Teacher Background

Stem Cells

Note: The Teacher Background Section is meant to provide information for the teacher about the topic and is tied very closely to the PowerPoint slide show. For greater understanding, the teacher may want to play the slide show as he/she reads the background section. For the students, the slide show can be used in its entirety or can be edited as necessary for a given class.

What Are Stem Cells and How Do They Relate To Development?

Stem cells are immature cells that have the potential to differentiate into specialized cells which have a distinct function. There are two types of human stem cells: those associated with the embryo (or embryonic) and those associated with the adult (or somatic). (1)

Stem cells associated with the embryo are normal karyotype pluripotent undifferentiated cells formed when the zygote (fertilized oocyte) begins to divide and forms the inner cell mass in the blastocyst stage of development. They have the ability to reproduce indefinitely in culture, thus are considered immortal. They can be propagated in an undifferentiated state and are able to differentiate *in vivo* or *in vitro* into any of the 220 types of cells in the human body. They can incorporate into host embryos and there is no rejection by the immune system since the cells are "self". (2)

Adult somatic stem cells are undifferentiated cells that reside in already developed tissue. These stem cells develop into specific types of adult cells to replace cells that have been damaged by injury or disease. (1, 2)

How Are Stem Cells Classified?

Stem cells can be classified by their potential to differentiate. Totipotent and pluripotent describe embryonic stem cells. Multipotent, oligopotent, unipotent, and nullipotent describe adult somatic stem cells.

a) Totipotent - Stem cells in a zygote and in the first few divisions of the zygote (2- to 8-cell embryo) are known as totipoten*t* because they can differentiate into embryonic and extraembryonic cell types. Each cell in the 2- to 8-cell embryo is known as a blastomere and is totipotent; each can become a blastocyst and eventually go on to form an individual. These blastomeres in the 2- to 8-cell embryos are the only cells that are totipotent. For example, after a zygote divides into a 2-cell embryo, it can grow into a complete human being. However, if the 2 blastomere cells become separated, each cell can grow into 2 complete, genetically identical

human beings and we call them identical twins. After a zygote divides twice to become a 4-cell embryo, it can grow into a complete human being. If the 4 blastomere cells become separated, each cell can grow into 4 complete, genetically identical human beings and we call them identical quadruplets. If 2 of the 4 cells became separated and 2 did not, then three genetically identical triplets would develop. The child formed from the 2 cells that did not separate would be 1 cell division older than the other 2 and would be indistinguishable from them. Identical triplets can also form if the 4 cells become separated and one of them fails to continue to develop.(3)

b) Pluripotent – Embryonic stem cells that are capable of developing into any type of cell which makes up the body of an individual are considered pluripotent. By the time the embryo has divided to form a blastocyst, the developmental fate is more limited. If a blastocyst were cut into two pieces, the development into two complete individuals would be less successful. The blastocyst consists of: a trophectoderm or outer layer of cells that surrounds the blastocyst and forms the placenta; a blastocoel, or hollow cavity inside the blastocyst, that will form the body cavity; and an inner cell mass, a group of about 30 cells at one end of the blastocoel that forms the 3 germ layers (ectoderm, mesoderm, and endoderm) of the fetus. Both the trophectoderm and the inner cell mass are necessary for successful development of each embryo and cutting a blastocyst precisely in half would be difficult. (2)

The inner cell mass has the potential to differentiate into all of the cells of the body derived from the three germ layers. These embryonic stem cells are pluripotent because they are capable of differentiating into ectoderm, mesoderm, and endoderm both *in vivo* and *in vitro*. The ectoderm becomes the external layer of the embryo and forms the skin cells of the epidermis, including pigment and hair cells, and the nervous system, including eyes and ears. The mesoderm becomes the middle layer of the embryo and forms muscle cells, the skeleton, adipose cells, heart, blood vessels, kidneys, and blood cells. The endoderm becomes the internal layer of the embryo and forms the lungs, the liver, thyroid, pancreas, bladder, and urethra cells, and the endothelial lining of the gastrointestinal tract. The inner cell mass stem cells have a normal karyotype, can be propagated in an undifferentiated state, have the ability to reproduce indefinitely in culture, and can incorporate into host embryos. Stem cells used in research which are harvested from the inner cell mass of the blastocyst of the developing embryo are called embryonic stem cells. (2, 3, 4)

The ultimate test for pluripotency has been the ability of mouse-cultured embryonic stem cells to contribute to chimeric (an individual, organ, or part consisting of tissues of diverse genetic constitution) tissues of the embryo after introduction of embryonic stem cells from several individuals into the preimplantation host blastocyst. In brown mice, the result is a mottled brown and white mouse in which the pigmented areas show the contribution of the embryonic

stem cells to the skin. All tissues in the brown and white mouse are also composed of a mixture of embryonic stem cells and host embryo cells. Similar chimeric animals have been produced in rats, rabbits, sheep, and cattle. Report of the first chimeric monkeys composed of a mixture of stem cells from at least 2 distinct genomes was published in the journal *Cell* on January 20, 2012. Shoukhrat Mitalipov at the Oregon National Primate Research Center found that stem-cell-injected blastocysts did not contribute to chimeras and the transplanted inner cell mass cells did not incorporate into host inner cell mass as they did in mice and instead formed separate fetuses. The key to producing chimeric monkeys was to mix the cells from different embryos in the very early stage when the cells were totipotent rather than pluripotent. As he said, "The cells never fuse but they stay together and work together to form tissues and organs." Aggregation of 3-6 embryos formed three chimeric monkey infants. (11, 17, 26)

Most adult cells are fully differentiated. If they do divide to form new cells, as in liver tissue, the differentiated cell divides by mitosis to produce two new differentiated cells. Tissues in babies, children, and adults are maintained and repaired by undifferentiated adult stem cells located in already differentiated tissue and they produce new replacement differentiated cells as older ones are damaged or sloughed off. Usually, when an adult stem cell divides, one daughter cell remains a stem cell and the other daughter cell develops into the specialized cell it is replacing.

Adult stem cells include i) hematopoietic stem cells which give rise to all of the types of blood, ii) mesenchymal stem cells which give rise to osteocytes, chondrocytes, adipocytes, and other connective tissue, iii) neural stem cells which give rise to neurons, astrocytes, and oligodendrocytes, iv) epithelial stem cells which give rise to cells lining the digestive tract such as absorptive cells, goblet cells, Paneth cells, and enteroendocrine cells, and v) skin stem cells which occur in the basal layer of the epidermis and at the base of the hair follicles and give rise to new epidermal layers of skin. Also included in the category of adult stem cells are cord blood stem cells in the umbilical cord of a baby which give rise to platelets, red and white blood cells, and mesenchymal cells. Use of umbilical cord stem cells in transplantation has the advantage that the recipient is less likely to reject the transplant since the cord stem cells have not yet developed the features that can be recognized by and attacked by the recipient's immune system and the blood in the umbilical cord lacks well-developed immune cells to attack the recipient's cells.

c) Multipotent - Adult or somatic stem cells can be classified as multipotent stem cells if they can differentiate into numerous but closely related cells. An example of a multipotent stem cell is the hematopoietic stem cells in bone marrow that give rise to many types of blood cells. Most blood stem cells are found in the bone, but a small number can be found in the bloodstream. These adult stem cells are called peripheral blood stem cells or PBSCs. PBSCs are easier to collect than bone marrow stem cells which must be extracted from within the bones but they are too few in number for transplantation.

d) Oligopotent – These stem cells can differentiate into only a few cells and include the myeloblast stem cells that produce 3 types of white blood cells – eosinophils, neutrophils, and basophils.

e) Unipotent – These stem cells can produce only one cell type but have the property of self-renewal that distinguishes them from non-stem cells. Examples of a unipotent stem cell are a germ line stem cell (producing sperm) and an epidermal stem cell (producing skin).

f) Nullipotent - Some cells are terminally differentiated and do not go through cell division. These cells are called nullipotent and an example of this is an erythrocyte (red blood cell). Sometimes the nullipotent state is reached by damage to the stem cells. Undifferentiated stem cells will continue to produce new differentiated cells as long as the basal cells themselves are not damaged. For instance, a 1st (reddened skin) or 2nd (blistered skin) degree burn will heal with new skin forming from the basal skin stem cells because the basal cell layer itself isn't permanently damaged. A 3rd degree burn (charred skin) doesn't heal by producing a new cell layer of skin because the basal cell layer has been destroyed. Skin grafts from other parts of the body are done to cover the burned surface to prevent fluid loss and infection. Eventually scar tissue will grow from connective tissue to cover the damaged area. Likewise, if the stem cell layer in the testes is damaged by chemotherapy or radiation therapy, no further sperm will be produced. (1, 3, 5)

   Damaged stem cells can be successfully replaced in some instances, such as in the case of bone marrow transplants, when a healthy, matching donor can be found. Human adult stem cells were first used for therapy in 1968 when the first successful bone marrow transplant was performed. The process involves irradiating the bone marrow to destroy the faulty (often cancerous) stem cells and replacing them with normal bone marrow stem cells from a healthy and immune compatible donor. Today, bone marrow is transplanted routinely to treat a variety of blood and bone marrow diseases, blood cancers, and immune disorders. More recently, peripheral blood stem cells and umbilical cord stem cells have been used to treat some of these same blood-based diseases.

<u>When Did the Study of Embryonic Stem Cells Begin?</u>

Embryonic stem cells (ES cells or ESC) were first isolated from mouse embryos in the early 1980s. The term 'ES cell' was first introduced in 1981 to distinguish mouse embryo-derived pluripotent (ES) cells from mouse teratocarcinoma-derived pluripotent embryo-derived carcinoma (EC) cells and much work has been done to understand how these mouse embryonic stem cells work. In 1995, the first non-human primate embryonic stem cells were cultured at the University of Wisconsin, Madison. However, it took two subsequent breakthroughs to fully focus attention on stem cell research. The first was in 1997 when Ian Wilmut, Keith Campbell, and colleagues published an article in *Nature* entitled "Viable

offspring derived from fetal and adult mammalian cells" which described the cloning of Dolly the sheep, by reprogramming an adult cell nucleus. By the end of 1998, James Thomson and his team at the University of Wisconsin published an article in *Science* entitled "Embryonic stem cell lines derived from human blastocysts", showing they had isolated stem cells from human embryos and coaxed them to grow into five 'immortal' cell lines. (4, 6, 7)

By late 1999, the National Institutes of Health (NIH) published a draft guideline for doing federally funded research on pluripotent human stem cells and the guidelines were published in 2000. Worldwide, 155 human embryonic stem cell lines were developed and 78 were approved for U.S. federal funding. In 2006, President George W. Bush amended the guidelines to allow only 22 of the established stem cell lines to be used in federally funded research. Most of these 22 cell lines did not grow or died easily. President Barack Obama in 2010 approved 14 additional human embryonic stem cell lines, 5 of which were new embryonic stem cell lines and 9 of which were previously known and were currently used only in non-federally funded research. (8, 9, 10, 11, 13)

<u>What Current Research Is Being Done on Stem Cells?</u>

Currently, work is being done to reprogram the DNA of human adult somatic cells, such as mature skin, liver, or stomach cells, to become more like embryonic stem cells which can give rise to the 3 germ layers and then to multiple cell types.

There are four experimental routes for nuclear reprogramming.

a. Nuclear Transfer to Eggs - The first method of nuclear programming is by nuclear transfer to eggs, which includes reproductive cloning and therapeutic cloning (somatic-cell nuclear transfer (SCNT)). In nuclear transfer to eggs, the DNA in an oocyte from a donor is removed and the DNA from a skin cell of the patient is inserted into the enucleated oocyte. The oocyte containing patient DNA develops into a cloned embryo. In reproductive cloning, this embryo can be implanted into a surrogate mother to produce a genetically identical younger version of the adult patient. However, this option is very controversial and largely prohibited when it pertains to human cloning. In therapeutic cloning, inner cell mass cells are harvested from the cloned embryo and encouraged to grow into differentiated cells of the type of tissue needed by the patient. This option is very desirable since the tissue will not be rejected by the immune system of the patient since the cells derived from the patient are seen as 'self' by the immune system. In addition, a wide variety of diseases that involve the loss or malfunction of a cell type can be addressed. These include diseases such as diabetes, Parkinson's, Huntington's, chronic liver failure, and osteoporosis.

   The first successful somatic cell nuclear transfer in non-human primates was done at the Oregon National Primate Research Center and Oregon Stem Cell Center in Beaverton, Oregon and an

article called “Producing Primate Embryonic Stem Cells by Somatic Cell Nuclear Transfer” was published in *Nature* in November, 2007. Shoukhrat Mitalipov’s team was successful in the nuclear reprogramming of adult rhesus macaque somatic cells into pluripotent embryonic stem cells and in demonstrating proof-of-concept for therapeutic cloning in primates. They used 304 rhesus macaque oocytes which yielded 35 blastocysts and 2 stem cell lines. In June 2013, Shoukhrat Mitalipov’s team published an article called “Human Embryonic Stem Cells Derived by Somatic Cell Nuclear Transfer” in the journal *Cell* showing how they created stem cells using skin cells from an 8 month old child where therapeutic cloning was the goal. Many scientists have tried to create human SCNT lines but until now, none had been successful. (18, 21)

b. Induced Pluripotency (iPS) -The second route for nuclear reprogramming is by induced pluripotency (iPS) and involves the introduction of embryonic genes into a donor cell to induce the DNA of the cell to move from a mature state to an embryonic state. Again the goal is to develop embryonic stem cells from the patient’s own cells to increase the chance of compatibility of tissues. Mouse iPS cells were developed in 2006. In 2007, Shinya Yamanaka’s team at Kyoto University, Japan integrated 4 genes, OCT3/4, SOX2, KLF4, and c-Myc, into the human adult fibroblast cell genome via a retrovirus to direct the differentiation of the adult cell into an embryonic-like cell. The team noticed that a small number of genes were active only in stem cells but not in differentiated cells. By introducing a mixture of 24 genes back into the adult cell, they found that they could turn differentiated cells into undifferentiated cells with only the 4 genes. The genes appeared to be remodeling the cells’ DNA by uncondensing (turning on) the genes that had been condensed (turned off) in the differentiated cell. In stem cells, the DNA is arranged loosely, with its genes ready to spring into action. As signals enter the cell and differentiation begins, genes that are not needed will be condensed (turned off) and those genes that are required for a specialized function will remain uncondensed and active. In 2012 Shinya Yamanaka won the Nobel Prize in Medicine or Physiology along with Sir John Gurdon “for discovery that mature cells can be reprogrammed to become pluripotent”. (12, 19)

   Also in 2007, James Thomson did similar experiments in human somatic cells integrating 4 genes, OCT4, SOX2, NANOG, and LIN28, via a lentiviral system. The OCT3/4 and SOX2 comprise a regulatory complex that controls the expression of genes important for the maintenance of the primitive state. The two research teams differed on the necessity of the other genes for maintenance, differentiation, and proliferation. (20)

   In mouse studies, in order to tell if the induced embryonic cells are able to make tissues from all 3 embryonic germ layers (endoderm, ectoderm, and mesoderm), the cells are transplanted subcutaneously under the skin of a mouse which is immunocompromised. The development of a teratoma would indicate the process worked since c-Myc and LIN28 genes are associated with

cancer. This does present a risk for cancer when introducing the cells back into the human and this is still being addressed. Unfortunately, not all iPS cells reprogram fully, but the promise is there. While more comparative study is needed to ensure iPS cells are the same embryonic stem cells derived from a blastocyst, iPSs may allow scientists to eventually move away from the controversial use of human embryonic stem cells.

On July 10, 2014, Shoukhrat Mitalipov published a paper in the journal *Nature* called "Abnormalities in human pluripotent cells due to reprogramming mechanisms" in which he showed that somatic cell nuclear transfer (SCNT) was more accurate at reprogramming human skin cells to become embryonic stem cells and produced fewer epigenetic abnormalities than inducing pluripotent stem cells (iPS) when compared side by side using the same set of skin cells from the start. The SCNT cells were almost identical to the embryonic stem cells while the iPS cells made from the same skin cells retained a large number of characteristics of skin cells. (22)

c. Lineage Switch - The third route of nuclear reprogramming is by lineage switching back to a branch point and out again in a different direction.

d. Direct Conversion - The fourth is by direct conversion of one cell type in an organ to another in the same organ. (16)

Once reprogrammed, the embryonic stem cells can be aggregated into an embryoid body (EB) (a ball-like embryo-like structure consisting of a core of mitotically active and differentiating human embryonic stem cells and a periphery of fully differentiated cells from all three germ layers), made to differentiate in culture, or transplanted to a blastocyst and from there, various types of adult cells can be formed.

Newer techniques also address the ethical issue of using human embryonic tissue and include removing a single cell from an embryo at the 8 cell stage. The one cell is cultured into a new stem cell line for therapeutic purposes and the other 7 are implanted into the uterus to develop into an individual. Another idea is to create embryos that cannot develop by modifying the growth genes. The modified genes are inserted into the nucleus of a fertilized egg and then the egg is allowed to develop into a blastocyst. The inner cell mass cells are harvested, the modified gene removed, and a new cell line is cultured. (20)

<u>What Are the Therapeutic Successes of Stem Cell Research?</u>

One of the main goals of human embryonic stem cell research is to create treatments for patients with genetic and degenerative disorders with a ready supply of transplantable cells generated in culture. Researchers from the Massachusetts Institute of Technology (MIT) in Boston teamed with researchers from the University of Alabama in Birmingham in 2007 to investigate the introduction of a corrected gene in iPS cells taken from fibroblasts in the tail of a mouse that was a humanized knock-in mouse

model of sickle cell anemia. In this mouse, the alpha-globin genes were replaced with human alpha-globin genes and the beta-globin genes were replaced with human sickle beta-globin genes. The mice, denoted as hβs/hβs, lived for up to 18 months and had the typical symptoms of sickle cell anemia, including severe anemia, urine concentration defects, spleen infarcts (areas of tissue necrosis or cell death by progressive enzymatic degradation caused by impairment of the arterial or venous blood supply due to blood clots or blood pressure alterations), and poor health. Fibroblasts from the tail of these mice were collected and treated with retroviruses containing OCT4, SOX2, KLF4 genes and a lentivirus containing a 2-LOX c-Myr cDNA and then these iPS cells were cultured. The sickle cell gene mutation was corrected and inserted in the fibroblast iPS cells and allowed to differentiate into embryoid bodies and then into hematopoietic progenitors. The latter were transplanted into irradiated hβs/hβs mice. Blood smears of the untreated mice showed an abundance of elongated cells typical of sickle cell anemia while smears from treated mice showed fewer elongated cells. Also, the treated animals had a high red blood cell count. In the future, it is hoped that a similar protocol can be done in human embryonic stem cells to replace genetic mutations and to screen iPS cells for effective disease-specific drugs. (15)

On June 4, 2012, it was announced in the British newspaper, *The Guardian*, that the first person in the United Kingdom to receive a transplant of human embryonic stem cells had recovered some of his central vision. Marcus Hilton is part of a human clinical safety trial which includes two people in the U.S. (and Hilton in the U.K.) with Stargardt's disease, a disease in which the retina begins to degenerate and thin affecting the central vision. Hilton was diagnosed when he was 10 years old and had only peripheral vision intact. In January, 2012 at the age of 34, he had 50,000 retinal pigment epithelium (RPE) cells injected into the space behind the retina in his right eye. The RPE cells were developed in the United States by the company Advanced Cell Technology (ACT) from human embryonic stem cells derived from embryos left over from fertility treatments. It was equally successful in the two American patients. Rejection of the cells by the patients isn't expected to be an issue since the eye is immune privileged and can tolerate a graft of human tissue. In 2014, an article published in *The Lancet*, entitled "Human embryonic stem cell-derived retinal pigment epithelium in patients with age-related macular degeneration and Stargardt's macular dystrophy: follow-up of two open-label phase 1/2 studies" stated that 13 (72%) of (by now) 18 patients "had patches of increasing subretinal pigmentation consistent with transplanted retinal pigment epithelium. Best-corrected visual acuity, monitored as part of the safety protocol, improved in ten eyes, improved or remained the same in seven eyes, and decreased by more than ten letters (on the eye chart) in one eye, whereas the untreated fellow eyes did not show similar improvements in visual acuity."(10, 23)

Recent breakthroughs in human medical applications have been very promising. A windpipe (trachea) has been made out of tiny plastic fibers seeded with stem cells from the person's own bone marrow. The tissue-engineered scaffold was placed in a container called a bioreactor and soaked in a solution that allowed the cells to be fully absorbed. When the engineered trachea was implanted, the cells

continued to grow. In 2008, the first trachea transplant was performed on a woman whose trachea had collapsed due to complications from tuberculosis. As of 2013 (five years later), she was in good health with good lung function and had no rejection issues. In July, 2011, and in January, 2012, the same surgeon, Dr. Paolo Macchiarini, transplanted a trachea made from self-stem cells to replace ones destroyed by cancer into a person in Eritrea and in a person in Baltimore. Sadly, both of these cancer patients did not survive. Tracheal transplants have also been tried in several children with mixed success. Similar work has been done to reconstruct the outer ear of patients where the outer ear was damaged or destroyed. (14, 24, 25)

There is still much more basic research to be done on human embryonic stem cells and induced pluripotent stem cells. These cells don't seem to be the same as embryonic stem cells obtained from mice. Mice embryonic stem cells seem to divide and differentiate more slowly than human embryonic cells. The human cells from the inner cell mass cells in a blastocyst have already moved to the next stage, when equivalent mouse cells have not. Since human cells, either from an embryo or generated by re-programming, haven't been successfully held at a ground state, it has been difficult to produce standardized cells to transplant as replacement cells. Some early experiments involving the transplant of fetal neurons into Parkinson's patients show improvement in some but not all and adverse side effects in some. In addition, ethical issues concerning when life begins, when parenthood begins, embryo rights, and embryo donor rights continue to be raised with respect to the use of human embryonic tissue. (10)

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