specific therapies for cancer treatment (73 - 75).

Delivery of stem cell

Clinical trial is going on delivery of stem cells in animal models as well as in human for regeneration of damaged tissue or organ. Tissue regeneration targets are mostly successful in skin, bone, cartilage. Stem cells are generally implanted or seeded into an artificial structure capable of supporting 3-D tissue formulation called scaffold allowing cells to influence the own microenvironment. Scaffold should be a) biodegradable in nature b) able to allow cell attachment and migrations c) delivers and retain cells and biochemical factors d) enable diffusion of vital cell nutrients e.g. polycaprolactone, polyglycolic acid, collagen or fibrin, glycosaminoglycans etc.

Ethical Aspects

Some scientists use ASCs due to the ethical and moral aspects involved in using human ESCs as a less controversial alternative. US Federal funding for stem cell research is restricted to the 64 cell lines. This ethical issue has divided the scientific community into two i) those that believe in the extension of stem cell research and thus do not believe in the ethical questioning, and ii) those who are apprehensive about the prospect and thus take a moral stand on research against using ESCs (76, 77). In India, wasted embryos available from IVF clinics only are permitted to be used by researchers after receiving consent from the donors. However, more recently, Indian Council of Medical Research, New Delhi has formulated guidelines for stem cell research in the country.

The main ethical issues for consideration are as follows:

i. Instead of producing a baby a human embryo, develops only into certain types of cells. Is it acceptable to reprogramme a human embryo?
ii. Are there likely to be viable alternative therapeutic methods, which could avoid using embryos?
iii. If the route to such alternative methods involved some limited embryo research, would such research be permissible?
iv. Would it be acceptable to perform the nuclear transfer of human cells into the enucleated egg of a cow, to produce a non-viable chimera, which would be reprogrammed to produce certain human cells?
v. Would the risks involved in cell replacement therapy be considered acceptable?

If a procedure with discerning ethical difficulties were to be pursued, it is also essential to be honest about its chances of success. There is a formidable list of experimental hurdles to overcome. No one knows how successful cloned cells would be on patients, or what risk there is of cultured cells becoming cancerous (20).

Concluding Remarks

Do cells with unpredicted stem cells hide in other parts of the body? Is it possible to induce ASCs to produce cell types other than their standard range of progeny? Stem cells taken directly from adult tissues promise to be useful in many ways for tissue repair. It should be possible to use adult tissues to derive ES cells with the same genome as the adult patient whose body is in need of repair. The cloning of Dolly, the sheep and of other mammals has indicated a way to do this. The nucleus of an egg cell can be artificially replaced by a nucleus derived from adult cell and the hybrid

cell can then go on to develop into an entire individual whose nuclear genome is identical to that of the adult donor. Serious ethical issues need be resolved and enormous technical problems overcome before such an approach can become a reality. Perhaps other, better ways will be found to restore adult cells to an embryonic state of versatility. But by one route or another, it seems that stem cell biology is beginning to open up new opportunities for improving on nature's mechanisms of tissue repair.

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