Figures adapted from ISSCR. Presentations of Drs. Martin Pera (Monash University), Dr. Susan Kadereit, Children’s Hospital, Boston and Dr. Catherine Verfaillie, University of Minnesota
Turning Blood into Blood: Cells Bearing Neuronal Antigens Generated in Vitro from Bone Marrow

Science 2000, 290:1779-1782
Mezey, E., Chandross, K.J., Harta, G., Maki, R.A., McKercher, S.R.

Turning Blood into Brain: A Hematopoietic Fate Adopted by Adult Neural Stem Cells in Vivo

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Stem cells are found in various organs where they participate in tissue homeostasis by replacing differentiated cells lost to physiological turnover or

Hematopoietic potential of stem cells isolated from murine skeletal muscle

PNAS 1999, 96: 14482-14486
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Edited by Irving L. Weissman, Stanford University School of Medicine, Stanford, CA, and approved October 6, 1999 (received for review September 1, 1999)

We have discovered that cells derived from the skeletal muscle of adult mice contain a remarkable capacity for hematopoietic differentiation. Cells prepared from muscle by enzymatic digestion self-renew in response to physiological stimuli (14-17). Therefore, satellite cells could represent stem cells capable of commitment to myogenesis as opposed to the right environment.

From Marrow to Brain: Expression of Neuronal Phenotypes in Adult Mice

Science 2000, 290:1775-1779
Brazelton, T.R., Rossi, F.M., Keshet, G.I., Blau, H.M.

Purified hematopoietic stem cells can differentiate in hepatocytes in vivo

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The characterization of hepatic progenitor cells is of great scientific and clinical interest. Here we report that intravenous injection of adult bone marrow cells in the FAH† mouse, an animal model of hepatic progenitor cell renewal, caused the mouse to recover the biochemical function of its fore
Stem Cell FAQs

Do you need to get one from an egg?
Must you sacrifice an Embryo?
What is an ES cell?
What about adult stem cells or cord blood stem cells
Why can’t this work be done in animals?
Are “cures” on the horizon?
Will this lead to human cloning – human spare parts factories?
Are we going to make a Frankenstein?
What is a stem cell?

A primitive cell which can either self renew (reproduce itself) or give rise to more specialised cell types.

The stem cell is the ancestor at the top of the family tree of related cell types. One blood stem cell gives rise to red cells, white cells and platelets.
Stem Cells Vary in their Developmental capacity

A **multipotent** cell can give rise to several types of mature cell.

A **pluripotent** cell can give rise to all types of adult tissue cells plus extraembryonic tissue: cells which support embryonic development.

A **totipotent** cell can give rise to a new individual given appropriate maternal support.
The Fertilized Egg

The “Ultimate” Stem Cell – the Newly Fertilized Egg (one Cell) will give rise to all the cells and tissues of the adult animal.

Truly Totipotent but difficult to use.
Properties of human ES cells

The defining feature of an ES cell is its ability to differentiate into a wide range of tissues.
Embryonic stem cells

Derived from five day old spare human embryos before specialised tissues of the body begin to form

May be grown indefinitely in culture in the primitive embryonic state

Retain the property of pluripotency during extended growth in vitro
Pluripotent cell populations

- Inner cell mass
- Epiblast
- Primordial germ cells
- Oocyte
- Embryonal carcinoma

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Properties of pluripotent stem cells

Derived directly and at high frequency from pluripotent cell populations in vivo

Grow indefinitely in vitro (express telomerase)

Maintain normal karyotype

Cloned lines capable of differentiation into a wide range of somatic and extraembryonic tissues in vivo and in vitro—at high frequency and under a range of conditions

Capable of colonising all tissues including germ line after blastocyst injection to give chimeric offspring
Where do human embryonic stem cells come from?

ES cell colony

Excess

Destroy

Embryo bank

IVF

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Early Stages of Human Development

Zygote

Blastocyst (pre-implantation)
Blastocyst stage of development

Body plan not yet apparent

Many cells will not form new human, but will give rise to tissue such as placenta which support pregnancy

Embryo does not yet necessarily represent a unique individual (twins can form up to 14 days)

No precursors of nervous system present yet

Not possible to predict whether embryo will be able to develop to term
Establishment of ES cells

Inner cell mass  ES colony 10-15 days later
Characterisation of primate pluripotent stem cells

Immunological markers: TRA1-60 and related epitopes, SSEA-3, SSEA-4

Gene expression: transcripts for generic markers of pluripotent stem cells

Biological properties: Differentiation into derivatives of all three germ layers
Human Embryonic Stem Cells (hESC)

Derived from five day old spare human embryos created during IVF.

Retain the property of pluripotency during extended culture growth:

► Unlimited supply for meaningful experiments.
► Can give rise to clinically relevant cell numbers.
What Actually Happens When Stem Cells Do “Their Thing”

ALL SORTS OF THINGS

LET’S TAKE A LOOK
Teratoma formed by human ES cells
ES cells give rise to disorganised growths called teratomas.

Teratomas do not display axis formation or segmentation. Unlike embryos, ES cells on their own are incapable of generating the body plan. This lack of organisation is also seen when ES cells differentiate in vitro.
ES cell differentiation

Cartilage, bone, skin, nerves, gut and respiratory lining form when ES cells are injected into host animals.
Spontaneous ES cell differentiation in vitro

Stop ES cell multiplication

ES cell

Mixture of differentiated Cells with some pancreatic cells
Spontaneous ES cell differentiation in vitro

Nerve and muscle cells are found in a complex mixture of many cell types

Cell differentiation in monolayer cultures of ES cells grown to high density. Aggregates of cells and cysts eventually form a multilayered structure when ES cells are left in situ and grown to high density from a monolayer culture. Multiple cell types and some organoid structures are seen in this section made by scraping the cell layer from the dish, embedding it, and staining with hematoxylin and eosin. The structures resemble those found in embryoid bodies formed by placing ES cell aggregates in suspension culture.
Neural precursors can be derived from ES cells
**Routes to differentiation**

- **ES cell**
- **Commitment Signal**
- **Selection and growth of committed precursor cell**
- **Specific signals for growth and differentiation**
- **Production of large numbers of mature cell in pure form**
How Do We Make A Stem Cell Into a Specific Cell Type?

Need to understand biology of differentiation

Much Data Can be Derived from Animal Experimentation

Use growth factors and “differentiation agents”

Key advantage is the ability to grow large quantities of “identical” cells
Studies of the mammalian embryo provide clues as to how embryonic stem cell differentiation might be controlled
The embryo and ES cells

Cell interactions between pluripotent cells and extraembryonic cells mediate patterning and fate decisions

Do the same cell populations exist in ES cell cultures?

Do the same molecules mediate fate decisions in ES cell culture?
Cell types derived from human ES cells in vitro

Nerve, astrocyte, oligodendrocyte
Hematopoietic stem cells
Insulin producing cells
Cardiomyocytes
Hepatocytes
Endothelial cells
Hematopoietic stem cells from human ES cells (Kaufman et al.)

Culture of ES cells on marrow stromal support lines leads to formation of CD34+ hematopoietic precursor cells

Will form myeloid erythroid and megakaryocytic colonies.

Frequency 1-2%
Embryonic stem cells have important applications in biomedical research

Basic studies of early human development and its disorders—birth defects, childhood cancers

Functional genomics in human cells

Discovery of novel factors controlling tissue regeneration and repair

In vitro models for drug discovery and toxicology

Source of tissue for transplantation medicine
Successful treatment of animal models of disease with mouse ES derived cells

Severe immune deficiency
Diabetes
Parkinson’s disease
Spinal injury
Demyelination
Myocardial infarction
Challenges for transplantation therapy

Production of required cell type in sufficient numbers and pure form

What cell to transplant

Delivery

Problems of tissue rejection
The immune rejection issue in ES cell based therapy

How immunogenic are embryonic or fetal derived grafts?

Some transplantation sites will be immunologically privileged

Interesting data to suggest embryonic cells can induce tolerance in hosts Fandrich et al Nat. Med. 8: 171, 2002
Solutions to the rejection problem

Large banks of ES cell lines

Manipulation of histocompatibility genes in ES cells

Replacement of hematopoietic/lymphoid tissue of patient with ES derived cells prior to transplant

Manipulation of T cell response with antibodies or drugs

Therapeutic cloning or related techniques
Therapeutic cloning

Combines cloning methods with embryonic stem cell technology to produce cells which are custom made for patient

A promising solution to problem of tissue rejection

Used to produce ES cells in mice and cure a severe immune disorder

More research may enable us to reprogram adult cells without going through embryo step

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Adult stem cells

Proper tissue organisation and response to demands of growth or repair require that there be restrictions on developmental potential of adult stem cells.

These limits are strictly imposed by powerful molecular restraints on gene expression and are heritable during many rounds of cell division.

An adult stem cell may show relaxation of these restrictions in an altered environment, possibly accounting for plasticity. Even so, plasticity is observed usually at low frequency.

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Bone Marrow Stem Cells

- isolated from human, mouse and rat.
- Appear only after 35 population doublings.
- Can be grown to 50-80 population doublings.
- Give rise to mesoderm, neuroectoderm and endoderm
- Can form chimeras with cells in all somatic tissues, when injected into blastocysts (multipotent).
Cord Blood Stem Cells

• rare cell, CD45⁻/ HLA⁻

• Grow robustly in vitro without differentiating.

• Give rise to mesoderm, neuroectoderm and endoderm.

• In vivo: differentiation into neural cells, bone and cartilage, blood, myocardial cells and Purkinje fibers, hepatic cells.
Nuclear Transfer

To produce cells which are custom made for patient.

A promising solution to problem of tissue rejection, as cells express the patients genes.

Embryonic stem cell lines created from patients with certain diseases, to study disease development and to develop drugs.
Therapeutic cloning in transplantation: necessary or feasible?

How severe will immune rejection problem be?

Other solutions exist to the problem

Is it practical? Where will the eggs come from? Can the procedure be turned around in the required time frame?

Is it safe? Is it easier to make normal cells from cloned ES than to make normal animals?
Nuclear Transfer
Problems with Nuclear Transfer (NT)

• Inefficient: 242 → 30 blastocysts → 1 cell line

• Time to derive therapeutic cells from NT blastocysts will take several weeks to months.
Somatic Cell Nuclear Transfer

Source of eggs: Self, Mother, Relative, Egg bank

Problems: Inefficient (may need hundreds of eggs)
Technically demanding - need to be available in many or all hospitals
Correction of a Genetic Defect by Nuclear Transplantation and Combined Cell and Gene Therapy

William M. Rideout III, Konrad Hochedlinger, Michael Kyba, George Q. Daley, and Rudolf Jaenisch

Diagram:
- Expand HSC culture and transplant
- Dissociate EBs and infect with HoxB4/GFP
- Differentiate into EBs
- Repair Rag2 gene in ES cells
- Isolate isogenic Rag2 +/- ES cells
- Activate and culture to blastocyst
- Nuclear transfer into enucleated oocyte
- Culture tail tip cells

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ARE THERE WAYS AROUND USING EGGS AND EMBRYOS?

POSSIBLY
Reprogramming without using an egg or making an embryo

Now

Pluripotent cell

Adult cell

Cell fusion

Hybrid cell shows activation of embryonic genome

In future

Adult cell

Inject proteins from pluripotent cell or egg

adult cell becomes pluripotent

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Do We Have the Ability to Repair Ourselves?

Endogenous Stem Cells

Need to learn to manipulate in the body

Indications from hematology that is possible
Cancer Stem Cells

• Not all the cells within a tumor can maintain tumor growth, most cancers are not clonal.

• Several long-known oncogenic pathways are pivotal to the maintenance of normal stem cell self-renewal.

• Techniques used in the stem cell field have identified self-renewing cells.

• By identifying the stem cells in tumors, it could be shown that only the cancer stem cells propagated the tumor.
Cancer Stem Cells

• In breast cancer, brain tumors, certain forms of leukemia, and gastric tumor.

• Unknown whether the tissue stem cell degenerates, or if a more differentiated cell reacquires stem cell phenotype.

• Despite preventive mechanisms adult stem cells may accumulate mutations over the years.
Why we need new stem cell lines

Panels of cell lines required for tissue matching in transplantation

Safety hazards with current cells derived using animal tissue

Current bona fide lines few in number; improvements to techniques will enable production of second generation ES cells with better properties;

Need cell lines in the public domain without commercial restrictions on use
Are We Making a Frankenstein?

Not That Dramatic

But, Beware of hype

Remember the Law of Unintended Consequences