Outline

• How can you identify Adult Stem Cells?
  • Cell Surface Antigens
  • Fluorescence-Activated Cell Sorting (FACS)
  • Magnetic-Activated Cell Sorting (MACS®)

• Pre-Clinical Model of Adult Stem Cell Transplantation for Muscular Dystrophy
  • Pathology & Current Hurdles

• Enhanced Stem Cell Transplantation Strategy
  • Host niche conditioning using chemotherapy with chemo-resistant donor cells

• Stem Cell Activation Signaling
  • New insights on systemic pro-regenerative signaling mechanisms.
Stem Cells - Therapeutic Applications

- Test Drugs on Human Cells in Culture
- Test drugs before conducting clinical trials
- Toxicity Testing
- Understand how to prevent and treat birth defects
- Study Cell Differentiation
- Generate Tissues and/or Cells for Transplantation
- Ectoderm: Neuron
- Mesoderm: Blood Cells
- Endoderm: Liver Cells
1. Generation of cells/tissues for Cell-Based Therapies

**Direct Delivery**

- Therapeutic gene
  - The therapeutic gene is packaged into a delivery vehicle such as a retrovirus
  - ...and injected into the patient

**Cell-based Delivery**

- Genetically modified ES cells (can block immune rejection from patient)
  - OR
  - ES cell HLA bank
  - OR
  - SCNT

- ES cells
  - in vitro differentiated stem cell
  - Adult stem cells
  - The therapeutic gene is packaged into a delivery vehicle such as a retrovirus and introduced into the cells.
  - The genetically modified cells are reintroduced into the patient.

**Target organ (e.g., liver)**
How to Identify & Isolate Stem Cells?

Cells in suspension are tagged with fluorescent markers specific for undifferentiated stem cells.

Labeled cells are sent under pressure through a small nozzle and pass through an electric field.

A cell generates a negative charge if it fluoresces and a positive charge if it does not.

Stem cell found
How to Identify & Isolate Stem Cells?

Figure E.1.1. Identifying Cell Surface Markers Using Fluorescent Tags.
Cell Surface Antigens - Lymphocyte Example

- CD34
- CD45
- CD15 (Granulocyte)
- CD14 (Monocyte)
- CD45
- CD4 (Helper T-lymphocyte)
- CD3
- CD25 (Activated T-lymphocyte)
- CD45
- CD19 (B-lymphocyte)
- CD61 (Thrombocyte)
- CD45
- CD3 (Suppressor T-lymphocyte)
Absence of Dystrophin in DMD Patients

- **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)
Dystrophin & Dystrophin-Glycoprotein Complex

- 4 Major domains
  - N-terminal
  - Central rod
  - Cysteine rich
  - C-terminal

- Crucial regions
  - ABDs
  - DgBD

Dystrophin and the dystrophin–glycoprotein complex

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Therapeutic Interventions for DMD

- **Viral vectors**
  - Adeno-associated virus
    - + High tropism for striated muscle
    - + Effective systemic delivery
    - + Low immunogenicity
    - - Low capacity
  - Lentivirus
    - + Permanent transduction
    - + Works well for ex vivo modification of cells
    - - Risk of insertional mutagenesis
  - hd-Adenovirus
    - + High capacity
    - - Modestly immunogenic
    - - Low tropism for skeletal muscle

Strategies for treating the muscular dystrophies

Muir & Chamberlain (2009)
Therapeutic Interventions for DMD

a. Viral vectors
   - Adeno-associated virus
     + High tropism for striated muscle
     + Effective systemic delivery
     + Low immunogenicity
     - Low capacity
   - Lentivirus
     + Permanent transduction
     + Works well for ex vivo modification of cells
     - Risk of insertional mutagenesis
   - hd-Adenovirus
     + High capacity
     - Moderately immunogenic
     - Low tropism for skeletal muscle

b. Exon skipping
   - Low immunogenicity
   - Effective systemic delivery
   - Potentially toxic byproducts

c. Plasmid DNA
   - Simplicity, relative ease/cost of synthesis
   - Full-length dystrophin
     - Potential toxicity and low transfection efficiency

(d) Cell therapy
   - Regeneration of muscle
     - Potentially costly
     - Isolation and expansion methods in autologous setting
     - Immune rejection in allogeneic setting

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Muscular Dystrophies (MD)

• Over 40 forms identified

• 4 Most common types are…
  • Duchenne Muscular Dystrophy (DMD) & Becker Muscular Dystrophy (BMD)
  • Myotonic Dystrophy (DM1)
  • Facioscapulohumeral Muscular Dystrophy (FSHD)
Cell Therapy - *Myoblast Transfer Therapy*

- **Aim:** donor cell engraftment and fusion to form muscle fiber heterokaryon.

- **Initial Limitations…**
  - >75% *donor cell death within 3 days following transplantation*
  - *Anoikis / Immune-rejection following ex vivo expansion*
Strategies for Enhancing Donor Cell Survival: Conditioning of the Host Niche

• Radiation & Cryodamage
  • Safety of use to achieve clinically meaningful level of ablation
  • Non-discriminatory against donor cells

• Chemotherapeutic Drug-Mediated Selective Survival of Donor Cells
  • Established in bone-marrow transplantation
Cell Therapy: Skeletal Muscle Biology

(a) Fusion of myoblasts into muscle fiber

(b) Muscle fiber

MUSCLE

GROUP OF MUSCLE FIBRES

- Epimysium
- Perimysium
- Fasciculus
- Endomysium
- Capillary