Embryonic Stem Cells:
Basic Science and Clinical Applications

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The tremendous potential of human embryonic stem cells and the resulting questions regarding their ethical use, have caught the attention and imagination of both scientists and the public. Until the ban on the use of these cell lines for federally funded research was lifted in August 2001, research in this important field was being conducted largely outside the United States or by biotech companies. Although the 78 federally recognized stem cell lines (http://escr.nih.gov) will not be sufficient for all the work scientists would like to do, researchers now have an important opportunity to begin to study a period of development that is otherwise inaccessible, to begin to determine methods to control the differentiation of these cells into various types of cells, including neurons, muscle, skin, bone and cartilage, and eventually to combine these cells to replace damaged organs.

What Is a Stem Cell?

Stem cells are present in the early embryo, as well as in adult tissues, where they are very rare. While definitions may vary, scientists agree that stem cells have both the ability to divide, and to produce progeny that are more limited in their developmental potential. It has been known for many years that CELLS IN certain adult tissues such as the skin, liver, blood and gut can divide and replace damaged tissue, but the recent demonstration that organs such as the nervous system contain populations of stem cells was initially viewed with skepticism. Regardless of its source, a stem cell is not determined to be a particular type of cell, but remains uncommitted until signaled by growth factors to begin to differentiate into the more than 200 different cell types that make up the body. Cells with unrestricted developmental potential are often termed "pluripotent." They are derived from embryos or fetal tissue and are called "embryonic stem cells" or "pluripotent stem cells."

A tissue stem cell, on the other hand, is an undifferentiated cell found in adult tissues, which on stimulation can differentiate into a limited number of tissue types present in the tissue of origin. Surprisingly, in recent years scientists have reported that stem cells derived from adult tissues can "transdifferentiate" into tissues they would not normally form. Cells from the adult bone marrow have been shown to integrate into the brain and express genes which mark neural tissue; stem cells from the nervous system have been introduced into early embryos, where they have, surprisingly, contributed to diverse cell types including muscle and bone. This concept of "stem cell plasticity" suggests that resident within all adult tissues are cells with multilineage potential, or the ability to form many different cell types. Why then, after a heart attack or stroke, does the body not mobilize these resident adult stem cells to repair the damaged tissue? This and many other scientific and ethical questions remain to be answered.

What Makes Stem Cell Research so Exciting?

Five days after fertilization, the growing embryo has developed into a blastocyst which contains an aggregate of pluripotent cells, the inner cell mass (ICM), which forms all of the tissues of the developing embryo. This mass of stem cells is essentially a *tabula rasa*, awaiting instructions regarding its ultimate fate. Embryonic stem (ES) cells are derived from these pluripotent cells. Because ES cells have the potential to form all the cells of the body, it should be possible to determine methods to control their differentiation into neurons to replace tissue damaged by stroke, by tumors or the treatment of tumors, by spinal cord injury, or by Alzheimer's or Parkinson's disease; into islet cells that would secrete insulin for the treatment of diabetes; into skin for grafting to burn victims; and into muscle tissue for replacement in muscular dystrophies or following heart attack. A full range of therapeutics awaits better understanding of the potential of both types of stem cells, embryonic and adult, and it is likely that each will have a unique niche. Clearly, a better understanding of their potential, how to control their differentiation, and the surrounding safety and ethical issues must first be addressed by carrying out careful research.

Sources of Pluripotent Stem Cells

Embryonal Carcinoma (EC) Cells

Before mouse embryonic stem cells were derived in 1981, and human embryonic stem cells became available in 1998, pluripotent cell types derived from germ cell tumors were employed as models of early development. These cells formed by malignant transformation of germ cells (future eggs and sperm) in the testis or ovary, the tumor was removed, and the cells taken from it were grown in culture. Although the study of these cells has significantly improved our understanding of many of the mechanisms involved in early embryonic development, and in the differentiation of specific types of cells, many of these tumor cell lines are partially differentiated, i.e., can only form certain cell types. These cells are known as embryonal carcinoma (EC) cells and, in early clinical trials, have been shown to improve function in patients recovering from strokes. There is, however, considerable concern that because they are derived from tumors, implantation of these cells may result in uncontrolled growth, and formation of a new tumor.

The Embryonic Germ (EG) Cell

The embryonic germ (EG) cell is a second source of pluripotent cells. These cells are derived from cells in the 5–9-week fetus that are destined to become germ cells—future eggs and sperm. When grown in culture, these cells proliferate and can form many other cell types. Although they have considerable developmental potential, concern that these cell lines have been derived from fetuses, as well as the fact that they appear to grow less well in culture, limit their usefulness.

Derivation of Embryonic Stem (ES) Cells

As early as the 1870s, scientists began to fertilize mammalian embryos *in vitro*. In 1968, the first human egg was fertilized *in vitro*, followed in 1978 by the birth of Louise Brown, the first IVF baby. In order to understand early development better, scientists have attempted to grow the pluripotent embryo cells in culture. In 1981, two groups of scientists reported growing these embryonic stem cells *in vitro*, setting the stage for the eventual derivation of similar stem cells from human embryos. Within 24 hours after fertilization, the fertilized human egg (zygote) divides to produce a two-celled embryo, followed by division into four-, eight- and 16-, 32- and 64-cell embryos. By day 3, when it contains approximately 8 cells, the embryo begins to control its own development, rather than relying on maternal RNA and proteins present in the egg. By the fifth day of development, an inner cluster of cells which will form all the cells of the body, the inner cell mass, and an outer ring of cells destined to form the placenta have been established. The formation of these two different cell types, stem cells of the ICM and the trophoblast (future placenta) are the first sign of cell specialization in the early embryo. Five days after fertilization, the blastocyst contains approximately 200-250 cells (approximately 40 are stem cells). This is the stage of development when an embryo would normally implant into the uterine wall, and just before there is differentiation of the embryo into specific tissue types. At this stage the embryo-forming cells are carefully removed from the blastocyst and grown in culture (arrow). These cells divide rapidly, forming ES cell lines. In fact, many of the cell lines derived in the 1980s are still growing normally.

Because human ES cells have only recently been derived, much of what is known about the characteristics of ES cells has come from studies in mouse embryos. Mouse embryonic stem cells have been widely used to study the role of a particular gene in development using a technique known as gene targeting (or knock out), where a mutation is introduced into a specific gene causing it to malfunction. This technique has allowed researchers to infer the normal role of the targeted gene in the growth and development of the embryo, as well as to create animal models of human genetic diseases. Embryonic stem cells have also allowed developmental biologists to study this very early period of development, which is not normally accessible for investigation, and to begin to understand the interplay between genetic and environmental factors in shaping the early embryo.

In addition to these basic science applications, ES cells are a rich source of cells for transplantation and eventual tissue bioengineering. Before this can be done, however, scientists must determine ways to control the

irreversible differentiation of these cells into the desired cell type. Because so very little is known about this early stage of development, this has been more difficult than anticipated. In the normal blastocyst staged embryo, the stem cells of the inner cell mass rapidly differentiate to form the embryonic ectoderm, which differentiates at gastrulation into the three primary germ layers: ectoderm (skin and nervous system), mesoderm (bone and muscle) and endoderm (digestive system, lungs).

To determine their differentiation potential, ES cells have been either injected into an adult animal where they form a small tumor or teratoma, or ES cells have been grown in suspension culture, where cells are not allowed to attach to the tissue culture dish, but are forced to aggregate. Because these small clusters of differentiating stem cells resemble an early embryo—they have a blood circulation and contain regions of beating cells—they have been termed "embryoid bodies." Under both these growth conditions, many different cell types form, including bone, teeth, intestine, hair, nervous tissue, etc., demonstrating that the cells are indeed pluripotent. Differentiation in these aggregates is not organized and they do not spontaneously form an embryo, only masses of differentiating cells.

Because of this unorganized differentiation, it has not been possible to determine precisely which combination of growth factors, contact with other cells, or which genes are responsible for cell-type specific differentiation. This is because the aggregate of cells is heterogeneous, and differentiation is chaotic rather than following the pristine organization that is typical of the early embryo. Differentiation of embryoid bodies has allowed the determination of the pattern of gene expression during differentiation, the role of certain growth factors; or particular genes in development when deletion of that gene is lethal to the early embryo. ES cell lines, both mouse and human, can form a wide variety of cell types including cells typical of yolk sac, cardiomyocytes, skeletal myocytes, smooth muscle cells, chondrocytes (cartilage), adipocytes (fat), endothelial cells, pigment cells of the skin, neurons, hematopoietic (blood and lymph) progenitors, pancreatic islet cells, primitive endoderm, bone, keratinocytes (skin), and glial cells. This work is important because it indicates that ES cells *can* follow many of the pathways involved in the normal formation of the embryo; what the critical factors actually *are* remains to be determined.

Controlling ES Differentiation

Instruction

Although it may seem a simple task to take a pluripotent cell and cause it to develop into the particular cell type desired, in fact, little is known regarding the critical genes and growth factors required to make, for example, a muscle cell from a stem cell. To derive specific cells for transplantation and "stem cell therapy," two basic approaches have been taken. In the first, a particular cell type, for example a blood cell, can be developed in culture by exposing undifferentiated ES cells to specific (hematopoietic) growth factors. Much of this research is based on what we know about the growth factors present in the early embryo in regions where specific cells will differentiate. For example, to cause ES cells to differentiate into primitive liver cells, the pattern of expression of molecules in the embryo in the region where the liver forms was determined, then ES cells were grown in the presence of those factors. Although this approach can be quite successful, the choice of growth factors is obviously restricted to those which are known (many are not).

If growth factors can be determined to promote the differentiation of ES cells into the desired cell types (e.g., neurons), this approach may be preferable to genetic selection techniques, in that growth factor differentiated cells may be less likely to form tumors when transplanted to damaged tissues. However, even when growth factors have been used to "instruct" stem cells to differentiate into the desired cell type, the resulting cultures are not pure and contain many other "contaminating" cell types that must somehow be removed from the cultures before they could be implanted.

Selection

To get around these limitations, genes that are thought to control the differentiation of specific cell types, such as muscle or neurons, have been expressed in embryonic stem cells to "force" them to differentiate into the desired type of cell. Because ES cells proliferate so extensively in culture, they can be transduced to express a lineage-restricted gene linked to a gene for resistance to antibiotics. Cells that incorporate the lineage gene can then be selected by growing them in high levels of antibiotic. Cells that express the lineage gene will be resistant to the antibiotic and will continue to grow, while cells that do not express the construct will be killed by the antibiotic. Using this technique, it has been possible to develop embryonic stem cell lines from strains of mice that normally fail to grow ES cells *in vitro*, to develop cardiac muscle cells, and to develop neurons. This approach can be used to generate highly purified cells, but there is concern that introduction of additional DNA into the cells may cause them to become malignant eventually.

Therapeutic Uses

While considerable basic science research remains to be done to understand early embryonic development, as well as the interplay of genetic and environmental factors that produce specific populations of cells, much work has already been carried out to determine the ability of mouse ES cells to differentiate in culture, and to integrate when transplanted to models of human disease.

Diabetes

One obvious use of stem cells is to replace missing hormones and growth factors. In diabetes, beta cells in the endocrine pancreas, which normally produce insulin to control blood glucose levels, are defective or destroyed. Insulin-secreting cells could be developed from stem cells and implanted near blood vessels, where they would be able to produce a continuous source of insulin, rather than daily injections of insulin to control blood glucose levels. Several groups have produced insulin-secreting cells from embryonic stem cells. In one study, ES cells were grown for several weeks in the presence of growth factors that promoted insulin secretion. The differentiated ES cells did produce insulin, and importantly, the cells were able to respond to changes in glucose levels by producing insulin. However, when they were implanted into animals with induced diabetes, the ES cells did not produce high enough levels of insulin to control blood glucose levels. In another approach to this problem, when the human insulin gene was placed in mouse ES cells, and the resulting insulin-secreting cells were implanted into a similar animal model of diabetes, the implanted cells did control blood glucose levels!

These two studies are important because they demonstrate that ES cells can be induced to secrete therapeutic molecules, and also because they illustrate a fundamental difference in the approaches researchers are using to differentiate stem cells. In the first example, combinations of growth factors were used to cause ES cells to produce insulin (instruction); in the second, an exogenous gene was introduced into them. Many researchers believe that it is preferable to cause cells to differentiate using growth factors, rather than introducing foreign genes into them because of the concern that the cells containing foreign DNA may transform, or become tumor-forming. While that is an important concern, implanted cells must secrete sufficiently high levels of the desired hormone to be useful. Another concern is that cells produced by growth factor exposure often contain clusters of undifferentiated stem cells that, once implanted, could divide. Clearly, methods to control the division of the cells must also be developed to prevent formation of tumors.

Spinal Cord Injury

It has been estimated that there are 10,000 new cases of spinal cord injury each year in the United States alone. High profile cases, such as Christopher Reeve's injury, have brought the critical need for treatments for these devastating injuries to the public view and have improved resources for funding. Much remains to be done, however, before this research can move to clinical trials. One major problem in using cell-based therapies in the spinal cord is that in addition to the primary damage to the neurons in the cord, the supporting cells respond to the

injury by dividing and secreting factors that actually *inhibit* healing of the injury. The most successful restoration of function to the spinal cord has come from studies in John McDonald's lab at Washington University in St. Louis. In these studies, the rat spinal cord was injured, then aggregates of ES cells were implanted into damaged zone. After only two weeks, animals regained some mobility in their hind limbs. In another study by this group, the ES cells were differentiated into the myelin-forming cells of the central nervous system, and *then* implanted. In these studies, cells survived for four weeks and produced myelin in both a chemical injury and in a genetic model, in which mice lack myelin proteins. After four weeks, ES cells were still present in the damaged spinal cord and had migrated up to 8 mm from the site of implantation and formed multiple layers of myelin.

These studies make several important contributions. First, implanted aggregates of neural cells can integrate and improve function after injury. Second, not all of the cells in the aggregates were differentiated into neurons or into glial cells, yet there were no tumors in the recipient animals. Since ES cells divide so extensively in culture, there has been concern that these cells would form tumors once implanted; and it will be important to study later time points. Finally, the ability to specifically derive the myelinating (insulating) cells of the nervous system from ES cells opens the possibility of treating de-myelinating conditions such as multiple sclerosis.

Reverse Genetic Disease

The concept, much discussed in the '90s, of replacing disease genes with normal genes, or gene therapy, has found new promise using stem cells. In fact, researchers have used neural stem cells to replace the gene (beta-hexosaminidase alpha-subunit) that when mutated causes the severe degenerative disorder Tay-Sachs disease. Myelin-forming cells are integrated into the shiverer mutant mouse lacking myelin-basic protein that causes the myelin sheath to compact (myelin basic protein). Oliver Brüstle has also implanted ES cells into a rat model of human myelin disease, successfully correcting that genetic defect.

Another potential use of stem cells is to study the cellular effects of genetic disease with the goal of developing and testing new treatments. One very severe neurological condition, Huntington's disease, is characterized by the insertion of trinucleotide repeats into certain genes. Dr. Matthew Lorincz, in a collaboration between our lab and Dr. Roger Albin's lab, has used a line of mouse ES cells that have been genetically altered to over-express these CAG repeats. When these cells are differentiated into neurons in culture, they fail to extend the normal cell process that is responsible for transmitting signals to other cells in the CNS. Importantly, the cells that contain the most repeats are the most abnormal. Because they differentiate gradually in culture, it will now be possible to test new ways of protecting cells from forming toxic products, which might ultimately develop into pharmaceutical treatments of this disease.

Heart

Cardiac muscle cells were one of the first cell types to be derived from embryonic stem cells. ES cells were transduced to express a gene found only in differentiated heart muscle cells linked to a gene for antibiotic resistance. When these ES cells were differentiated in culture, then exposed to high levels of antibiotics, only cardiac muscle cells survived. The muscle cells were functional and were able to incorporate into the heart when implanted into rats. This approach of using a gene expressed in the desired tissue with antibiotic killing of all other cell types in the culture is known as "lineage selection" and is very powerful in producing very pure cultures, consisting of the desired cell type (>98% of the surviving cells were cardiac muscle). However, the differentiated cell type (muscle cells) represent only a small proportion of the total number of cells present in the cultures, so it is often difficult to obtain large numbers of cells for grafting.

Parkinson's Disease

In Parkinson's disease, degeneration of specific neurons that produce dopamine in the midbrain (striatum) results in characteristic motor disturbances such as tremor. One promising treatment for Parkinson's disease is replacement of the lost neurons by dopaminergic neurons from fetal tissue, which *do* provide symptomatic relief.

However, the many ethical questions in obtaining and using fetal tissue, in combination with the very limited quantity of cells available, has led investigators to search for other sources of cells.

It has recently been possible to derive dopaminergic neurons from embryonic stem cells using growth factors that are present in the region of the brain that produces these neurons during development. In the case of dopaminergic neurons, embryonic stem cells were exposed to a cocktail of growth factors (sonic hedgehog protein, fibroblast growth factor-8 and ascorbic acid) over a long time in culture (24-43 days), and at the end of that time a high percentage (72%) of the cells were neuronal, and a smaller percentage (5%) were dopaminergic neurons. These cells have electrical properties typical of neurons, and some synthesize dopamine, but it is not yet known if when implanted, they will ameliorate the symptoms of Parkinson's disease. In this example, the small number of dopaminergic neurons must somehow be separated from the other neurons in the cultures before implantation.

In a very different approach, another group took pluripotent ES cells and, without any differentiation, implanted these cells into the midbrain of rats that had a chemical lesion that produces symptoms similar to Parkinson's disease. Surprisingly, the ES cells differentiated into dopaminergic neurons and were able to reverse the Parkinson's-like symptoms in the animals. This study is remarkable in that it implies that after injury, there may be growth and differentiation factors in the brain that direct the ES cells to differentiate into the missing cell type.

Alzheimer's Disease

Progressive neurodegenerative disorders, of which Alzheimer's disease is perhaps the most prevalent, are projected to produce an increasing burden on the health care system as the aged population grows. Despite the critical need, there is currently limited potential to alleviate the progressive dementia, memory loss and behavioral changes which characterize this disease. Treatment will be complicated by the fact that even if neurons were available for transplantation, the diseased brain contains deposits of amyloid protein, which are likely to damage newly implanted neurons. Stem cell technologies offer the ability to co-transplant new neurons to replace the damaged neuronal cells, and additional supporting cells that might express an inhibitor or scavenger of the amyloid deposits. Alternatively, it should be possible to engineer stem cells to both secrete inhibitors *and* differentiate then into replacement neurons. Our lab has recently been funded to develop neurons from ES cells for transplantation in this devastating condition. We are using a combination of genetic and growth factor treatment to differentiate ES for these studies.

Liver

Although the liver is an organ with considerable natural regeneration capacity, transplants for hepatic failure and metabolic disease are required constantly. When ES cells are exposed to growth factors present near the septum transversum of the early embryo (developing liver field), they differentiate and express markers of mature liver cells. To date, cell replacement therapies have used primary hepatocytes (taken from livers then grown in culture), which are effective in animal models of liver disease, so the ability to produce quantities of hepatocytes for implantation and tissue engineering could provide an important source of this critical cell type.

Other Tissue Types

Many other cell types have been produced in culture from mouse ES cells. Although these studies provide important information on gene expression in development, the role of specific factors in the formation of particular cell types, the full potential of these cells has not yet been recognized. This is due to the concerns below, to our lack of basic knowledge regarding the growth factors and genes required, but also due to the fundamental complexity of the organs to be engineered. Therefore, it is likely that initial success using stem cell "therapies" will come from studies to replace a single growth factor or hormone, such as insulin in diabetes, to replace a single cell type such as dopaminergic neurons in Parkinson's disease. Other tissues such as bone, that forms by appositional growth, and has few cell types should also be an important target, particularly for osteoporosis.

Issues Surrounding Stem Cell Implantation

Tumor Formation

A significant concern using ES cells for transplantation is that because of their tremendous ability to divide, they will continue to proliferate when implanted, forming tumors. There are a number of approaches that could be employed to inhibit cells re-entering cycle and dividing. One that we are investigating is to link the expression of a particular cell cycle gene to a toxi-gene, so that if the implanted cell begins to divide, and expresses a gene (cyclin) involved in cell division, it will also express the toxic gene, and self-destruct. Recently, dopaminergic neurons were implanted into patients with severe Parkinson's disease in a phase I clinical trial. Unfortunately, the implanted cells secreted too much dopamine and produced unwanted side effects (tardive dyskinesia) in the patients. Using ES cells, it should be possible to develop dopaminergic neurons that also express an inducible suicide gene; when too many cells express dopamine, some of the cells could be selectively killed by treatment with a drug that causes the toxi-gene to be expressed.

There has been some success using stem cells to reverse the effects of stroke. Layton Biosciences has used human teratoma stem cells (tumor stem cells), differentiated these cells in culture into neuronal cells, implanted them into the damaged region of the brain, and has reported no tumor formation, and improvement in symptoms of the stroke. They are now in phase II clinical trials and are experimenting with many different types for cells for implantation. Their position is that when cells have been terminally differentiated into neurons, they will not "de-differentiate" and begin to divide. If all the neurons are, in fact, terminally differentiated, that may well be the case. However, starting with a transformed (tumor) cell rather than a normal cell (such as an ES cell) would seem to increase this risk. In the case of transplanting aggregates of undifferentiated ES cells into the midbrain (for Parkinson's disease, above), and finding that cues in the region promoted differentiation to dopaminergic neurons, as predicted these studies did report the presence of large (lethal) tumors in 5 of 25 animals.

Transplant Rejection

Although there has been considerable progress in transplantation medicine, there are number of hurdles before transplantation becomes routine. One major problem has been the need to suppress the natural rejection of the transplant using massive quantities of immunosuppressive drugs. It was initially hoped that because ES cells were derived prior to implantation of the blastocyst in the uterus, they might be immunologically privileged, or not express MHC antigens that are responsible for an immune response. Unfortunately, this does not appear to be the case, and it has been suggested that it should be possible to genetically engineer ES cells to escape the host immune system. However, when skin cells were transplanted to mice in which these genes had been deleted, there was still a robust rejection response, likely because additional cells play a role in the recognition of "self." Another alternative would be to "bank" large numbers of ES cells to match donor and recipient of the cells. In the case of human transplantation medicine, that would require the development of many additional human ES cell lines, or using genetic approaches to create "universal donor" ES cells.

Alternatives

Therapeutic Cloning

Although the term "cloning" evokes Frankenstein-like images, transplantation of nuclei from a recipient into embryonic stem cells to develop genetically matched cells for implantation is a powerful way to create tissues and organs without the concerns of rejection. This technique is different from reproductive cloning, where the goal is to produce an individual, in that a cell line is the final goal. This technique is often called "somatic cell nuclear transplantation" or, in translation, the transplantation of a nucleus from a differentiated adult cell into an embryonic cell, or an egg. The nucleus of an adult somatic cell (often taken from skin, or in the case of Dolly, the cloned sheep, from a mammary gland epithelial cell) is removed from that cell and placed into the oocyte (egg). Factors that are present in the cytoplasm of egg cause the DNA of the donor nucleus to "re-program" (to de-

condense) and allow normal gene expression and development to occur. The embryo is then grown in culture to the blastocyst stage when the inner cell mass is removed and grown to produce ES cells. A concern with using an egg cell as a nucleus recipient is that an embryo is produced using this approach, and it is destroyed to produce the ES cells. While it is highly unlikely that these blastocysts are healthy enough to develop normally if they were placed in a uterus, an embryo *has* been created. A good alternative to using an oocyte to receive the transplanted nucleus would be to use an existing ES cell.

The nuclear reprogramming step is poorly understood, and if it fails to happen, development will not be normal. This is a major concern with reproductive cloning; that the donor nucleus will not be reprogrammed and development will not be normal, producing severe developmental defects in the offspring. Thus, Ian Willmut's group in Scotland who created the sheep Dolly using this technique, tried over 400 times before they produced a "normal" sheep. The concern, naturally, is that since we do not fully understand nuclear re-programming, many abnormal offspring will be produced.

If the goal is to produce cell lines to differentiate, for example, into liver cells for transplantation, complete reprogramming is probably not required, and other sources (other than an egg) of cells to receive the nucleus should be explored. Nuclear transplantation will also allow us to understand better how genes are expressed and repressed during differentiation, and how they might offer approaches to de-differentiate cells for autologous transplantation.

Harnessing the Body's own Stem Cells

Observations that most adult tissues contain populations of stem cells has created considerable interest in determining methods to cause resident stem cell populations to divide, migrate specifically to a region of tissue damage, differentiate and repair the damaged tissue. Surprisingly, there are examples of the body doing just this.

One study examined the ability of stem cells to colonize heart transplants. In this work, the transplanted heart was from a female donor, and the recipient was male, making it possible to determine if stem cells from the recipient colonized the donor heart, using markers of the Y chromosome. Surprisingly, at autopsy, numerous muscle cells from the male recipient were present in the donor heart, suggesting that it may be possible to motivate tissue stem cells to repair damage from heart attack.

The heart is an organ that might be expected to re-colonize more easily than other tissues because it is constantly in contact with the blood and with stem cells carried in the blood. However, it has also been shown that in a genetic condition with chronic muscle degeneration—the mdx mouse model of human Duchenne muscular dystrophy—cells from the bone marrow migrate, differentiate and contribute to the repairing muscle fibers. Because of their complexity, other tissues such as the brain are likely to be more difficult to target for repair by endogenous stem cells. Still, a number of investigators have reported that neural stem cells can migrate to regions of injury.

It is difficult to specifically destroy cells to create animal models of disease without creating a large injury and the release of multiple growth factors. Dr. Jeff Macklis at Harvard University has developed a technique in which he can specifically kill cells in one layer of the brain (without damaging the many connections above and below this layer of cells), and study the activation of neuronal cells in the damaged layer. This research is based on the idea that dying cells release factors that stimulate endogenous stem cells to divide and replace them. They have demonstrated that regions of the adult brain that are not thought to contain neural stem cells, can form new neurons. They are now trying to identify the factors released by degenerating cells that stimulate the addition of new neurons.

It has recently been shown that cord blood contains mesenchymal stem cells (bone, muscle-forming cells), and many hospitals are now storing cord blood for future transplants. Other sources of stem cells, and whether the "plasticity" exhibited by adult tissue stem cells is real, remain important questions. In the short term, it will be

easier to use stem cells to replace missing growth factors and hormones, or for cell therapies where a single cell type has been destroyed. Although there has been considerable progress in developing cultures of synchronously beating heart cells, and scaffolds of liver cells, the complex innervation and blood vascular requirements of tissues and organs will require scaffolds of biomaterials and collaboration with tissue engineers to obtain the full range of organs to meet transplantation needs.

Research Using Human Embryonic Stem Cells

Studies of the directed differentiation of human ES cells are just beginning. Neuronal cells have been developed by two groups of researchers, and another group is beginning to examine the ability of growth factors to promote the differentiation of these cells. Additional unpublished studies regarding the differentiation of hES are described in the recent NIH publication on stem cells.

Jamie Thomson's group recently reported that human ES cells could differentiate into hematopoietic (blood-forming) cells when cultured on bone marrow cells. They also reported one very interesting difference between the human and mouse cell lines that, somewhat surprisingly, the human cell lines can form extra-embryonic tissue—placenta cells. This unexpected observation may be important for placental insufficiencies, and will also allow new studies of this critical organ.

Conclusions

The field of stem cell biology is poised to make real contributions to treatment of human diseases. There are tremendous opportunities in this field, including the use of these cells as drug targets for the pharmaceutical industry, as alternatives to animal testing, which are only beginning to be tapped. Scientific questions of tumor formation, the real potential of adult stem cells, how to differentiate the cells, however, pale against societal questions regarding the role in the managed health care environment of stem cell transplants, their cost, their availability, use of nuclear transfer techniques to generate genetically matched stem cells for transplantation.

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Glossary of Terms

Adult (or somatic) stem cells: stem cells present in adult organs that typically form only the cells characteristic of the tissue in which they are found.

Blastocyst: the mammalian embryo 4-5 days after fertilization, prior to implantation in the uterine wall. Undifferentiated cells from the blastocyst are grown in culture to form embryonic stem cells.

Cloning: the creation of an identical cell or embryo. This can be done by splitting an embryo, or by nuclear transplantation. It is important to distinguish the goals of cloning: in **reproductive cloning** the goal is to create a copy of the individual, a *doppelganger*. In **therapeutic cloning** the goal is to create an embryo that will be used to derive cells for transplantation.

Differentiation: formation of a more mature cell type from an uncommitted (stem) cell.

Embryonic germ cells: cells that would normally form sperm and eggs. These cells can be grown in culture like ES cells.

Embryonic stem cells: undifferentiated cells derived from the blastocyst inner cell mass that divide continuously in culture.

Growth factors: natural substances that promote differentiation of stem cells.

Multipotent: capable of forming several cell types.

Nuclear transplantation: removal of the nucleus from a recipient cell (often an egg), and replacement of that nucleus with another nucleus, often from an adult tissue cell.

Plasticity: the ability of cells to change their characteristics and form another type of cell.

Pluripotent: capable of forming many types of cells. In the case of the embryonic stem cell, the ability to give rise to all the somatic cell types of the organism.

Stem cell: cells that have the ability to proliferate widely, produce exact copies of themselves (self-renewal), and to form differentiated cell types.