Stem cells and SCI: Where are we?

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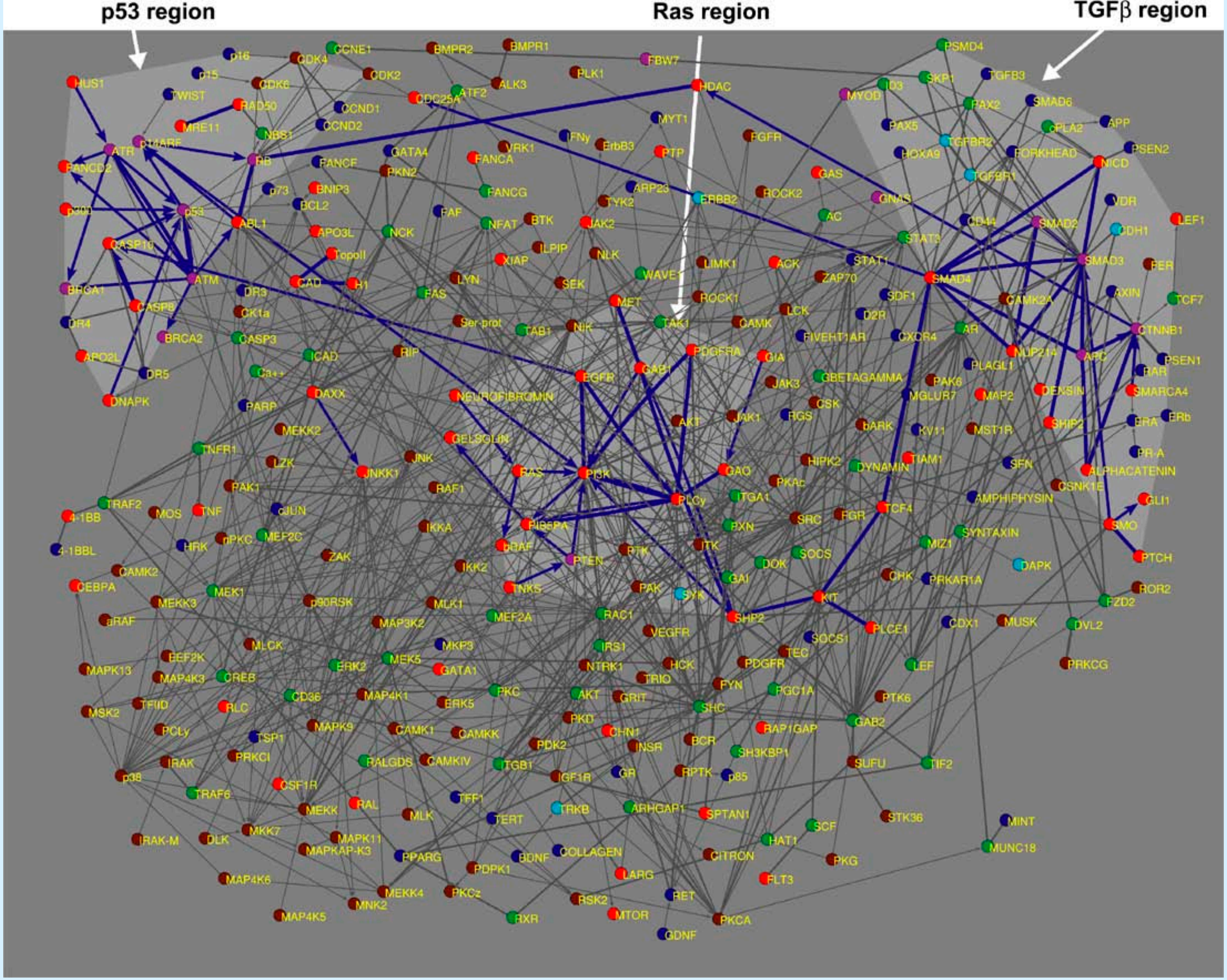
May 2009.



Human cancer signaling map – a network of more than 300 proteins and 900 signaling relations.

Three Major Phases of Stem Cells.

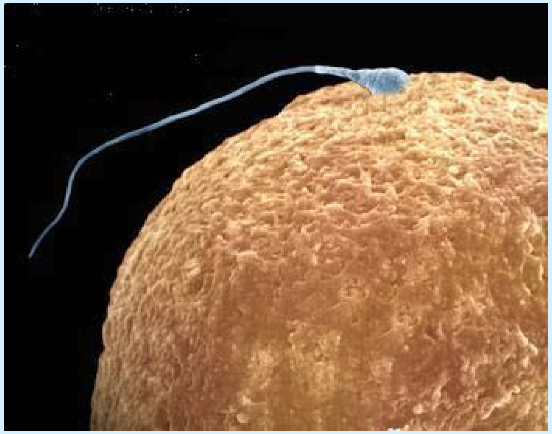
- Phase 1: Basic science – learning how stem cells operate, learning how to produce them in purified form and to control / manipulate them as required.
- Phase 2: Early clinical applications, used in combination with other treatments. This will be a relatively long period of research, gathering data and experimentation into what techniques work best.
- Phase 3: Very specific and very safe applications to address spinal cord injuries.

Cells

- Humans are a multicellular organism made up of trillions of cells. A cell is our smallest living subunit.
- Over 200 types of cells are found in humans.
- Cells nourish themselves, produce energy, exchange information with other cells, multiply, and eventually die – most are continually being replaced in the body.
- Cells work individually to do many biological tasks.
- Cells also work together to build larger units, for example, to form different types of tissues and organs.
- Stem cells are critical in initial development and in maintaining our cells over our lifetime.

Fertilization

- To understand stem cells, we need to first look at development.
- An ovum (egg) is the largest cell in the human body.
- A single sperm is the smallest cell found in the body.
- Fertilization (conception) takes place inside the fallopian tube.



- Upon fertilization, one egg cell and one sperm cell join together to form one new cell, beginning the development of an individual.
- This first single cell is called a zygote [ZYE-goat].

Early Cell Division

6 blastomeres

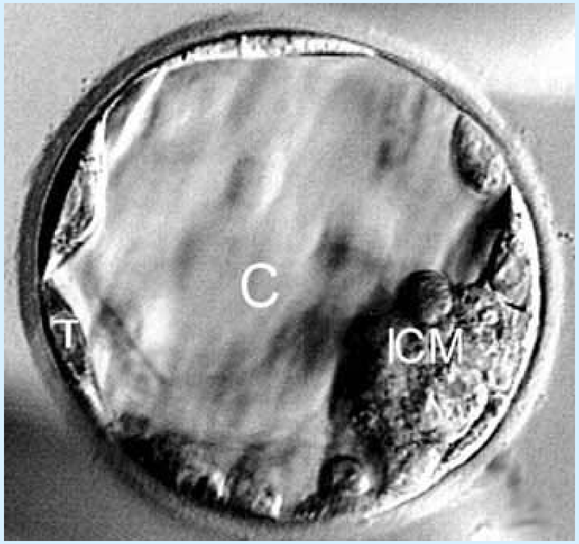
- The zygote begins a series of cell divisions going through 2-cell, 4-cell, 6-cell and 8-cell stages.
- These first 8 or so cells, called blastomeres, are very special "stem cells" because each one is essentially identical and each has the ability to create <u>any</u> <u>type</u> of cell found in the human body: any one of these early cells could go on to develop into a complete individual.
  - If all 8 cells were removed & implanted individually, we would get octuplets – 8 identical siblings.

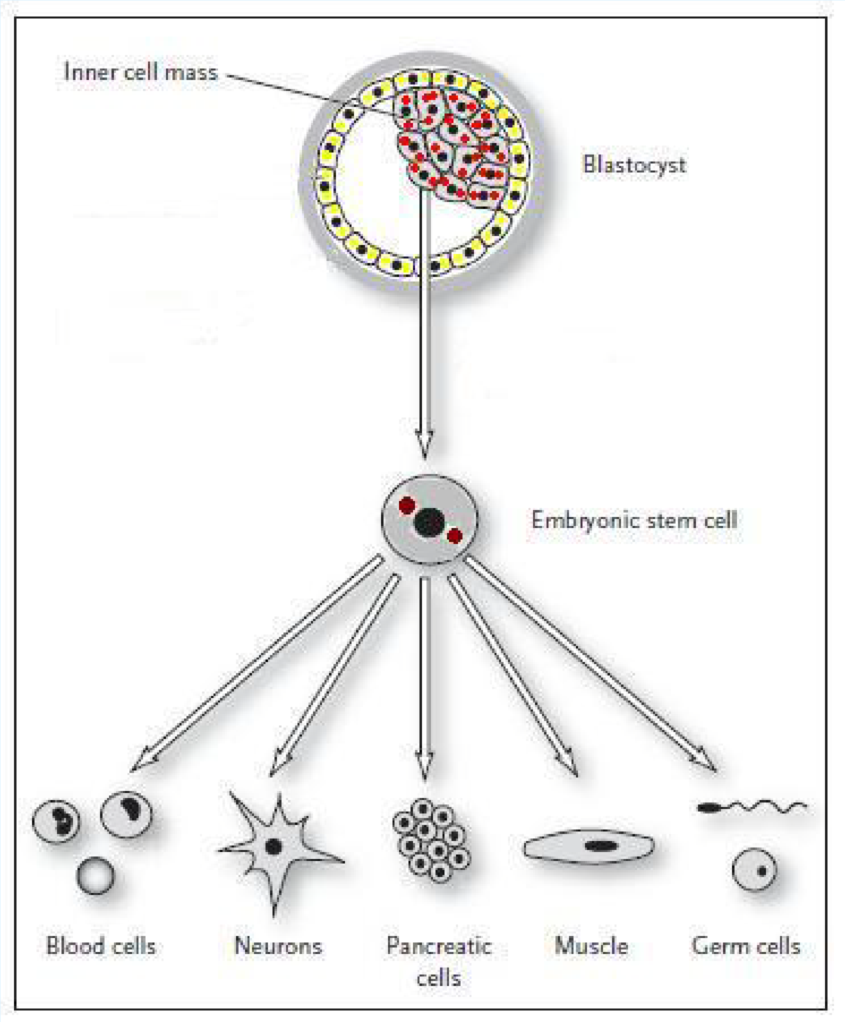
Early Cell Division Continues

- All of the different cells of the body are derived from the zygote's single cell and its first few divisions.
- Removal of a blastomere or two at this stage does not affect subsequent development (this is how preimplantation genetic testing is done).
- As cell divisions continue past the eight cell stage, the cells begin to differentiate and specialize: different cells emerge to do different jobs.
- A ball of cells called a blastocyst [BLAST-oh-cyst] emerges with an outer wall and an inner cell mass.

Inner and Outer Layers

- By about day 4 or 5, the cells of the blastocyst have differentiated into two distinct cell types, inner & outer:
  - The outer wall of cells that will form the extraembryonic membranes (e.g., the placenta). These cells also produce the hormone measured by pregnancy tests and they are responsible for implantation of the embryo into the uterus.
  - The inner cell mass – a core of cells that will form the embryo. These cells are the primary source of embryonic stem cells.
- By day 5, the blastocyst has about 200-300 cells.





Panno, 2005, p. 6

Pregnancy Begins

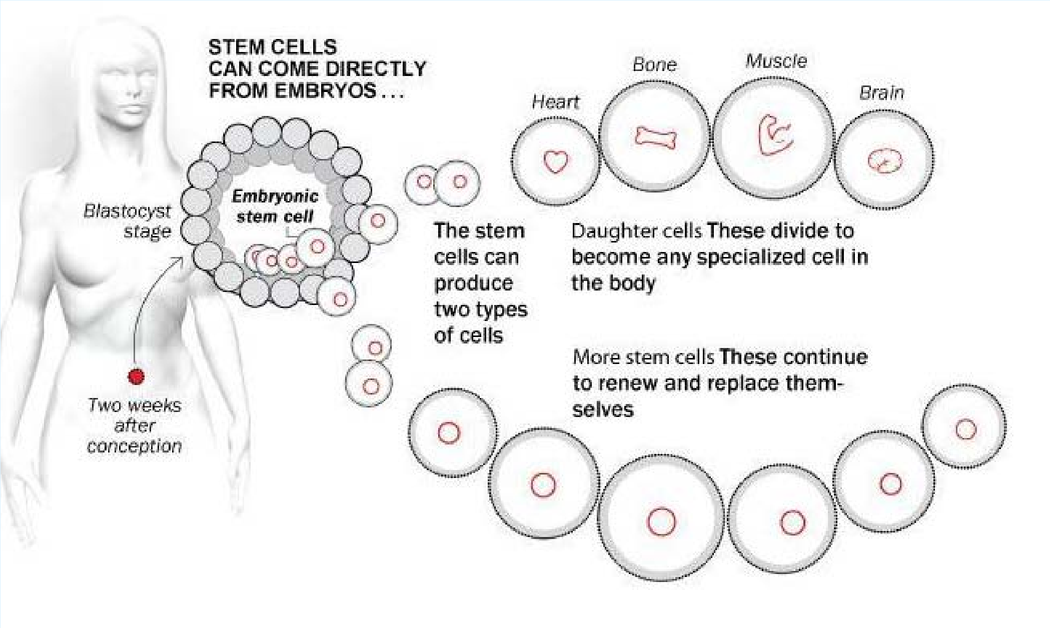
- About 7 – 9 days after fertilization, the blastocyst (‘embryo’) embeds itself into the wall of the uterus – a process called implantation, and the pregnancy is established.
- From implantation to eight weeks the term embryo is used. From eight weeks until birth at about 38 weeks, the term fetus is used.

Stem Cells are Special

- Stem cells are central to three processes:
  - Differentiation and development of cells,
  - maintenance and repair of adult tissue,
  - and in cancer.

- Stem cells are vital in initial development but also a small number of stem cells are always present in different tissues and when called upon, stem cells differentiate to maintain and repair tissue.

Stem Cells Divide In a Special Way



←differentiated

←undifferentiated

- Each stem cell is able to keep on dividing to form:
  - one daughter cell that differentiates into a specific type of cell, and
  - one undifferentiated daughter cell that remains a stem-cell.

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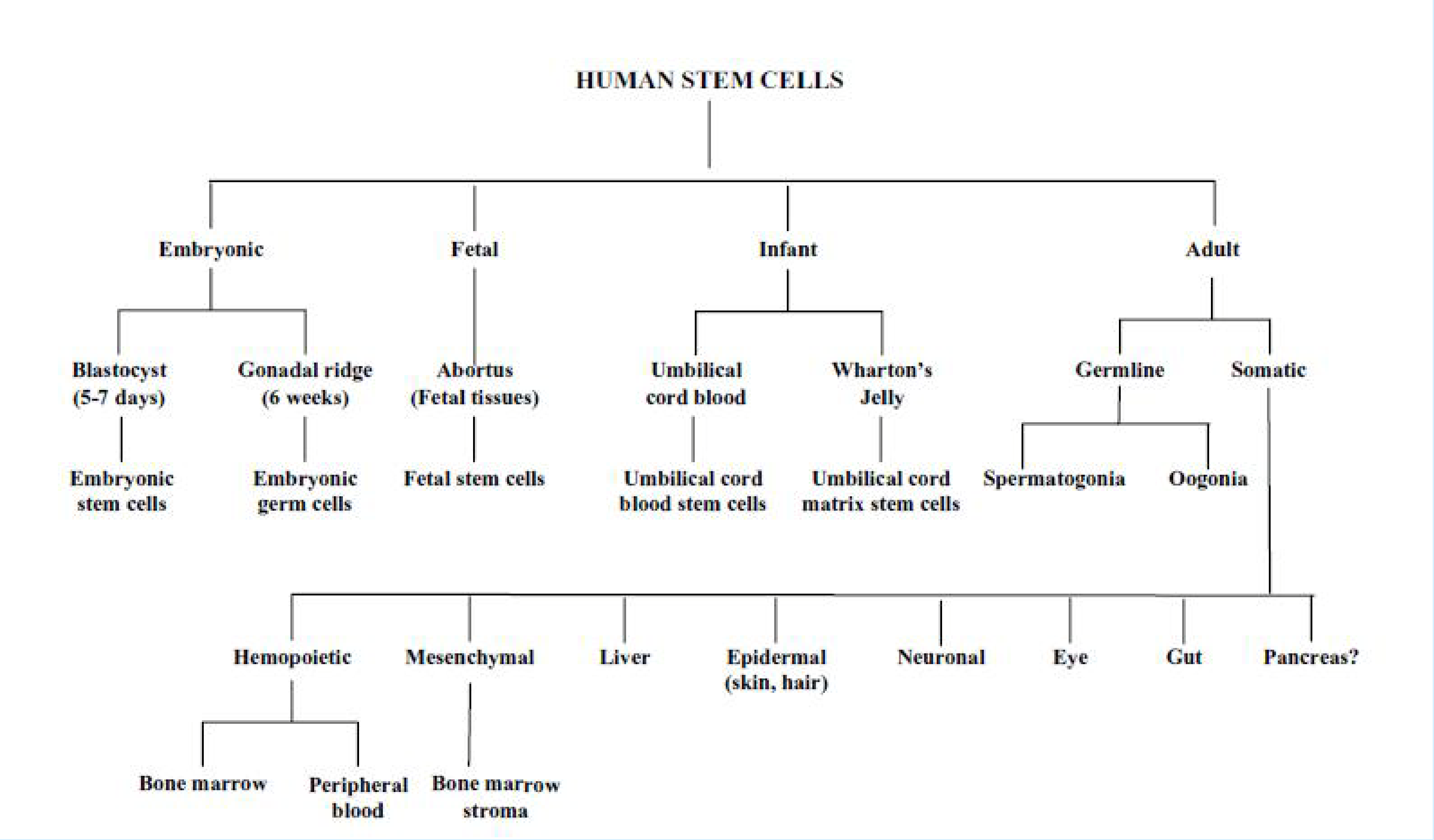
Types of Stem Cells



Bongso and Lee, 2005, p. 4

Two Basic Types of Stem Cells

- There are two basic types of stem cells:
  - Embryonic stem cells:
    - The most undifferentiated, greatest potential
    - Primarily found in the inner cell mass of the blastocyst.
  - Adult stem cells:
    - Already somewhat differentiated, potential limited (?)
    - Found in the umbilical cord and in differentiated tissues.
    - By birth, these cells are present in various tissues (although they are called “adult,” they are present as soon as cells begin to differentiate).
- These two types of stem cells have different characteristics that need to be understood.

Totipotent Cells

- In mammals, the zygote and the first 8 or so cells produced by division are totipotent [TOE-tea-potent]:
  - Each of these cells can become any type of cell in the body (including the extraembryonic membranes, e.g., the placenta, amniotic fluid, & umbilical cord).
- Think of totipotent as stem cells with TOTAL potency – these cells can make <u>any</u> cell needed by the developing individual (with one of these cells, you could theoretically reproduce an individual).
- These are one type of “embryonic stem cells.”
- These cells are very important in basic research.

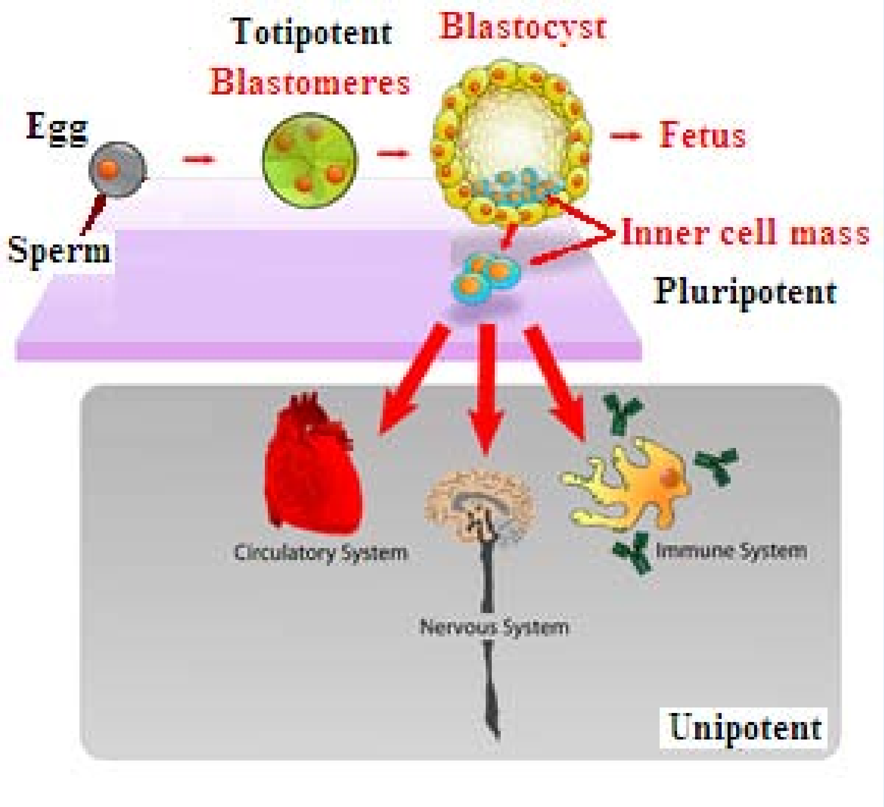
Development Limits Potential

- As cells differentiate and become more specialized, they quickly lose their totipotency and they become pluripotent [PLUR-eee-potent]:
  - Each pluripotent cell has the potential to make any differentiated cell in the body, but it can no longer make the extraembryonic membranes. (one of these cells could not reproduce an individual).
  - Think of pluripotent as cells with SEVERAL potencies.
- Pluripotent stem cells are primarily found in the inner cell mass of the blastocyst and are the primary type of embryonic stem cells we hear about.

Further Limits on Potential

- As pluripotent stem cells further specialize, they become multipotent (or unipotent) stem cells.
- These stem cells are found in various body tissues.
- The traditional view was that multipotent cells could only become the type of tissue they are found in, e.g., bone stem cells can only give rise to bone cells.
- Think of multipotent as cells with SINGLE potency.
- These cells have several names:
  - Adult stem cells.
  - Postnatal stem cells (postnatal means after birth).
  - Umbilical stem cells (the umbilical cord is rich with them).
  - Somatic stem cells (somatic means found in the body).

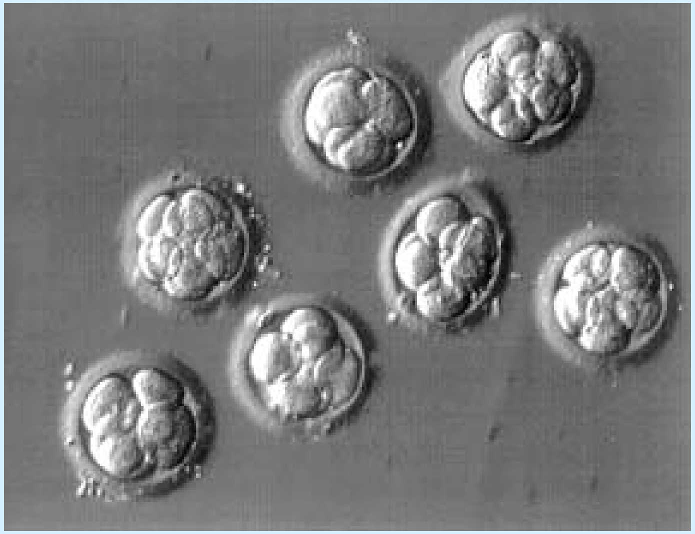
Summary



Totipotent
Blastocyst
Blastomeres
Egg
Fetus
Sperm
Inner cell mass
Pluripotent
Circulatory System
Immune System
Nervous System
Unipotent

In Vitro Fertilization (IVF)

- Infertility affects about 10% of men & women of reproductive age in the United States. Just less than 5% of them use *in vitro* fertilization (IVF).
- *In vitro* fertilization involves first stimulating a woman's egg production and collecting several eggs.
- The eggs are fertilized with sperm in the laboratory (*in vitro* means outside of the body) producing zygotes.
- The zygotes are grown for a few days becoming blastocysts, then several are transferred into the woman's uterus. Ideally, a normal pregnancy will result.



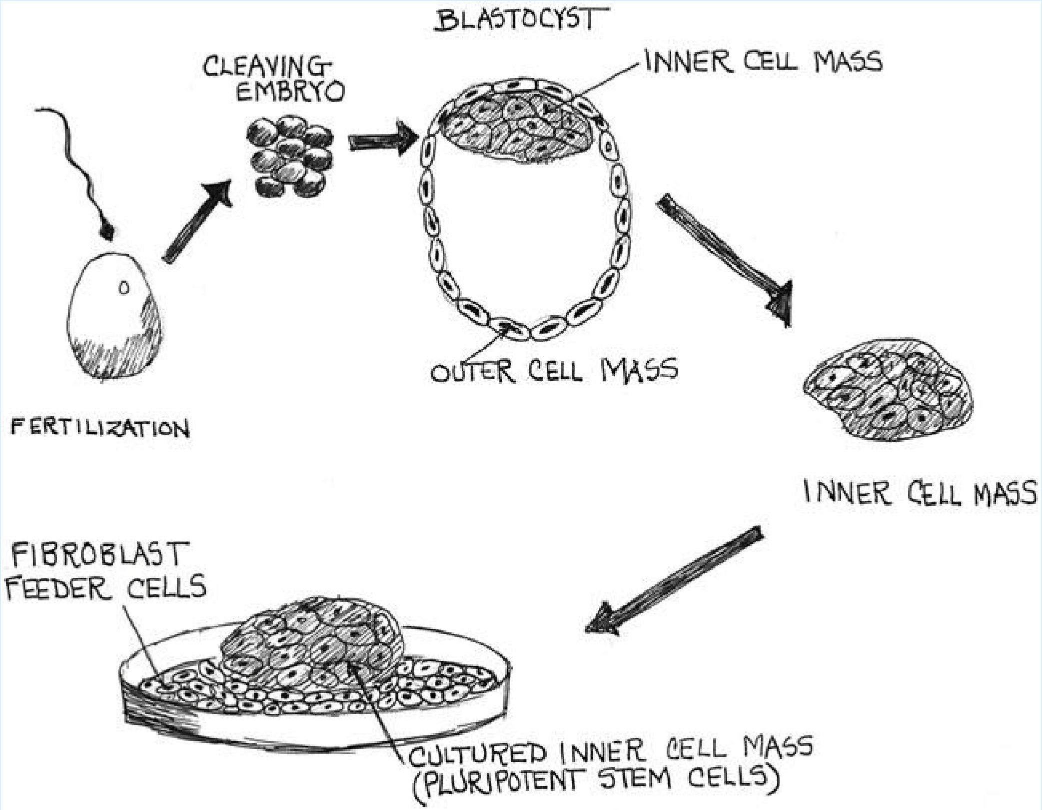
How Scientists Obtain ESCs

- There are usually extra blastocysts left over after the couple has had the children they want. These extra frozen “embryos” are usually either simply discarded or are stored indefinitely.
- With informed consent, many couples donate these extra embryos for use in stem cell research.
- Most embryonic stem cells (ESCs) used in stem cell research are obtained from four or five day old blastocysts created through *in vitro* fertilization.
- The use of ESCs is controversial because the procedure destroys the unimplanted blastocysts.

Embryonic Stem Cells

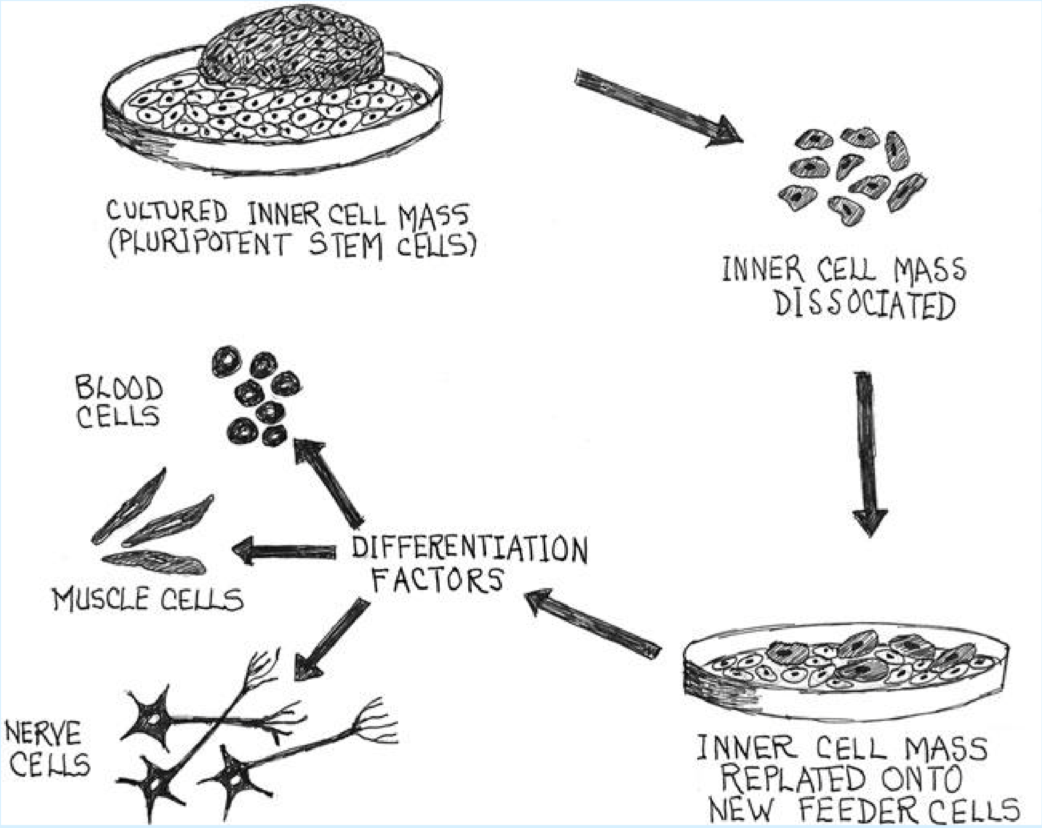
- In the laboratory, ESCs can be grown in special conditions & remain undifferentiated.
- If cells are allowed to clump together, they spontaneously begin to differentiate: they begin to form muscle cells, nerve cells and other types of cells.
- Scientists are learning how to control the differentiation of ESCs into specific cell types:
  - If they can reliably control this process, they will be able to produce differentiated cells in the laboratory to treat certain diseases.

Producing Stem Cells



Cohen 2007, p. 20

Producing Stem Cells



Cohen 2007, p. 22

Embryonic Stem Cell Lines

- Researchers remove a cell from a blastocyst before the cells differentiate, and grow it in the laboratory:
  - The undifferentiated stem cells keep dividing (for years), creating millions of identical cells. This is called a cell line. These cells are used in research.
- Each of these millions of cells is pluripotent – they can go on to grow into any type of cell in the body.
- One major challenge is that conditions must be perfect – one mistake here would be repeated over & over and multiplied – e.g., just one skin cell in a batch of a million muscle cells would create a cancer.

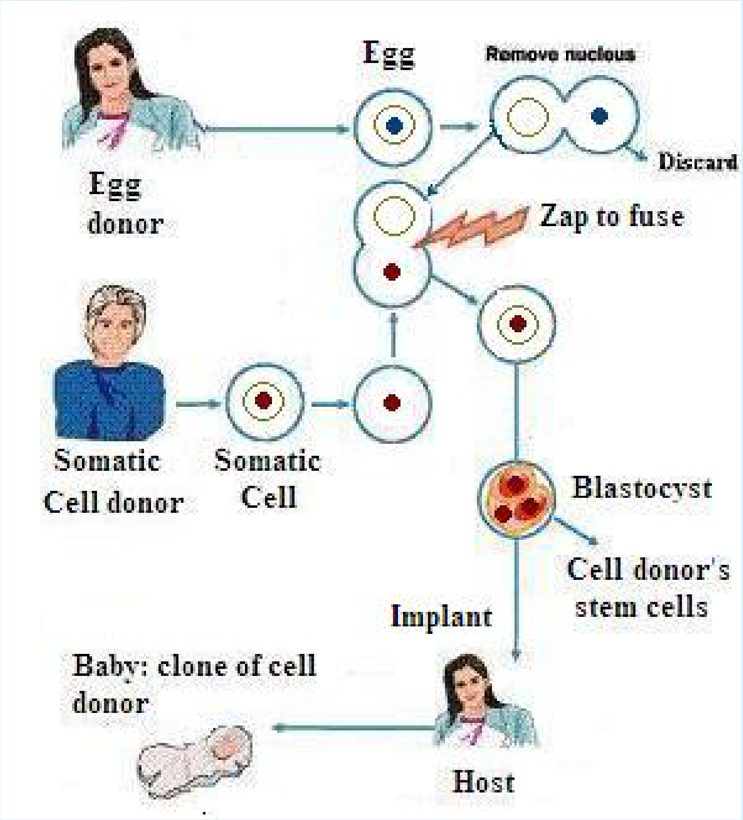
ESCs From SCNT Cloning

- Scientists are developing a type of cloning (often called therapeutic cloning) to create ESCs:
  - Also called somatic cell nuclear transfer (SCNT), this procedure creates stem cells genetically matched to the patient.
- The nucleus (center) of a cell contains its DNA. The nucleus is removed from a donated, “fresh,” unfertilized (human) egg. This “hollow” egg now cannot be fertilized or develop normally.
- The nucleus is taken out of a somatic cell (e.g., a skin cell) obtained from a patient & is inserted into the egg.

Somatic Cell Nuclear Transfer

- The cell now reverts to a totipotent state and the new "egg" starts to develop into a normal blastocyst.
- Embryonic stem cells are removed at day 5 or 6.
- The stem cells are grown in the laboratory and are allowed to differentiate into different types of cells.
- These differentiated cells – whatever tissue or organ needed to treat the patient – would be transplanted back into the patient, ideally curing his or her disease.
- This procedure is controversial as it destroys human genetic material (the DNA of the unfertilized egg).
- This method is used to clone animals (Dolly).
- This SCNT procedure is still in the very early stages.

The Future of SCNT?



- This procedure may be possible in humans, but due to technical issues, may not be practical to do clinically.

Cautionary Note # 1 Korea

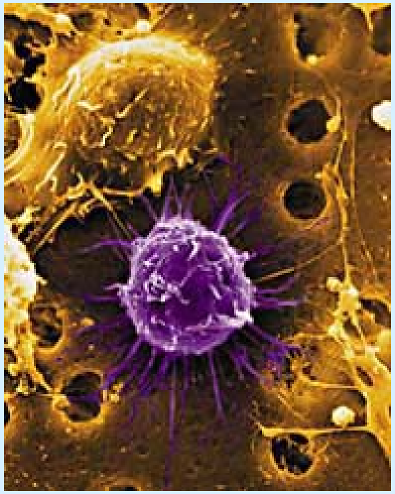
- In May 2005, Korean researcher Woo Suk Hwang said they had used somatic cell nuclear transfer to create human embryonic stem cells from patients – this would have been a huge step ahead in the research.
- The claims were later found to be fraudulent.
- Today, other research teams are still trying to use SCNT in humans to create embryonic stem cells.

Adult Stem Cells

- Adult stem cells are undifferentiated cells found along with the differentiated cells in a tissue or organ.
- An adult stem cell can renew itself, and can differentiate to yield the specialized cell type of the tissue or organ it is found in (multipotent).
- The primary roles of adult stem cells are to maintain and repair the tissue in which they are found:
  - Example, adult stem cells in the blood help to maintain and repair the blood.
- Only a fraction of the cells are adult stem cells: e.g., 1 in every 15,000 bone marrow cells is a blood stem cell. Scientists have had a hard time isolating them.

Types of Adult Stem Cells

- Adult stem cells are thought to stay in a specific area of each tissue where they may remain silent (non-dividing) for many years until they are activated by disease or tissue injury.



- About 20 types of adult stem cells have been found – here is a picture of an adult bone marrow cell (colored purple to stand out).

- Tissues now discovered to contain stem cells include; brain, bone marrow, blood, blood vessels, skeletal muscle, heart, fat, teeth, skin and the liver.

A Tantalizing Theory

- Theory: Because most cells contain a full set of DNA, any cell can be "induced" to become any type of cell.
- Cells differentiate based upon the genes that "turn on" inside them. For example, in skin cells, a certain set of genes switches on to tell the cell to become a skin cell while other genes remain off: in nerve tissue, the "skin cell genes" stay turned off and a different set of "nerve" genes are activated to form a nerve cell.
- Scientists are slowly learning how to control these mechanisms -- it now appears possible to make somatic cells dedifferentiate back to totipotency and then trigger them to redifferentiate into other kinds of cells. DOI: 10.1126/science.1116447, doi:10.1038/nature07863(Toronto)

Induced Pluripotent Stem Cells

- Researchers insert genes responsible for embryonic pluripotency into skin cells returning them to a state resembling embryonic stem cells.
  - Example: In 2005, researchers were able to manipulate adult skin stem cells into developing into bone, fat and muscle cells. doi:10.1089/scd.2005.14.337
- This approach holds the promise that adult cells may be a source of stem cells, but much more research needs to be done.

Autologous (self-to-self) Transplantation

- Autologous [awe-TAH-la-gus] transplantation uses the patient’s own cells, avoiding major problems linked to rejection and the need for immunosuppression. Because the cells belong to the patient to begin with, the body accepts them and there is no immune response to them.
- Adult stem cells/cells are taken from a patient (at any age) and are grown & differentiated in the laboratory to create healthy cells that are then reintroduced back into the patient to treat his or her disorder.
- This is an ideal scenario – using autologous cells, possibly with genetic enhancements or repairs, to treat or replace a patient’s damaged tissues.

Cautionary Note # 2 Moscow

- A boy with an inherited neurodegenerative disease, was treated in Moscow with intracerebellar and intrathecal injection of human fetal neural stem cells. Four years later, growths in his brain and spinal cord were found. The spinal growth was removed and has not reappeared but the brain tumor continues to grow slowly and appears benign.
- Analysis showed that the spinal tumor was derived from the transplanted donor neural stem cells.
- This is the first example of a donor-derived tumor.
- doi:10.1371/journal.pmed.1000029

Moscow

- This case illustrates the risks of participating in unregulated stem cell transplant procedures.
- There were no previous studies showing this technique would work in animals.
- American researchers note that the child received an inordinate number of transplanted cells.
- American researchers comment that these results where predictable based upon the procedures used.
- This case highlights the perils of participating in unregulated and unmonitored procedures in foreign countries.
- doi:10.1038/stemcells.2009.34

Neurological Cells

- The brain and the spinal cord contain two main cell types: neurons, which transmit electrical signals to and from the brain, and glial [GLEE – al] cells, which support and protect the neurons.
- If these essential neural cells become damaged or diseased, the body cannot readily replace them.
- Although neural stem cells have been found in the nervous system, for some reason, these cells do not participate in the repair of damaged or diseased neuronal tissue.

Adult Neural Stem Cells

- Human neural stem cells (hNSCs) as well as neuronal precursors (neuroblasts) that divide to give rise to nerve cells (neurons), can be isolated from the adult central nervous system:
  - These neural stem cells can generate several types of nerve cells.
  - Neuroblasts spontaneously multiply and move toward areas of brain damage, but, for some reason, natural regeneration and repair of tissues is not very efficient in the CNS.
- The ultimate goal is to someday control and use the neural stem cells present in the nervous system to repair and replace cells damaged by CNS injury.

Embryonic Neural Stem Cells

- Neurons do not divide and show little capacity to regenerate after injury to the central nervous system.
- Research has shown that human embryonic stem cells can develop into precursor CNS cells and then further differentiate into neurons and astrocytes, the cell types that populate the different regions of the brain and spinal cord.
- In 2005 scientists learned how to induce human embryonic stem cells to become spinal motor neurons, capable of surviving in the lab.
  doi:10.1001/jama.293.9.1047
  - This creates a laboratory model that will allow drugs to be safely screened and make research easier.

Restoration of Lost Spinal Function

- General strategies to restore function:
  - 1). Adaptations to the loss of function (using an orthosis, e.g. a brace or electromechanical device).
  - 2). Reorganization of neuronal circuits through retraining sessions aimed at improving limb movements.
  - 3). Regeneration or repair of existing cells through manipulation of the bodies natural repair mechanisms (e.g. growth factors).
  - 4). Replacement of damaged neuronal cells (e.g. stem cells).

Combination Therapy

- A 2005 study showed that combining stem cells with gene therapy promoted the growth of new myelin around nerve cells in damaged spinal cords of rats.
- Researchers injected genetically enhanced precursor cells into the damaged spinal cords of rats. Studies of the cord tissue showed that many transplanted cells survived and migrated within the cord and that about 30% developed into myelin-producing cells. (oligodendrocytes) [ALL-a-GO-den-dro-sights]
- The study demonstrated that producing myelin can lead to functional improvements in animals with SCI.

DOI:10.1523/JNEUROSCI.1065-05.2005

An Important Step (2009)

- Transplantation of ependymal [eh-PEN-de-mal] stem cells taken from the lining of the spinal cord reverses paralysis in rats with spinal cord injuries.
- The transplanted cells proliferated after spinal cord injury and were recruited by the injured area.
- In animals with spinal cord injury, these cells were found to regenerate ten times faster than similar cells taken from healthy control animals.
- The presence of these stem cells in the adult human spinal cord suggests that stem cell-associated mechanisms might be exploited to repair human spinal cord injuries. DOI: 10.1002/stem.24

The Latest Step

- On January 23, 2009, the first clinical trial of therapy using human embryonic stem cells was announced.
- Geron, a California company, received approval for a phase 1 safety study of a therapy for spinal cord injury.
- The application included data from more than 24 studies, involving nearly 2,000 animals with injured spinal cords and requiring the production and injection of more than 5 billion cells.
- The trial approved by the FDA consists of injecting oligodendrocyte precursor cells into the spinal cord of patients immediately after injury, a procedure shown to help in animals. http://www.geron.com/media/pressview.aspx?id=1148

Cautionary Note # 3

- "At this moment, the full promise of stem cell research remains unknown and it should not be overstated."

- "I cannot guarantee that we will find the treatments and cures we seek."

- President Barack Obama, March 9, 2009, Signing of Stem Cell Executive Order and Scientific Integrity Presidential Memorandum.

Additional Sources

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NIH Stem Cell Information Home Page:
http://stemcells.nih.gov/info/resources.asp

- doi: 10.1038/sc.2009.24