ANAT 3231 Cell Biology
Lecture 21 - Stem Cells

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Outline
• What are Stem Cells?
  • Totipotency - Pluripotency - Multipotency
• What are different sources of Stem Cells?
  • Embryonic vs Adult
  • Pros and Cons for each type of stem cell?
• What makes Stem Cells, "Stem Cells"?*
  • Self-Renewal & Differentiation
• What can Stem Cells be used for?*
  • Therapeutic Applications for Stem Cells


Stem Cells in the News

Stem Cells Discovery Timeline
What is a Stem Cell?

What is a Stem Cell? - Pluripotency

1 Cell (Zygote - fertilised egg)

6,000,000,000 cells (230 different cell types)

Where does it all begin? - Human

Human
Where does it all begin? - Mouse

Development of Primordial Germ Cells

Totipotency vs Pluripotency

What are Different Types of Stem Cells?

1. Embryonic Stem (ES) Cells
2. Embryonic Germ Cells
3. Adult Stem Cells
4. Umbilical Cord-Blood Stem Cells

Plus
1. Somatic Cell Nuclear Transfer (SCNT)
2. Induced-Pluripotent Stem (iPS) Cells
1. Embryonic Stem (ES) Cells

1. Pluripotent - can generate any cell type in the body.
2. From 5-7 day-old blastocyst - ICM.
3. Can be cultured as cell lines or frozen for future use.
4. In Australia, plenty of embryos are donated from IVF treatments.
5. Ethical issues?

2. Embryonic Germ Cells (EGCs)

1. Primordial germ cells (PGCs) destined to become gametes (oocytes / sperm cells)
2. From 5-7 week-old embryos developing into fetus.
3. EGC-lines share many properties with ES cells.
4. But in vitro, PGCs show spontaneous differentiation, thus limited in isolation of pure clonal lines.

3. Adult (Somatic) Stem Cells

1. More specialised than embryonic stem cells.
2. Found in the majority of tissues & organs (for maintenance & repair).
3. Have restricted ability to produce different cell types (multipotent not pluripotent).
4. Identification/isolation can be difficult.
5. Only small numbers may be present requiring expansion ex vivo.
6. More adult stem cell types are being discovered from variety of tissues with plasticity - ability to form cell type of a completely different tissue.
7. Autologous (one's own) use can avoid immune-rejection problems in following transplantations.
8. Ethical issues?

3. Adult (Somatic) Stem Cells - Bone (Marrow)

1. Haematopoietic Stem Cells for Blood Cells
2. Stromal Stem Cells for other Mesodermal Tissues
3. Adult (Somatic) Stem Cells - Plasticity?

1. Neural Stem Cells for Blood.
2. Bone-Marrow Stem Cells for Liver, Skin and Neurons.

4. Umbilical Cord Blood Stem Cells

1. Adult Stem Cells collected from a newborn baby shortly after birth.
2. Can generate all blood cell types including immune system.
3. Potential to generate other tissue types is currently being explored.
4. Can be stored (at cord-blood bank) for future use; matched-siblings, matched but unrelated-individual or autologous-use.
5. ‘Saviour Siblings’ - using donor baby’s cord blood to treat a matched ill sibling.
6. Ethical issues?

5. Somatic Cell Nuclear Transfer (SCNT) - Therapeutic Cloning

1. Nucleus of oocyte is removed and replaced with foreign nucleus from any somatic cell that is ‘reprogrammed’ in the environment of the egg.
2. Artificial activation of egg and 5-7 days in culture allows development of ES cell-like, SCNT pluripotent stem-cell line.
3. Allows patient-specific cell therapies avoiding immune-rejections following transplantation.
4. Normal Zygote (fertilised egg) vs SCNT-derived egg - ‘Dolly’ died prematurely with signs of arthritis and lung infection.
6. Ethical issues?

6. Induced Pluripotent Stem Cells (iPSCs)

1. Mature somatic cells were genetically engineered by virus to achieve pluripotent, ES-like state (Nov 2007).
2. Forced expression of genes (including Oct3/4 and SOX family) in adult mouse/human cells led to a ‘reprogramming’ into pluripotent status.
3. Rapid development of strategies for gene delivery (non-integrating viruses, chemicals and small molecules) to prevent permanent / harmful changes.
4. Allows patient-specific cell therapies avoiding immune-rejections following transplantation.
5. Possible clinical use may be distant due to concerns on genetic stability.
6. Ethical issues?
Embryonic vs Adult Stem Cells - Pros & Cons

### Embryonic Stem (ES) Cells

<table>
<thead>
<tr>
<th>Advantages</th>
<th>Disadvantages</th>
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<tr>
<td>Pluripotency - ability to differentiate into any cell type.</td>
<td>Unstable - difficult to control differentiation into specific cell types.</td>
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<td>Immortal - one cell can supply endless amounts of cells.</td>
<td>Immunogenic - potential immune-rejection when transplanted into patients.</td>
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<td>Easily available - human embryos from fertility clinics.</td>
<td>Teratomas - tumor composed of tissues from 3 embryonic germ layers.</td>
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### Adult Stem Cells

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<th>Advantages</th>
<th>Disadvantages</th>
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<tbody>
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<td>Already specialised - induction of differentiation into specific cell types will be easier.</td>
<td>Minimal quantity - number of isolatable cells may be small.</td>
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<td>Plasticity - Recent evidence suggests wider than previously thought ranges of tissue types can be derived.</td>
<td>Finite life-span - may have limited life-span in culture.</td>
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<td>No Immune-rejection - if used in autologous transplantations.</td>
<td>Ageing - stem cells from aged individuals may have higher chance of genetic damage due to ageing.</td>
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<td>No Teratomas - unlike ES cells.</td>
<td>No Ethical Controversy - sourced from adult tissues.</td>
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<td>No Ethical Controversy - sourced from adult tissues.</td>
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What makes Stem Cells, ‘Stem Cells’?

**Self-Renewal**
through Asymmetric Cell Division

**Differentiation**
via Multipotent Progenitors

Self-Renewal vs Differentiation

Skeletal Muscle Biology

Myogenic Lineage

**Paired-box transcription factors**

**bHLH transcription factors**

Myogenic Regulatory Factors (MRFs)

MyoD, Myf-5, myogenin
**Myogenic Lineage**

Stem Cell

Pax3$^+$ and/or Pax7$^+$

Myogenic Specification

Myf5$^+$, MyoD$^+$

Myogenic Differentiation

MyoD$^+$, Myogenin$^+$

Terminal Differentiation

Myogenin$^+$, MHC$^+$

- Modified from Tajbakhsh (2003) Current Opinion in Genetics & Development

**Developing Muscles from Rat Hindlimb**

- Cryostat Section
- Isolated Cells

- Triple Immuno-labeling: Pax3$^+$, Pax7$^+$, MRFs$^+$ (MyoD$^+$, Myf-5$^+$, myogenin$^+$)

- Grid

**Adult Muscle Stem (Satellite) Cells**

- Symmetric vs Asymmetric Division

**Symmetric Division**

- Stem Cell

- Differentiated Cell

- Symmetric Division

**Asymmetric Division**

- Stem Cell

- Symmetric Division
Symmetric vs Asymmetric Division

Asymmetric Cell Division - Stem Cell Hallmark

Symmetric vs Asymmetric Division

Divisional Asymmetry

A. Extrinsic Regulation
- adding growth factors & cytokines.
- changing surface properties of the culture dish.
- co-culture with 'feeder' cells.
- co-culture with scaffolding or matrix.

B. Intrinsic Regulation
- activation of transcription factors.
Cancer Stem Cells?

Stem Cells - Therapeutic Applications
1. Generation of cells/tissues for Cell-Based Therapies

2. Drug discovery/screening through safer and cheaper testing using human cells.
3. Study the mechanisms of human development, stem cell differentiation and function.
4. Study the mechanisms to understand and treat birth abnormalities.

Summary
- Stem Cell Definition?
- Totipotency - Pluripotency - Multipotency
- Various sources of Stem Cells?
- Advantages & Disadvantages
- Unique feature of “Stem Cells”?
- Self-Renewal vs Differentiation and how this could be achieved
- Therapeutic applications of Stem Cells?
  - Find out examples that spark your interests from the news and media for discussion in Lab 12.
<table>
<thead>
<tr>
<th>Lab 11 - Information &amp; Overview</th>
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<tr>
<td>Please ensure that you read and bring the Laboratory Handout for the Laboratory Session tomorrow.</td>
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<tr>
<td>Questions?</td>
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