ANAT2341: lecture overview

Stem Cells

Resources:
http://php.med.unsw.edu.au/cell_biology/
Essential Cell Biology – 3rd edition Alberts

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ANAT2341: lecture overview

**Stem Cell Biology**

Tissue development and regeneration
- Stem cell biology
- Stem cell niches
- Stem cell regulation
- Stem cells and cancer
- Regenerative medicine
- Stem cell sources
- Future of regenerative medicine
Prenatal development

Figure 20-32 Essential Cell Biology 3/e (© Garland Science 2010)
Tissue homeostasis

Maintenance of the organism’s internal environment in response to internal and external conditions
Tissue renewal in higher vertebrates
Stem cells divide to self renew and to produce terminally differentiated cell types

- Mostly quiescent
  - High proliferation rates
  - No function
- Limited proliferation
  - Highly functional cell

Assymmetric cell division:
- Stem cell
  - Self-renewal
  - Transit-amplifying cell
  - Dividing precursor cells
  - Terminally differentiated cells

Figure 20-35 Essential Cell Biology 3/e (© Garland Science 2010)
Stem cells potential

**Totipotency:**
capacity to generate all cell types within the body + extraembryonic tissue

**Pluripotency:**
capacity to generate all cell types within the body

**Multipotency:**
capacity to give rise to more than 1 cell type

**Unipotent stem cell:**
tissue precursor cells, capacity to give rise to one cell type only
Stem cells potential

Examples:
- Circulatory System
- Immune System
- Nervous System

Types of stem cells:
- Totipotent
- Pluripotent
- Multipotent
- Unipotent
Adult stem cells

Locations of Somatic Stem Cells in the body

- brain
- blood vessels
- skeletal muscle
- bone marrow
- peripheral blood
- teeth
- skin
- liver
- heart
- gut
Stem cell niche:
Keeps stem cells in an undifferentiated state

A Cellular niche

- Supporting cell
- Stem cells
- Regulated proliferation and subsequent differentiation

B Non-cellular niche

- ECM
- Stem cells
- Regulated proliferation and subsequent differentiation

Key:
- Secreted signals from niche
Epidermal Stem Cell Niches
The Intestinal Crypts Stem Cell Niche
Descendants of Crypt Base Columnar Stem Cells live up to 48-72 hours

(Radtke and Clevers, Science 2005)
The Haemopoietic Stem Cell Niche

approximately $10^{11} - 10^{12}$ new blood cells are produced daily

HSC: haemopoietic stem cell
MPP: multipotent progenitor cell
CLP: common lymphoid progenitor
CMP: common myeloid progenitor cell
GMP: granulocyte/monocyte precursor cell
MEP: Megakaryocyte-erythroid precursor cell

Figure 5.1. Hematopoietic Stem Cell Differentiation (2001 Terese Winslow, Lydia Kibiuk)
Seminiferous Tubules
Spermatogenesis: 2 months life span
Regulation of Stem Cells

Should I stay quiescent?

Should I die?

Should I proliferate?

Should I self-renew?

Should I generate transit amplifying cells?

Should I generate differentiating daughter cells?
Stem cell niche: Keeps stem cells in an undifferentiated state

A Cellular niche

B Non-cellular niche

Key:  ➥ Secreted signals from niche
Regulation of Stem Cells

Signalling pathways

Transcriptional response

Cellular response
Regulation of Stem Cells
The Mechanosensor YAP

A. Cell shape / size
   - spread cell
   - confined cell

B. ECM stiffness
   - stiff ECM
   - soft ECM

C. Substrate rigidity
   - rigid substrate
   - bendable substrate

D. Stretching
   - stretched cell
   - relaxed cell

E. Shear stress
   - fluid flow
   - static

YAP inactivation

Cytoplasm

Nucleus
Regulation of Stem Cells
The Mechanosensor YAP

YAP and TAZ = Cytoplasmic localization and proteasomal degradation

YAP and TAZ = Nuclear localized and transcriptionally active

Small adhesive areas
Soft ECM

Large adhesive areas
Stiff ECM

Apoptosis
Growth arrest
Adipocyte differentiation
Mesenchymal stem cells → Adipocyte

Proliferation
Epithelial or endothelial cells
Osteoblast differentiation
Mesenchymal stem cells → Osteoblast
What happens if cell renewal regulation goes wrong?
Mutations in Wnt pathway result in Cancer
Adenomatous Polyposis Coli

hyperproliferation
Mutations in Hedgehog pathway result in cancer

Basal Cell Carcinoma
Regenerative medicine
the clinical application of stem cells

"process of replacing or regenerating human cells, tissues or organs
to restore or establish normal function"
Stem Cell Sources for Regenerative Medicine

Stem cells derived from embryos

Stem cells derived from adults
Embryonal Carcinoma Cells are pluripotent

1964 – Pierce and Kleinsmith isolate EC cells from teratocarcinomas

Source: gametes

Pluripotent
In vitro culture and expansion
Genetic abnormalities
Embryonic Stem Cells are pluripotent

1981 – Martin Evans, Matthew Kaufman and Gail Martin

- Pluripotent
- No genetic abnormalities
- In vitro culture and expansion
- Ethical issues
Embryonic Stem Cells are pluripotent

1981 – Martin Evans, Matthew Kaufman and Gail Martin

**blastocyst**

- cells inside = ‘inner cell mass’
- outer layer of cells = ‘trophectoderm’

---

embryonic stem cells taken from the inner cell mass

---

fluid with nutrients
culture in the lab
to grow more cells
Embryonic Stem Cells are pluripotent

1981 – Martin Evans, Matthew Kaufman and Gail Martin

Embryonic stem cells → differentiation → all possible types of specialized cells

PLURIPOTENT
Embryonic Stem Cells are pluripotent

1981 – Martin Evans, Matthew Kaufman and Gail Martin

Embryonic stem cells

- grow under conditions A → skin
- grow under conditions B → neurons
- grow under conditions C → blood
- grow under conditions D → liver

Pluripotent
No genetic abnormalities
In vitro culture and expansion
Ethical issues
Adult stem cells

“An undifferentiated cell, found among differentiated cells in a tissue or organ that can renew itself and can differentiate to yield some or all of the major specialized cell types of the tissue or organ”

No ethical issues
Restricted plasticity
Limited quantities
Hard to identify
Adult stem cells

Haematopoietic Stem Cells

MULTIPOTENT

blood stem cell

found in bone marrow

differentiation

only specialized types of blood cell:
red blood cells, white blood cells, platelets

No ethical issues
Restricted plasticity
Limited quantities
Hard to identify
Somatic Cell Nuclear Transfer
John Gurdon, 1958

The developmental potential of nuclei of differentiated cells
Reproductive/Therapeutic Cloning

- **Reproductive Cloning**
  - Remove nucleus from a cell from the body
  - Take the nucleus (containing DNA)
  - Remove nucleus and take the rest of the cell
  - Clone identical to the individual that gave the nucleus
  - Example: Dolly the sheep

- **Therapeutic Cloning**
  - Remove skin cell from patient
  - Remove DNA from unfertilized egg
  - Fuse cells
  - Early embryo with donor DNA
  - Cloned embryo
  - Infant clone of patient

**Pluripotent (totipotent?)**
- Low success rate
- Genetic/phenotypic abnormalities
- Ethical issues
Somatic Cell Nuclear Transfer

“mature, differentiated cells can be reprogrammed to become pluripotent”
Nuclear Reprogramming
Induced pluripotency (iPS), Yamanaka, 2006

“mature, differentiated cells can be reprogrammed to become pluripotent”

‘genetic reprogramming’
= add certain genes to the cell

cell from the body

induced pluripotent stem (iPS) cell behaves like an embryonic stem cell

culture iPS cells in the lab

differentiation

Advantage: no need for embryos!

all possible types of specialized cells
Nuclear Reprogramming
2012 Nobel Prize

The Nobel Prize in Physiology or Medicine 2012 was awarded jointly to Sir John B. Gurdon and Shinya Yamanaka "for the discovery that mature cells can be reprogrammed to become pluripotent"
## Stem Cell Sources

### Embryonic vs Adult Stem Cells

<table>
<thead>
<tr>
<th>Attributes</th>
<th>Embryonic Stem Cells</th>
<th>Adult Stem Cells</th>
<th>iPS Cells</th>
</tr>
</thead>
</table>
| **In Vitro Fertilization** | - can produce all cell types  
- relatively easy to identify, isolate, maintain, and grow in the laboratory  
- large source of "excess" blastocysts from IVF clinics | - can produce all cell types  
- relatively easy to identify, isolate, maintain, and grow in the laboratory  
- stem cells may be genetically matched to patient | - Can generate any cell type  
- Easy to generate, maintain and grow in lab  
- Perfect genetic match to patient |
| **Nuclear Transfer** | - demonstrated success in some treatments  
- stem cells may be genetically matched to patient | - produce limited number of cell types  
- not found in all tissues  
- difficult to identify, isolate, maintain, and grow in the laboratory | - May retain age of parental cell  
- Inheritance of mutations: teratomas |
| **Adult Tissues** | - limited number of cell lines available for federally funded research  
- risk of creating teratomas (tumors) from implanting undifferentiated stem cells | - destruction of human blastocysts  
- donation of eggs requires informed consent  
- concern about misapplication for reproductive cloning | - no major ethical concerns have been raised |
| **Ethical Concerns** | - destruction of human blastocysts  
- donation of blastocysts requires informed consent | - destruction of human blastocysts  
- donation of eggs requires informed consent  
- concern about misapplication for reproductive cloning | - No major ethical concerns |

### Table: Comparison of Stem Cell Sources

<table>
<thead>
<tr>
<th>Source</th>
<th>Characteristics</th>
<th>Limitations</th>
<th>Ethical Concerns</th>
</tr>
</thead>
</table>
| Embryonic Stem Cells | - In Vitro Fertilization  
- Nuclear Transfer | - limited number of cell lines available for federally funded research  
- risk of creating teratomas (tumors) from implanting undifferentiated stem cells | - destruction of human blastocysts  
- donation of blastocysts requires informed consent |
| Adult Stem Cells | - Adult Tissues | - produce limited number of cell types  
- not found in all tissues  
- difficult to identify, isolate, maintain, and grow in the laboratory | - destruction of human blastocysts  
- donation of eggs requires informed consent  
- concern about misapplication for reproductive cloning |
| iPS Cells | - Easy to generate, maintain and grow in lab  
- Perfect genetic match to patient | - May retain age of parental cell  
- Inheritance of mutations: teratomas | - No major ethical concerns |
The Future of Regenerative Medicine
Future of Regenerative Medicine

1- how we can induce and maintain pluripotency?
2- how we can direct differentiation?
3- how we can cure diseased cells?
4- how we can repair mutations in cells?
Future Stem Cell Technologies

How can we direct differentiation?

- Uncontrolled differentiation
- Directed differentiation
Future Stem Cell Technologies
Directed differentiation of cardiomyocytes

Mummery et al., Circ Res 2012
Future Stem Cell Technologies
Directed differentiation of motor neurons

Dong et al., Nature 2014
Directed differentiation of pluripotent stem cells

Future of Regenerative Medicine
Future of Regenerative Medicine

How can we cure disease?

Disease Modeling and Drug discovery
Future of Regenerative Medicine

How can we repair mutations in cells?

Gene Therapy:
CRISPR/CAS9 genome editing
CRISPR/Cas9 Genome Engineering
(Clustered Regularly Interspaced Short Palindromic Repeats)
Guide RNA and Cas9

http://www.youtube.com/watch?v=Edx9L0Sasoc
## CRISPR/Cas9 Genome engineering

### Repair

<table>
<thead>
<tr>
<th>Non-homologous end joining:</th>
<th>Homology-directed repair:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small insertion/deletion</td>
<td>Provide donor template with homology arms</td>
</tr>
<tr>
<td>gene disruption</td>
<td>Gene mutation/correction/addition</td>
</tr>
<tr>
<td>(and occasional errors)</td>
<td>(Cas9 D10A mutant)</td>
</tr>
</tbody>
</table>

**Diagram:**
- **DSB** (Double Stranded Break) created by Cas9.
- **gRNA-Cas9** guides Cas9 to the target site.
- **NHEJ-mediated repair** (Non-Homologous End Joining): Insertion and Deletion.
- **HR-mediated repair** (Homology-directed repair): Nucleotide substitution and Sequence insertion.
CRISPR/Cas9 Genome engineering
Applications in Stem Cells

[Diagram showing the process of stem cell reprogramming using CRISPR/Cas9 technology.]
Repair of Cystic Fibrosis Gene CFTR by CRISP/CAS9

(cystic fibrosis transmembrane conductor receptor)

CF: accumulation of viscous mucus in pulmonary and gastrointestinal tract
Life expectancy: 40 years
F508del in CFTR (anion channel essential for fluid and electrolyte homeostasis of epithelia)

Lgr5+ intestinal stem cells -> organoids

In vitro assay in intestinal organoids:
Forskolin -> CFTR -> expansion

Schwank et al., Cell Stem Cell 2013
The Future of Regenerative Medicine

[Diagram showing the process of regenerative medicine involving skin biopsy, screening for therapeutic compounds, disease-specific drugs, and transplantation of genetically matched healthy cells.]

- Treatment with drugs
- Transplantation of genetically matched healthy cells
- Disease-specific drugs
- Screening for therapeutic compounds
- Affected cell type
- Skin biopsy
- Patient-specific iPS cells
- In vitro differentiation
- Repaired iPS cells
- Use gene targeting to repair disease-causing mutation
- cMYC, OCT4, KLF4, SOX2
The Future of Regenerative Medicine

Very hopeful and promising,

but are we there yet?

http://iview.abc.net.au/programs/head-first/DO1333V001S00
ANAT2341: lecture overview

Stem Cell Biology

Tissue homeostasis and regeneration
  Stem cell biology
  Stem cell niches
  Stem cell regulation
  Stem cells and cancer
Regenerative medicine
  Stem cell sources
  Future of regenerative medicine
ANAT2341: Stem Cell Lab

Stem cell generation: Orvin Atthi
Stem cell differentiation: James Isaac, Tony Wang
Regenerative Medicine: Anuj Chavan, Elisa Gill

Group Marianne Daher?

Duration: 15 minutes max!
Do not discuss M&M in detail
Do not improvise your lines, take time to rehearse