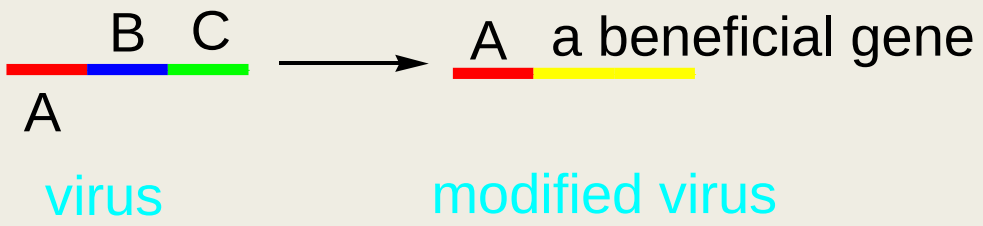
GENE THERAPY AND STEM CELLS

Gene Therapy-Definition

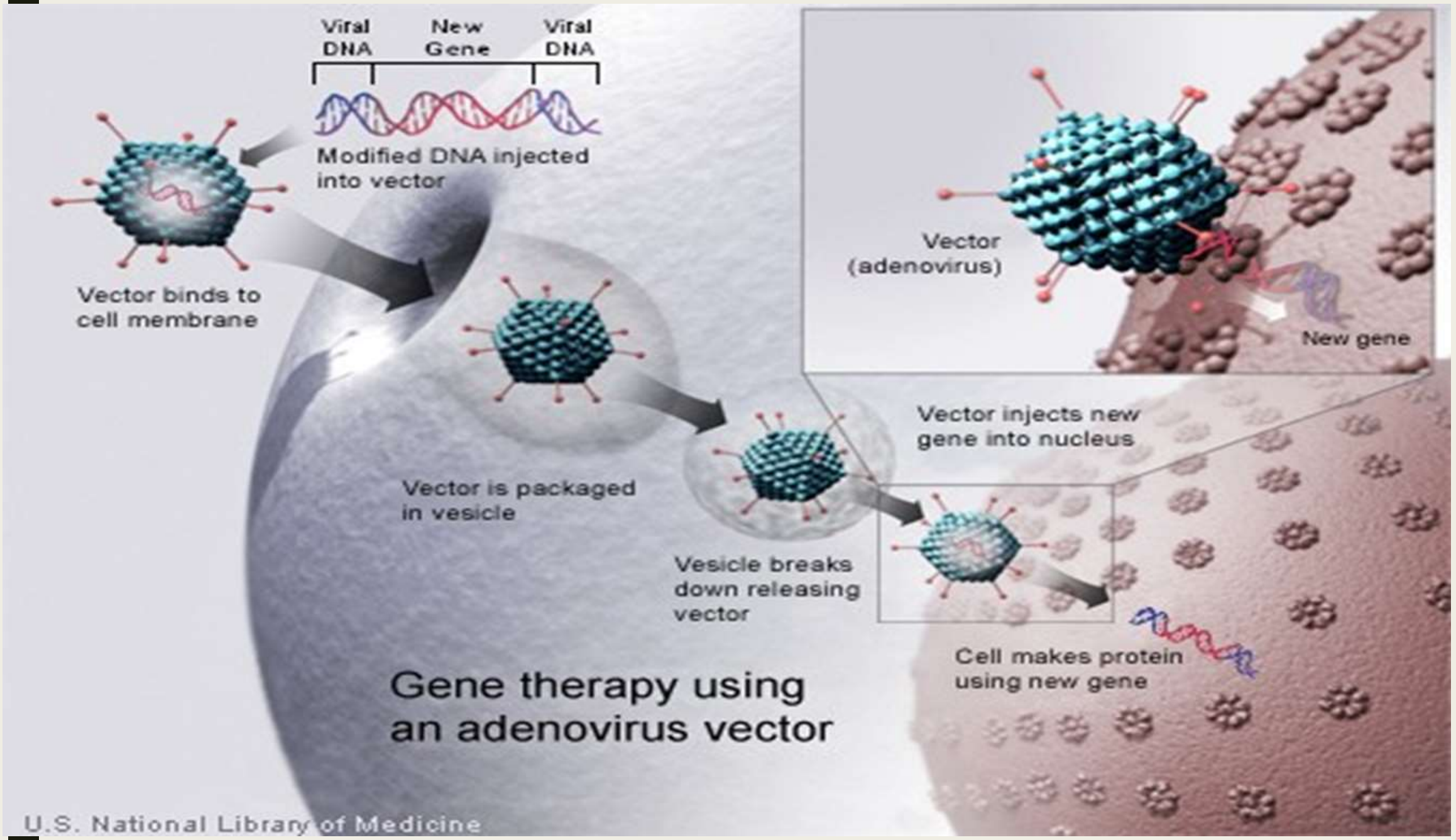
- Normal gene inserted into the genome to replace non-functional gene
- Trials began in 1990
- Scientists focused on diseases caused by single-gene defects, such as **cystic fibrosis, hemophilia, muscular dystrophy and sickle cell anemia, optic nerve disease, wound repair and regeneration, and cardiovascular disease**
- Cystic fibrosis gene was moderately successful

Vectors

- Viruses eg retro viruses, adenoviruses (commonly used)
- Direct introduction (“golden bullets”)
- Liposomes
- Endocytosis of DNA bound to cell surface receptors (low efficiency)
- Artificial chromosome (under development)



- A virus is found which replicates by **inserting its genes into the host cell's genome**. This virus has three genes: **A, B** and **C**.
- Gene A encodes a protein which allows this virus to insert itself into the host's genome.
- Genes **B** and **C** actually cause the disease this virus is associated with.
- Replace **B** and **C** with **a beneficial gene**. Thus, the modified virus could introduce your 'good gene' into the host cell's genome without causing any disease.
- So we use the modified virus to fix the damaged gene


Viral DNA
New Gene
Viral DNA
Modified DNA injected into vector
Vector binds to cell membrane
Vector is packaged in vesicle
Vesicle breaks down releasing vector
Vector injects new gene into nucleus
Vector (adenovirus)
New gene
Cell makes protein using new gene
Gene therapy using an adenovirus vector
U.S. National Library of Medicine

The main issue

- Acute immune response to viral vectors
- Repeated treatment needed
  Genes “lost” when the cell goes through mitosis
- Viral vectors could become pathogenic
- Genes spliced at random into the genome could upset other genes
- Multigene disorders too complex to treat

Applications of gene therapy

- Curing genetic diseases
- Correcting cancer genes
- Inducing cancerous cells to make toxins so they kill themselves
- Blocking viral genes (e.g. HIV)
- Creating stem cells from somatic cells

Future plans

- Gene therapy on sex cells of carriers
- Gene therapy on fertilised egg cells

Ethical issues

- Gene therapy for serious genetic diseases is fine but for other health problems?
- Somatic cell treatment stays with the individual, germ cell treatment passes down the germ line (becomes immortal)
- Very costly. Who pays? Who is eligible?

Adenosine deaminase deficiency

- The first clinical gene therapy was given to a 4-year old girl with adenosine deaminase (ADA) deficiency.
- ADA deficiency or SCID (Severe combined immunodeficiency) is an autosomal recessive metabolic disorder. It is caused by the deletion or dysfunction of the gene coding for ADA enzyme. In these patients the nonfunctioning T-Lymphocytes cannot elicit immune responses against invading pathogens. The right approach for SCID treatment would be to give the patient a functioning ADA which breaks down toxic biological products.
- In some children ADA deficiency could be cured by bone marrow transplantation, where defective immune cells could be replaced with healthy immune cells from a donor.
- In some patients it can be treated by enzyme replacement therapy, in which functional ADA is injected into the patient.

Adenosine deaminase deficiency

During gene therapy the lymphocytes from the blood of the patient are removed and grown in a nutrient culture medium. A healthy and functional human gene, ADA cDNA encoding this enzyme is introduced into the lymphocytes using a retrovirus. The genetically engineered lymphocytes are subsequently returned to the patient.

Since these cells are not immortal, the patient requires periodic infusion of such genetically engineered lymphocytes. The disease could be cured permanently if the gene for ADA isolated from bone marrow cells are introduced into the cells of the early embryonic stages.

GENE THERAPY CURES BLINDNESS

- Cure blindness of inherited condition
- Leber's conginetal amaurosis - inherited disease caused by an abnormality in a gene called RPE65. - The condition appears at birth or in the first few months of life and causes progressive worseness and loss of vision.
- HOW IT WORKS??
  - *used harmless viruses*
  - *enable access to the cells beneath the retina of patients*
  - *By using a very fine needle -safe in an extremely fragile tissue and can improve vision in a condition previously considered wholly untreatable.*

GENE THERAPY REDUCES PARKINSON'S DISEASE SYMPTOMS

- It has significantly improved the weakness of the symptoms such as tremors, motor skill problems, and rigidity
- Done with local anesthesia, used a harmless, inactive virus [AAV-2]

Cystic Fibrosis (CF)

- Cystic fibrosis was first described as a disease in the late 1930s

- The gene of CF is located on the seventh chromosome
- Research has found over 1000 different mutations that may cause CF, however **ΔF508** accounts for approximately 70% of CF patients in Europe (this percentage varies regionally).
- CF is an autosomal recessive disease and is the most common lethal genetic disease among whites. There are 30,000 cases in the United States, 3,000 cases in Canada, and 27,000 cases in Europe.

Current treatment

- Modern treatment now includes

①the intake of digestion enzymes, nutritional supplements,

②percussion and postural drainage of the lungs, improved antibiotics

③inhalation of aerosols containing medication.

- The most visible gene therapy drug under development is inhaled complementary DNA to treat CF.

A typical breathing treatment for Cystic Fibrosis, using a nebulizer and the ThAIRapy Vest

Gene Therapy for Cystic Fibrosis

- Cystic fibrosis should be an ideal candidate for gene therapy, for four main reasons:

1. it is a single gene defect;
2. it is a recessive condition, with heterozygotes being phenotypically normal (suggesting gene dosage effects are not critical);
3. the main pathology is in the lung, which is accessible for treatment;
4. it is a progressive disease with a virtually normal phenotype at birth, offering a therapeutic window.

Choices of Vectors

- Viral vectors:
- Retrovirus
- Adenovirus
- Adeno-associated virus
- Herpes Simplex Virus

Non-viral vectors:
Liposome
DNA–polymer conjugates
Naked DNA

The ideal vector system would have the following characteristics:

(1) an adequate carrying capacity;

(2) to be undetectable by the immune system;

(3) to be non-inflammatory;

(4) to be safe to the patients with pre-existing lung inflammation;

(5) to have an efficiency sufficient to correct the cystic fibrosis phenotype;

(6) to have long duration of expression and/or the ability to be safely re-administered.

1993 vector used: Adenovirus

- The **first** cystic fibrosis gene therapy clinical trials used **an adenovirus vector** to deliver the full-length CFTR (cystic fibrosis transmembrane regulator) gene to cells.
- Adequate doses of adenovirus vector will probably cause an **immune response**. If the adenovirus is to be useful, researchers need to find ways to both improve the virus's ability to enter cells and reduce the chances of immune response.

1995 liposome

- Trials using liposome-mediated CFTR gene transfer began in 1995.
- Non-viral vectors have the potential to avoid some of the critical problems observed with **viral** vectors, such as **the immune response, limited packaging capacity, and random integration**.
- **Liposomes** may be **mildly effective**, but their activity does not last. For this approach to work, researchers need to figure out how to improve delivery, make the effects more permanent and reduce the adverse side effects.
- To date, only cationic liposome-based systems have been tested in clinical trials in cystic fibrosis subjects.

1998 adeno-associated virus

- Trials using adeno-associated virus to deliver the CFTR gene began in 1998.
- Because it is safe, the adeno-associated virus holds promise for being a good way to deliver the CFTR gene to patients' airway cells.
- But researchers need to learn more about how the virus infects cells in order to make it an effective delivery method.

Mode of delivery

- The majority of experience in terms of vector delivery to the lungs has involved the instillation of large volumes of vector-containing fluid into the lung via the nose.
- However,

I. this mode of delivery poses safety problems because of the **potential for aspiration**.

II. In addition, the instillation of large volumes of fluid leads to **enhanced alveolar exposure**, as a result of bulk flow into the lung parenchyma. This exposure is undesirable because it may induce adverse reactions.

III. At the same time, it is likely that **airway epithelial cells**, rather than alveolar epithelial cells, are the **appropriate target** for CFTR gene transfer.

- Another mode of lung delivery for vector-containing fluid is by oral inhalation of aerosolized vectors.
- However, aerosolization of a fluid is typically achieved by means of a **nebulizer**, and most nebulizers have been designed to generate small particles. This is because most nebulizers have been developed to deliver drugs to treat patients with asthma, and in asthma the target region of the lungs is often the peripheral airways. Small particles enhance delivery to the **peripheral airways and the alveolar region** of the lung, but this is again undesirable for gene vector delivery because of the possibility of inducing adverse effects.

- One way to avoid alveolar deposition is to generate an **aerosol** that is composed primarily of **large droplets**.
- Delivery of the vector by means of a **spray device** that is inserted into a bronchoscope may have another advantage over nebulization.
- Research suggests that spray delivery of the vector could provide a means of targeting the larger, central airways, avoiding deposition in the smaller airways and alveolar region, which is more likely with nebulizers that generate small aerosol particles.
- Studies using spray technology indicate that efficient and targeted delivery of aerosolized gene vectors to the lungs may be possible in the future.

challenges

- The goal of developing an effective genetic therapy for CF lung disease has led to the attainment of several milestones in the larger field of gene therapy. These include:
- the first published *in vivo* gene transfers with adenovirus (Ad), and with recombinant adeno-associated virus (rAAV), and
- the first phase I clinical trials using each of these vector systems.
- **Choice of vector, mode of delivery to the airways, translocation of genetic information, and expression of normalized CFTR in sufficient amounts** to correct the CF phenotype in the lungs of CF patients continue to be hurdles in the development of gene therapy for CF.
- A few attempts at gene therapy were initially successful, but failed to produce acceptable long-term results.

STEM CELLS

What are stem cells?

- the body is made up of about 200 different kinds of specialised cells such as muscle cells, nerve cells, fat cells and skin cells
- all cells in the body come from stem cells
- a stem cell is a cell that is not yet specialised
- the process of specialisation is called differentiation
- once the differentiation pathway of a stem cell has been decided, it can no longer become another type of cell on its own

Unique characteristics of Stem Cells

- Stem cells can regenerate
  - *Unlimited self renewal through cell division*
- Stem cells can specialize
  - *Under certain physiologic or experimental conditions*
  - *Stem cells then become cells with special functions such as:*
    - Beating cells of the heart muscle
    - Insulin-producing cells of the pancreas

Unspecialization

- Stem Cells are unspecialized
  - *They do not have any tissue-specific structures that allow for specialized function*
  - *Stem cells cannot work with its neighbors to pump blood through the body (like heart muscle cells)*
  - *They cannot carry molecules of oxygen through the bloodstream (like RBCs)*
  - *They cannot fire electrochemical signals to other cells that allow the body to move or speak (like nerve cells)*

Self - Renewal (Regeneration)

- Stem cells are capable of dividing & renewing themselves for long periods
  - *This is unlike muscle, blood or nerve cells – which do not normally replicate themselves*
  - *In the lab, a starting population of SCs that proliferate for many months yields millions of cells that continue to be unspecialized*
    - These cells are capable of long-term self-renewal

Specialization of Stem Cells: Differentiation

- Differentiation: unspecialized stem cells give rise to specialized (differentiated) cells in response to external and internal chemical signals
  - *Internal signals: turn on specific genes causing differential gene expression*
  - *External signals include:*
    - Chemicals secreted by other cells such as growth factors, cytokines, etc.
    - Physical contact with neighboring cells

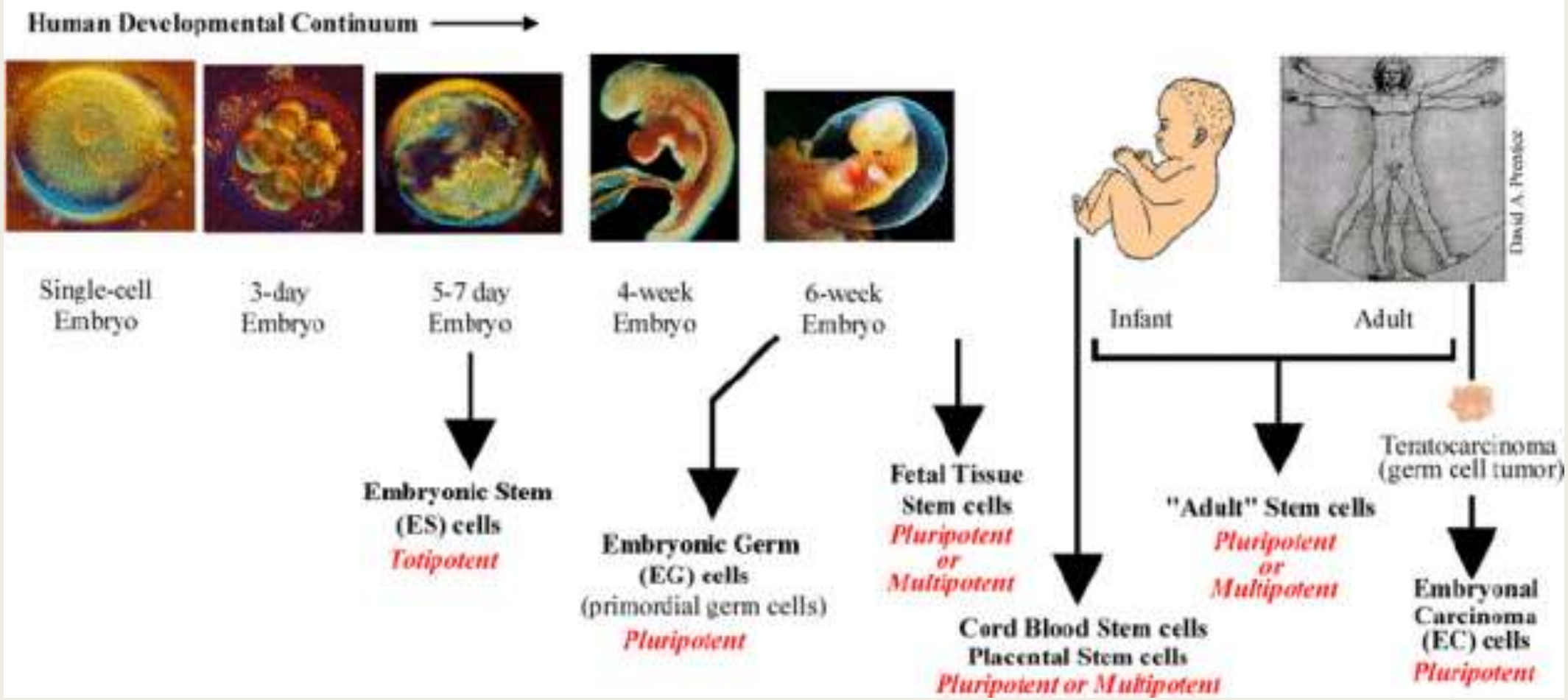
Potential of Stem Cells

- Totipotent (total):
  - *Total potential to differentiate into any adult cell type*
  - *Total potential to form specialized tissue needed for embryonic development*
- Pluripotent (plural):
  - *Potential to form most or all 210 differentiated adult cell types*
- Multipotent (multiple):
  - *Limited potential*
  - *Forms only multiple adult cell types*
    - Oligodendrocytes
    - Neurons

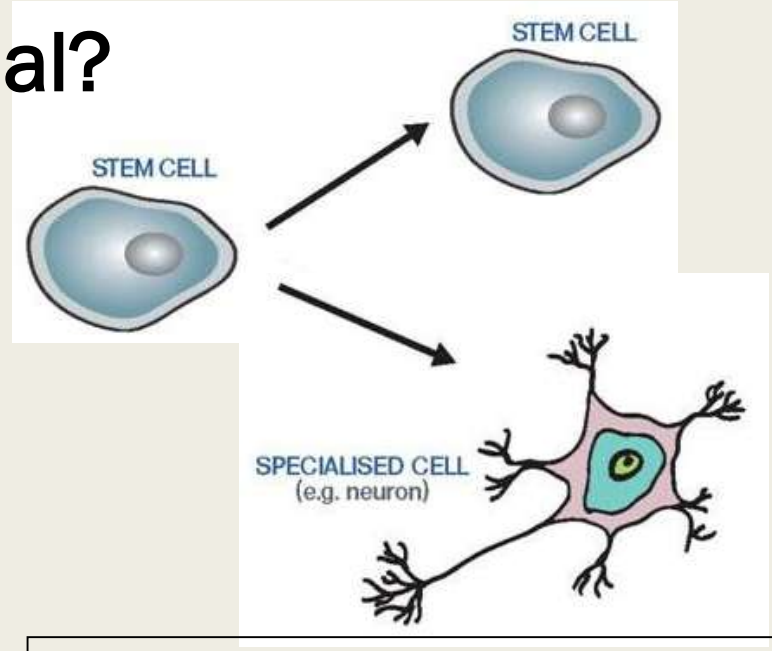
Adult Stem Cells

- Adult or somatic stem cells have unknown origin in mature tissues
  - *Unlike embryonic stem cells, which are defined by their origin (inner cell mass of the blastocyst)*
- They typically generate the cell types of the tissue in which they reside
  - *Stem cells that reside in bone marrow give rise to RBC, WBC and platelets*
  - *Recent experiments have raised the possibility that stem cells from one tissue can give rise to other cell types. This is known as PLASTICITY*
    - Blood cells becoming neurons
    - Liver cells stimulated to produce insulin
    - Hematopoietic (blood cell producing) stem cells that become heart cells

Stem Cells


Human Developmental Continuum
Single-cell Embryo
3-day Embryo
5-7 day Embryo
4-week Embryo
6-week Embryo
Infant
Adult
David A. Prentice
Embryonic Stem (ES) cells
Totipotent
Embryonic Germ (EG) cells
(primordial germ cells)
Pluripotent
Fetal Tissue Stem cells
Pluripotent or Multipotent
Cord Blood Stem cells
Placental Stem cells
Pluripotent or Multipotent
"Adult" Stem cells
Pluripotent or Multipotent
Teratocarcinoma
(germ cell tumor)
Embryonal Carcinoma (EC) cells
Pluripotent

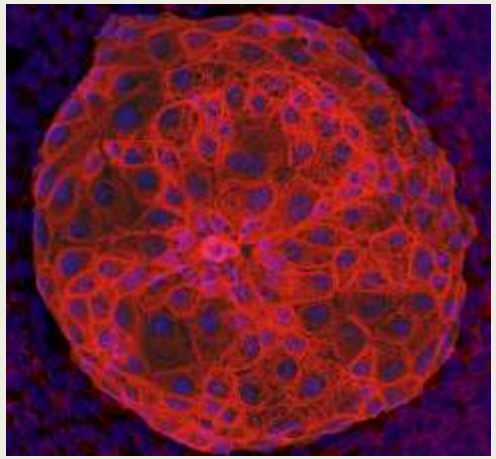
Why are stem cells special?



Stem cells can:

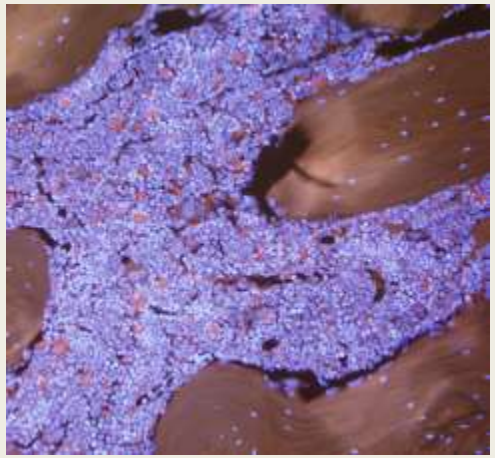
- self-renew to make more stem cells
- differentiate into a specialised cell type

Stem cells that can become <u>many</u> types of cells in the body are called **pluripotent**



Embryonic stem cells (pluripotent)

Stem cells that can become only <u>a few</u> types of cells are called **multipotent**



Tissue stem cells (multipotent)

Stem cells obtained according to the type

- Embryonic Stem Cells (ESC): received from:
  - *Embryos created in vitro fertilization*
  - *Aborted embryos*
- Adult Stem Cells (ASC): can be received from:
  - *Limited tissues (bone marrow, muscle, brain)*
    - Discrete populations of adult stem cells generate replacements for cells that are lost through normal wear and tear, injury or disease
  - *Placental cord*
  - *Baby teeth*

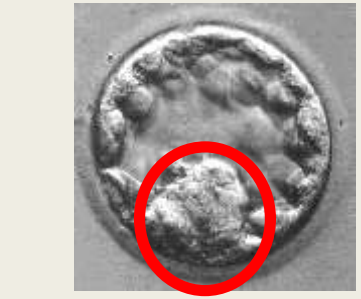
Source of ESC

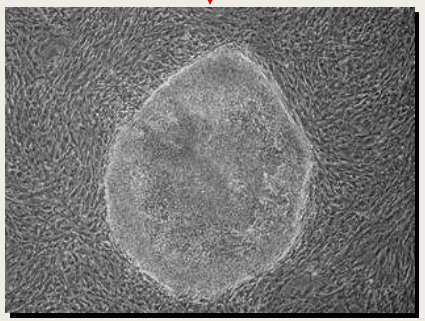
- Blastocyst
  - *“ball of cells”*
  - *3-5 day old embryo*
  - *Stem cells give rise to multiple specialized cell types that make up the heart, lung, skin, and other tissues*
- Human ESC were only studied since 1998
  - *It took scientists 20 years to learn how to grow human ESC following studies with mouse ESC*

How are embryonic stem cells harvested?

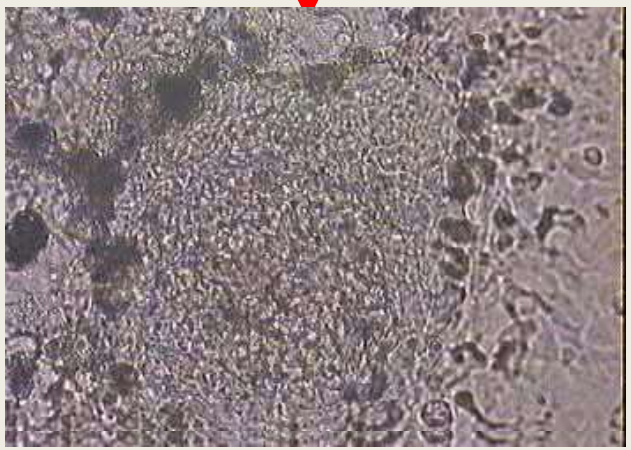
- Human ES cells are derived from 4-5 day old blastocyst
- Blastocyst structures include:
  - ***Trophoblast:*** *outer layer of cells that surrounds the blastocyst & forms the placenta*
  - ***Blastocoel:*** *("blastoseel") the hollow cavity inside the blastocyst that will form body cavity*
  - ***Inner cell mass:*** *a group of approx. 30 cells at one end of the blastocoel:*
    - Forms 3 germ layers that form all embryonic tissues (endoderm, mesoderm, ectoderm)

Embryonic stem cells





human embryonic stem cells



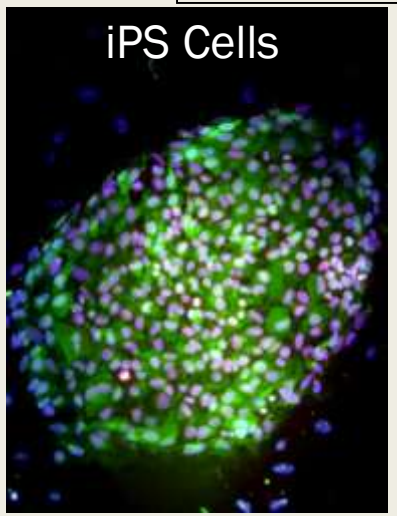
- derived from donated IVF embryos
- can be grown indefinitely in the laboratory in an unspecialised state
- retain ability to specialise into many different tissue types – know as **pluripotent**
- can restore function in animal models following transplantation

Human embryonic stem cells can become any cell in the body including these beating heart cells

Induced pluripotent stem cells (iPS)

Starting cells from donor tissue

Induced change in gene expression

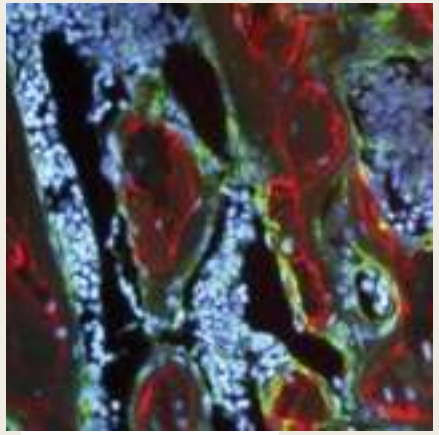


pluripotent stem cells

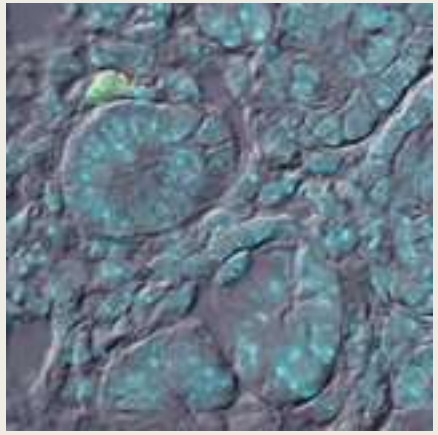
- derived from adult cells in 2007 - very recent discovery!
- can be grown indefinitely in culture in an undifferentiated state
- similar properties to embryonic stem cells as can differentiate into many different tissue types - **pluripotent**
- can create stem cells directly from a patient for research

Tissue stem cells

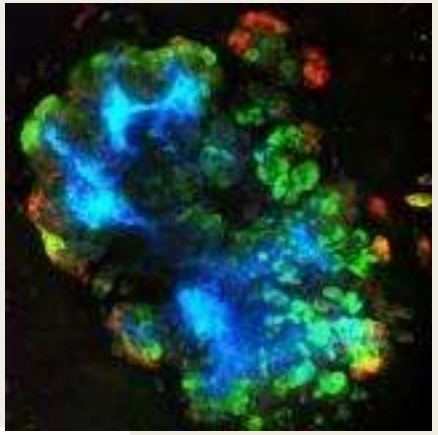
- often known as adult stem cells
- also includes stem cells isolated from fetal and cord blood
- reside in most tissues of the body where they are involved in repair and replacement



Bone marrow



Kidney



Lung

- generally very difficult to isolate
- already used to treat patients (haematological malignancies, diseases of the immune system)

Potential Uses of Stem Cells

- **Basic research** – clarification of complex events that occur during human development & understanding molecular basis of cancer
  - *Molecular mechanisms for gene control*
  - *Role of signals in gene expression & differentiation of the stem cell*
  - *Stem cell theory of cancer*

Potential uses of stem cells

- **Biotechnology**(drug discovery & development) – stem cells can provide specific cell types to test new drugs
  - *Safety testing of new drugs on differentiated cell lines*
  - *Screening of potential drugs*
    - Cancer cell lines are already being used to screen potential anti-tumor drugs
    - Availability of pluripotent stem cells would allow drug testing in a wider range of cell types & to reduce animal testing

Potential uses of stem cells

- **Cell based therapies:**
  - *Regenerative therapy to treat Parkinson's, Alzheimer's, Amiotrophic lateral sclerosis (ALS), spinal cord injury, stroke, severe burns, heart disease, diabetes, osteoarthritis, and rheumatoid arthritis*
  - *Stem cells in gene therapy*
    - Stem cells as vehicles after they have been genetically manipulated
  - *Stem cells in therapeutic cloning*
  - *Stem cells in cancer*

Embryonic vs Adult Stem Cells

- Totipotent
  - *Differentiation into ANY cell type*
- Known Source
- Large numbers can be harvested from embryos
- May cause immune rejection
  - *Rejection of ES cells by recipient has not been shown yet*

- Multi or pluripotent
  - *Differentiation into some cell types, limited outcomes*
- Unknown source
- Limited numbers, more difficult to isolate
- Less likely to cause immune rejection, since the patient's own cells can be used

Claims against ESC (unsubstantiated thus far!)

- Difficult to establish and maintain *
- Difficulty in obtaining pure cultures from dish*
- Potential for tumor formation and tissue* destruction
- Questions regarding functional differentiation
- Immune rejection
- Genome instability
- Few & modest results in animals, no clinical treatments
- Ethically contentious

* = same problem with ASC

Areas of community concern

- How come there are excess IVF embryos?
- Why do the embryos have to be destroyed for stem cell research? Isn't this the same as taking a life?
- Wouldn't it be better to donate the excess IVF embryos to other infertile couples?
- Could women be forced to sell eggs or embryos for research?
- Won't doing therapeutic cloning lead to cloning humans?
- Why do we need to keep using embryos in research when we have new iPS cells?