Therapeutic Use of Stem Cells
Practical Hurdles & Ethical Issues

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Stem cells in Development

- Blastocyst
- Cord blood

UNSW Embryology
http://anatomy.med.unsw.edu.au/cbl/embryo/Notes/week2_10.htm
Pluripotent Stem Cells

NIH Stem Cells: Scientific Progress and Future Research Directions
What is a stem cell - Pluripotent

• Pluripotent
  – to describe stem cells that can give rise to cells derived from all 3 embryonic germ layers
  – Mesoderm
  – Endoderm
  – Ectoderm

• layers are embryonic source of all cells of the body
What is a stem cell- Definition

• Stem cell is a cell that has the ability to divide (self replicate) for indefinite periods – throughout life of organism
• Under the right conditions, or given the right signals, stem cells can differentiate to the many different cell types that make up the organism
Amplifying Cells

- Stem cells in many tissues divide only rarely
  - give rise to transit amplifying cells
  - daughters committed to differentiation that go through a limited series of more rapid divisions before completing the process.
  - each stem cell division gives rise in this way to eight terminally differentiated progeny
(Ab)Normal Stem Cell Production

- (A) normal strategy for producing new differentiated cells
- (B and C) 2 types of derangement that can give rise to unbridled proliferation characteristic of cancer
Stem Cell Daughter Fates

- **environmental asymmetry**
  - daughters are initially similar
  - directed into different pathways according to environmental influences that act on them after they are born
  - number of stem cells can be increased or reduced to fit niche available

- **divisional asymmetry**
  - stem cell has an internal asymmetry
  - divides in such a way that its two daughters are already endowed with different determinants at time of their birth
Possible Therapeutic Uses

• Neural
  – Parkinson’s, ALS, spinal cord injury……..
  – Cell Replacement
    • cell death, loss of function
  – Grafting
    • where host-graft rejection normally requires substantial ongoing immunosuppression
  – Repair
    • Spinal cord and brain injury

• Other Diseases
  – Diabetes, muscular dystrophies, cardiac, vital organs…….
Current research on stem cells

• How to:
  – Isolate
  – Grow
  – Maintain, store
  – Differentiate
  – Therapeutic uses
Stem Cell Therapy: Current Limitations on Cell Transplantation

Competition from Endogenous Cells!!

Hostile Niche for Donor Stem Cells

Cell Type?
- Skin
- Bone marrow

Cell Number?
- Spinal cord / Brain
- Liver
- Heart

Route of Delivery?
- Hormones / enzymes replacement

Adult Stem Cell Transplantation

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Enhancing Muscle Stem Cell Transplantation using Chemotherapeutic Drug Selection

- **Alkylation Chemotherapy + Drug Resistant Donor Cells** - *based on mechanisms established for Bone Marrow Transplantation*
  - Efficient Elimination of Endogenous Cells
  - Creating Receptive & Favourable Niche for Donor Cells
  - Selective *in vivo* Expansion of the Protected Donor Cells
  - Feasibility in the Skeletal Muscle as a Solid Organ?
Skeletal Muscle during Injury

Normal Muscle

- Muscle fibers and myonuclei are post-mitotic
- Muscle stem / satellite cells remain quiescent

Injured Muscle

- Muscle stem / satellite cells are activated and rapidly proliferate
- Differentiated cells align and fuse to form new muscle fibres
Muscle Stem Cell Transplantation - Improved Strategy
Selective Enrichment: The Mechanism

Wild-Type cell

- BCNU
- Alkylates DNA
- Cell survives

MGMT-P140K * Expressing Cell

- BCNU
- Alkylates DNA
- Cell death

* Anthony Pegg
Selective Enrichment: The Mechanism

**SELECTIVE ENRICHMENT**

- Wild type
- MGMT-P140K

BCNU + O6BG
Muscle Stem Cell Transplantation: Protocol

**Day -3**
- **Donor Cells**
  - Notexin into TA (0.4ug) & EDL (0.1ug) : i.m.
  - 3 Days of regeneration

**Day 0**
- **Host Environment & Cell and Drug Delivery**
  - EDL on both hindlimbs
  - Donor cells + Notexin (10 ul) : i.m.
  - BCNU : Restricted i.v.
  - O6BG upon recovery : i.p.

**Selection of Donor Cells**
- CD34(+ve) cells using magnetic cell sorting (6hrs) with no expansion
- 6 x 10⁴ donor cells per injection

**Tissue Collection**

**Examination**
- Q-PCR, Histology & FISH

[UNSW Logo]
Higher Engraftment of MGMT(P140K)$^{+ve}$ Donors in Chemo-Ablated Recipient Muscle Bed

A

Wild-Type Donor

Wild-Type Recipients

B

Wild-Type Donor

C

MGMT(P140K) Tg Donor

D7 post-transplantation

% of donor DNA within recipient muscle

Group A

Group B

Group C
De Novo Muscle Fibre Formation by MGMT(P140K)$^{+ve}$ Donors

D14 post-transplantation

Wild-Type Donor

MGMT(P140K) Donor

Y-Chromosome FISH / DAPI
Absence of Dystrophin in the Duchenne Muscular Dystrophy (DMD) Patients and *mdx* Mice

- **Duchenne Muscular Dystrophy (DMD)**
  - X-linked disorder with defects in Dystrophin gene
  - 1:3500 live Male Birth (20,000 babies / year)
  - Confined to wheelchair by 12 yrs and death by 30 yrs
  - Several mouse models exist including *mdx* mice (Dystrophin KO)
Restored Dystrophin Expression by Engrafted MGMT(P140K)$^+$ Donors in the Recipient mdx Muscle.

Dystrophin – Wild-type EDL

Dystrophin – Untreated mdx EDL

Dystrophin – Treated mdx EDL

14 Days Post-Transplantation

Notexin + BCNU + O6BG

MGMT-P140K$^+$ Cell Injected

Dystrophin/DAPI

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