Science Highlights

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PATIENT-SPECIFIC, PLURIPOTENT STEM CELLS -- TESTIS IS A NEW SOURCE

Improving treatments for damaged organs and tissues is the promise of human pluripotent stem cells. The power of pluripotent stem cells to alleviate damage to organs, a form of regenerative medicine, has been amply demonstrated in many animal and laboratory model systems (see: State of the Stem Cell). In some studies, the pluripotent stem cells need to differentiate into the type of cell needed for normal function prior to transplantation, whereas in other studies, the presence of the transplanted stem cells themselves appears to alleviate damage and help restore organ function (1). It is not hype to assert that pluripotent stem cells are the foundation upon which regenerative medicine will grow.

Pluripotent stem cell: cell with unlimited potential to multiply and differentiate into all the tissues in the body

Several problems are currently hampering advances in stem cell therapy, one of which is the lack of readily available sources of patient-specific, pluripotent stem cells. Bedford Research scientists have focused on deriving pluripotent stem cells from unfertilized eggs, termed parthenote stem cells, for the past decade (2,3). Parthenote stem cells are patient-specific cells for the woman whose eggs were activated for their derivation, a highly promising source, but current protocols are too inefficient for routine therapeutic use. Bedford Research scientists are currently developing milestones during egg activation to improve the efficiency of parthenote stem cell derivation (4,5).

The challenge is not only producing pluripotent stem cells, but *patient-specific* pluripotent stem cells

Recent reports of the derivation of patient-specific, pluripotent stem cells from testis biopsies indicate a readily available source of stem cells for men (6,7,8). Bedford Research scientists will begin testing the efficiency of pluripotent stem cell derivation from testis biopsies in 2010, as soon as funding is available. Once the stem cell lines are derived, they need to be tested for pluripotency, stability and safety. They will be compared with all other known sources of human pluripotent stem cells.

BACKGROUND

Pluripotent stem cells derived from eggs: embryonic stem cells derived from fertilized eggs (usually left-over embryos), and parthenote stem cells derived from artificially activated, unfertilized human eggs (9), are undeniably the most robust and stable human stem cells currently known. They are the "gold standard" against which all other pluripotent stem cells are judged. They continue to divide and expand for years in culture, in sharp contrast to stem cells derived from tissues, such as cord blood and adult bone marrow.

But the clinical value of egg-derived stem cells as therapeutic agents is debated.

First Clinical Trial with Human Embryonic Stem Cells -- Spinal Cord Injury

On the one hand, trillions of identical embryonic stem cells can be grown under the careful laboratory conditions specified by the Food and Drug Administration for cell therapies. This characteristic is termed "stable cell line," meaning they maintain a constant, correct number of chromosomes after each cell division, and they remain pluripotent, capable of differentiating into any cell desired.

These stable, reproducible, reliable characteristics hold the promise of the development of off-the-shelf reagents to treat diseases. Indeed, the first embryonic stem cell therapy clinical trial approved in 2008 by the U.S. Food and Drug Administration (FDA) will use early stage nerve cells derived from human embryonic stem cells to treat acute spinal cord injury. The cells were developed in Geron corporation laboratories from one of the first lines of embryonic stem cells derived in 1998. According to the study protocol (www.geron.com/patients/clinicaltrials/hESC.aspx), the cells will be injected into the cord just below the injury site. The clinical study protocol was under review by the FDA for 3 years before its approval. The FDA review included detailed information about the cell culture procedures, precisely how the cells will be delivered into the spinal cord, extensive animal studies of safety, and approval of the devise developed for cell delivery to ensure all participating neurosurgeons treat the acute spinal cord injury patients uniformly. The approved study protocol is designed to assess safety of the cell delivery treatment. One concern is that the cells placed at the site of the spinal cord injury may not be 100% "early stage nerve cells" designed to replace the protective coating around the injured spinal cord nerve pathways, but may also include undetected embryonic stem cells that could develop into a tumor at the injury site. Many trials with rats and mice have indicated this will not happen, but it will not be known for certain until the human clinical safety trial is conducted.

On the other hand, the early stage nerve cells differentiated from the embryonic stem cell line may not be compatible with the patient's immune system. Just like blood type, every person has a unique repertoire of proteins on the surface of every cell. These proteins help the body's immune system distinguish between "self" and "foreign", such as bacterial or viral

invaders. This important protection causes the immune system to attack cells from other people as foreign, along with bacteria and viruses. This is the reason people needing kidney or liver transplants must wait for an organ that "matches" most of their proteins.

The term for this is "histocompatibility," meaning the two tissues can get along with each other. If the histocompatibility match

> **Histocompatibility:** the genetic match of cellular proteins between the patient and the stem cells.

is not good, a condition termed "host versus graft disease" results and the patients will need to take drugs that suppress their immune systems for the rest of their lives.

> **Host versus graft disease:** the attack of the stem cells (the "graft") by the patient's immune system (the "host")

Because there is only one line of embryonic stem cells being used for the first spinal cord clinical trial, the histocompatibility of the cells with each and every person entering the trial is unknown. There is evidence that embryonic stem cells may have fewer histocompatibility problems than adult organs such as a kidney, possibly due to their embryonic nature. But it will not be known until the trial is conducted if the spinal cord victims participating in the trial need to take immunosuppressive drugs for life, although it seems likely that at least some patients will. An immunosuppressive drug regimen has a number of side effects, including increased susceptibility to infections with both bacteria and viruses. One caveat to this is the possibility that the positive effect of the stem cells may not be needed forever, but only during the period of healing of the spinal cord injury. If this is the case, immunosuppressive therapy may only be needed for a few months or years. These considerations will not be understood until the trial is conducted.

Proposed Clinical Trial with Umbilical Cord Blood Cells -- Spinal Cord Injury

Another clinical trial approach being developed takes advantage of a growing body of information from other countries, such as China, that umbilical cord blood cells delivered to the site of the spinal cord injury has beneficial effect in reducing the severity of the injury (www.scinetusa.org). In this instance, the cord blood stem cells are not treated in the laboratory to become immature nerve cells before being injected into the spinal cord at the injury site. This clinical trial design has the advantage that cord blood stem cell banks already exist, and the histocompatibility type of each cord blood sample is known, so a match may be found for the spinal cord victim. The additional advantage is research that has demonstrated umbilical cord blood cells do not develop into tumors (www.scinetusa.org). Moreover, some of the studies using umbilical cord blood cells have treated chronic spinal cord injury, injuries more than one year old. The surgical approach has been to open the spinal cord at the site of the injury, remove the cellular debris that has accumulated

because of the large scar that forms at the injury site, and instill umbilical cord blood cells into the scar cavity. Although controversial, reports of improved function have appeared.

Sources of Patient-specific pluripotent stem cells

There is no way to know at this time which is more important, being able to coat the damaged spinal cord with new protective nerve cells, or supporting the re-growth of the spinal cord by inhibiting the damage and the scar tissue that forms. What is clear, however, is that if the stem cells being used for therapy were derived from the patient's own body, the problem of histocompatibility would not exist.

The question is, how to create patient-specific stem cells?

Bone marrow pluripotent stem cells

If bone marrow stem cells prove therapeutically useful for acute injuries, such as heart attack, stroke, spinal cord injury and severe burns, they could be harvested from the patient at the time of the injury. Recent studies are promising (10,11), but require the isolation and expansion of a specific sub-population of cells that requires several days to weeks to accomplish, rather than cardiac injection of an entire sample of bone marrow cells.

For chronic conditions, such as diabetes, Parkinson's disease, chronic spinal cord injury, deafness, congestive heart failure, kidney failure, Huntington's disease and Lou Gherig's disease, there is time to derive stable, pluripotent stem cells from the patient's own tissues.

Induced pluripotent stem cells from tissue biopsies

The recent reports of developing pluripotent stem cells, ("induced pluripotent" stem cells) from biopsies of patient's skin or liver are exciting and may prove broadly applicable to people of all ages. At this time the cell manipulations necessary to achieve pluripotency render the cells not suitable for therapeutic use, but many laboratories are working to circumvent this problem (1).

Egg-derived (parthenote) pluripotent stem cells

As described above, for younger women still producing eggs every month (before menopause), stem cells could be derived from artificially activated eggs, termed parthenotes, for their own use. The eggs could be collected by procedures that are routine for women undergoing assisted reproductive therapies for infertility. More than 80,000 women undergo hormone stimulation and egg collection every year in the U.S., so the risks and side effects are well documented. Hormone administration ensures the collection of 10 to 20 eggs, rather than the one or two normally produced by a women's ovaries each month. Studies in animal models have demonstrated that parthenote stem cells are histocompatible when transplanted back into the egg donor (12). For this to become clinically useful, however, the efficiency of deriving parthenote stem cell lines must reach at least 10% of eggs to

ensure the derivation of one stem cell line for each cycle of egg collection.

Research into this possible source of stem cells for therapy cannot be conducted with federal dollars in the U.S., according to the new National Institutes of Health guidelines for stem cell research. The rationale for the moratorium on government funding is thought to relate to the "Dickey" amendment which is a rider attached to the annual congressional budget for the National Institutes of Health that specifically prohibits federal funding for research on fertilized or artificially activated human eggs. This restriction is surprising, because parthenotes are not capable of development into offspring, and as such would seem to be less controversial for research than left-over embryos from fertility treatments. The restriction may relate to an over-arching concern about the ethics of asking women to donate eggs for research purposes. In my view, although the concern is real, and egg donation cycles must be carefully conducted to ensure safety to the egg donor (see: BSCRF egg donor program), the federal moratorium is an unnecessary restriction of research funding for women interested in supporting stem cell research.

Concerns over egg donation for stem cell research were fueled by the research scandal of a few years ago that fraudulently reported the derivation of another form of patient-specific stem cells, "nuclear transplant" stem cells. These stem cells were touted by scientists for several years as being the best source of patient-specific stem cells (13). A South Korean research team reported deriving several lines of patient-specific stem cells by this method, which was greeted by major enthusiasm all over the world, only to be quickly exposed by South Korean scientists as a fraudulent report. To date, no such nuclear transplant stem cells have been reported for human eggs, although this is a relatively common procedure in lower animals.

New: testis-derived pluripotent stem cells

The recent reports of deriving pluripotent stem cells from testis biopsies is an exciting new development in the field of patient-specific stem cells for men. No genetic manipulations of the cells are necessary, the efficiency appears to be high, and it may only take a few weeks to grow sufficient cells in the laboratory for therapeutic use.

First reported by a German research team, it was a surprise to many scientists that pluripotent stem cells existed in the testis. For several decades, it has been known that sperm are abundantly produced (billions per week) in the adult male testis for life, and that the sperm arise from "sperm stem cells" termed spermatogonia. There are several stages to sperm cell maturation, analogous to the several stages in blood cell maturation, and ultimately each sperm precursor cell gives rise to four adult sperm.

The new reports indicate that in addition to the spermatogonia, there is a less committed stem cell within the testis, a pluripotent stem cell, that may be called upon to divide only in extreme circumstances. It is the pluripotent testis

stem cell that has been shown to be as versatile as embryonic stem cells, potentially capable of developing into all tissues of the body. How stable it is in culture long term will take more time to learn, but results to date indicate this is a highly promising source of patient-specific stem cells that does not require genetic manipulation and can be derived from men of all ages in a relatively short time.

Bedford Research scientists will begin the Testis Stem Cell Project in 2010, as soon as funding is available.

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13. The research involved the collection of over 2,000 human eggs from South Korean women, removing all the chromosomes from the eggs, and transplanting into the eggs a cell nucleus containing all the chromosomes of the patient in need of stem cells. The reconstructed entity was activated artificially, by methods similar to parthenote egg activation, and stem cells would be derived from the resulting egg divisions. The South Korean research team was skilled in the relevant animal work, so it remains a mystery why they were not successful with 2,000 eggs.