Bone Marrow Stromal Cells Generate Muscle Cells and Repair Muscle Degeneration

By Stephanie Aracena & Roynan Krebs

Bone Marrow Stromal Cells

- What are they?
- Where are they located?
- Why not stem cells?
  - Small populations
  - Ethical reasons
  - Histocompatibility
  - Difficult to get
What are they planning on doing with them?

- Therapy for degenerative diseases
  - Muscles
  - Neurons in Parkinson’s Disease
- Possibility of finding cure for muscle degenerative disorders

The Experiment

- Induce skeletal muscle lineage cells from MSCs
- Results
  - MSCs behave like stem cells
  - Pax7-positive satellite cells
    - Muscle stem cells
MSCs induced with growth factors and transfected

- bFGF, FSK, PDGF, neuregulin

- “C-MSCs”
  - Transfected with DNA containing NICD and G418 resistance
  - Selected for only cells with NICD expression by use of G418 antibody to kill cells lacking G418 resistance
    - CN-MSC’s
    - Treated with MSC supernatant → M-MSC

M-MSC fusion induction

- Single cells become multinucleated by fusing with each other
M-MSC Single-cell clonal culturing

- 89% of clones formed multinucleated cells at 14 days
  - This means they have potential to become muscle
  - Control: Refutes existence of possible nonmuscle elements
M-MSCs: present certain muscle specific genes
RT-PCR

- Beta-actin used as a positive control for cDNA presence
- C2C12
  - Mesodermal cell line that differentiates into muscle
  - Importance of cytokine growth factor and NICD order
Western Blot

Muscle precursor gene expression compared to a known muscle cell lineage

Transplantation of cloned human M-MSCs in rat gastrocnemius muscles

- Damaged rat gastrocnemius muscle with cardiotoxin treatment
- Transplanted GFP-labeled human M-MSCs
  - Do they repair damaged muscle?
YES!

Satellite Cells

In normal muscle when repair is needed, signals draw satellite cells, which proliferate and fuse with muscle fiber nuclei. More nuclei leads to protein synthesis and the myofibril becoming bulkier.
Do these “satellite cells” regenerate muscle?

- Second injection of cardiotoxin damages muscle without transplantation of any new cells
The Dream

- Human M-MSCs transplanted into mdx-nude mice genetically lacking dystrophin
- Restoration of dystrophin expression is evidence for possible dystrophin expression restoration in humans with genetic muscle degenerative disorder.

Future Research

- Alternative to embryonic stem cells
- Avoid rejection and immunosuppressive treatments
- Genome defective: Need to fix it first
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

“Bubble Mouse”
A review of antibodies
Recombination Activating Genes

Knockout genes
TARGETING VECTOR
Because of the way the targeting vector was constructed, we can easily distinguish between the two scenarios. First, neomycin selection kills any cells that have not integrated the transgene.

Then, gancyclovir, an antiviral drug, kills cells that have integrated the thymidine kinase gene during nonhomologous recombination.

Only cells that have homologously integrated the targeted transgene survive the double selection and reproduce on the culture plate.

These cells are inserted into a blastocyst, and the blastocyst is integrated into a surrogate mother that will carry the chimeric embryo to term.
Mutant mice were treated with repaired ES cells in two ways:

1. Hematopoietic precursors were derived by in vitro differentiation from the repaired ES cells and engrafted into mutant mice.

2. Immune-competent mice were generated from the repaired ES cells by tetraploid embryo complementation and were used as bone marrow donors for transplantation.
CMV-HvgTK

Puromycin N-acetyltransferase (pac)

Cre
A brief review of the loxP technique

Cre is a protein that eliminates DNA between two sites called loxP. Each loxP contains a 13-base pair sequence at the 5’ end, and an inverted version of this sequence at the 3’ end. There are eight base pairs in the middle.

5’ ATAACTTCGTATAATGTATGCTATACGAAGTTAT
    TATTGAAGCATATTACATACGATATGCTCCAATA 3’
One molecule of Cre binds to each of the 13-base pair sequences. The four Cre molecules then form a tetramer that removes the DNA between the loxP sites. One loxP site is left in the chromosome.
Hematopoietic Stem Cells

- Multipotent hematopoietic stem cell (hemocytoblast)
  - Common myeloid progenitor
    - Erythrocyte
    - Megakaryocyte
    - Platelet
    - Granulocyte
      - Neutrophil
      - Basophil
      - Eosinophil
    - Macrophage
    - Monocyte
    - Plasma cell

- Common lymphoid progenitor
  - Small lymphocyte
    - T lymphocyte
    - B lymphocyte
  - Natural killer cell (Large granular lymphocyte)
a  ntESC derivation

Nuclear transfer

ICM

Blastocyst

TE

ES cells
Mutant mice were treated with repaired ES cells in two ways:

1. Hematopoietic precursors were derived by invitro differentiation from the repaired ES cells and engrafted into mutant mice.
2. Immune-competent mice were generated from the repaired ES cells by tetraploid embryo complementation and were used as bone marrow donors for transplantation.

**Tetraploid complementation**

*Figure 4* Efficiency of tetraploid complementation with ES cells derived from fertilized (fESCs) and embryos cloned by nuclear transfer (iESCs). (a) IVF in the mouse (control). (b) Tetraploid complementation: electrofusion of two-cell embryos (4N) followed by culture to blastocyst stage and injection of ES cells (2N) derived from fertilized embryos (fESCs). The resulting pup is 2N; the placenta is 4N. (c) Tetraploid complementation with injection of iESCs, showing similar efficiency to fESCs. (d) Nuclear transfer results in only 1%–2% term development, most have large placentas (98%–99% die in utero).
Table 1. Mice Derived from Tetraploid Embryo Complementation with Rag2\(^{-/-}\) and Rag2\(^{+/R-}\) ntES Cell Lines

<table>
<thead>
<tr>
<th>ES Line</th>
<th># 4n Blastocysts Injected</th>
<th>Live Pups (Number of Neonatal Death)</th>
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<tbody>
<tr>
<td>Rag2(^{-/-})</td>
<td>14</td>
<td>4 (0)</td>
</tr>
<tr>
<td>Rag2(^{+/R-})</td>
<td>226</td>
<td>38 (9)</td>
</tr>
</tbody>
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A
1. T cell receptors taken from the thymus of tetraploid embryo complementation derived mice show rearranged alleles.
   • Therefore, the "recombination" function of Rag appears to be restored.

2. The relative numbers of B and T cells detected in Rag2+/R/ ntES mice were comparable to the B and T cell populations in wild type mice.
   • In contrast, blood from mice derived from the parental Rag2/ ntES line showed essentially no mature B and T cells.
   • This indicates that the repaired ntES cells gave rise to normal bone marrow that restored the lymphoid system after transplantation into Rag2 mutant mice.
Summary Slide (cont.)

- FACS analysis detected a low level of GFP-positive, mature B cells by IgM staining and GFP-positive, mature T cells by CD4 and CD8 staining.

- The serum of ntES cell-engrafted mice showed the presence of IgM, IgG, and IgA.

Future Gene Therapy

- Treatment Method- “chimeric method”
  - Remaining Issues
    - Tissue rejection/immune suppression
    - Differentiation of cells
  - Hope for the field
    - ALD treatment
      - Modified HIV virus used as vector
      - Corrected ABCD1 gene was inserted