β-Cell Regeneration Mediated by Human Bone Marrow Mesenchymal Stem Cells

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Background
Mesenchymal Stem Cells

• First isolation from bone marrow 30 ys ago
• Isolation from: spleen, heart, skeletal muscle, synovium, amniotic fluid, dental pulp, bone, umbilical cord, adipose tissue
• Expansion in culture while maintaining multipotency
• (Trans-)differentiation into different cell types: osteoblasts, chondrocytes, adipocytes, myocytes, cardiomyocytes, hepatocytes, epithelial cells, endothelial cells, neurons
• Heterogeneity
  – International Society for Cellular Therapy:
  – Plastic-adherent in standard culture conditions
  – Expression of CD105, CD73, CD90
  – Lack of CD45, CD34, CD14, CD11b, CD79a, CD19, HLA-DR
  – Must be able to differentiate into osteoblasts, adipocytes and chondroblasts in vitro

Background

- BMSCs injected into diabetic animals reversed diabetic phenotypes and improved glucose control
- Poor direct β-cell differentiation -> other possible roles of BMSCs in pancreatic islet regeneration
- Introduction of transcription factor genes into cultured human BMSCs
  - Activation of genes related to the development and function of β-cells
- PDX1:
  - Master gene in pancreas development
  - Crucial for early pancreas differentiation
- VEGF-A:
  - Important for intra-islet angiogenesis
  - Vascular membrane is a niche for insulin gene expression and β-cell proliferation
- 3 treatment groups:
  - hBMSCs
  - hBMSCs expressing PDX1
  - hBMSCs expressing VEGF
Methods 1

- Human BMSC Culture and expansion
  - hBMSCs from a single donor, passage #7

- Adenovirus production and cell transfection
  - cDNAs for human PDX1 and mouse VEGF165 were subcloned into AdenoX viral DNA vector
  - hBMSCs were transfected with adenovirus 2 days before transplantation

- Animal model and stem cell transplantation
  - NOD/SCID mice
  - 3 i.p. injections of streptozotocin
  - hBMSCs / hBMSCs-VEGF / hBMSCs-PDX1
  - Injection of 1x10^6 cells (on day 7) intracardially

- Blood glucose and serum insulin measurements
  - non-fasting mice daily for 1 week, then twice a week
  - Mouse insulin ÉLISA, human insulin ELISA
Methods 2

• Immunohistochemical analyses
  – Mouse pancreatic tissues harvested 6 weeks after stem cell injection
• β-cell count
• Phase contrast and confocal microscopy analyses
• rtPCR arrays
  – Pancreatic tissue
Results 1.1
hBMSCs-VEGF

- Mice treated with STZ developed hyperglycemia 6-7 days after STZ-injection
- High mortality rate of diabetic mice
- Reversion of hyperglycemia due to hBMSC-VEGF injection
Results 1.2
hBMSCs-VEGF

Histological examination of the pancreatic islet morphology (6w after TX)

- Reduction of the number of insulin-expressing cells in STZ-induced diabetic mice
- Similar staining pattern in control mice and hBMSC-VEGF treated mice
Results 1.3
hBMSCs-VEGF

Engraftment and survival of hBMSCs-VEGF in the mouse pancreas (6w after TX)

<table>
<thead>
<tr>
<th>Animal/Cells</th>
<th>Pancreas</th>
<th>Kidney</th>
<th>Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/hBMSC-VEGF</td>
<td>0.2 ± 0.05</td>
<td>ND</td>
<td>NA</td>
</tr>
<tr>
<td>2/hBMSC-VEGF</td>
<td>0.18 ± 0.07</td>
<td>ND</td>
<td>NA</td>
</tr>
<tr>
<td>3/hBMSC-VEGF</td>
<td>0.025 ± 0.005</td>
<td>0.004 ± 0.001</td>
<td>ND</td>
</tr>
<tr>
<td>4/hBMSC-VEGF</td>
<td>0.03 ± 0.007</td>
<td>0.015 ± 0.007</td>
<td>ND</td>
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<tr>
<td>1/hMSC</td>
<td>0.008 ± 0.0005</td>
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<td>NA</td>
</tr>
<tr>
<td>2/hMSC</td>
<td>0.0048 ± 0.001</td>
<td>ND</td>
<td>NA</td>
</tr>
<tr>
<td>3/hMSC</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>4/hMSC</td>
<td>ND</td>
<td>ND</td>
<td>NA</td>
</tr>
<tr>
<td>1−3/no cells</td>
<td>ND</td>
<td>ND</td>
<td>NA</td>
</tr>
</tbody>
</table>
hBMSCs-VEGF were able to differentiate into vessels and β-cells
Results 1.5

hBMSCs-VEGF

- Reduction of VEGF expression in the β-cells after induction of diabetes
- Restoration of VEGF expression after treatment with hBMSCs-VEGF
Results 2.1
hBMSCs-PDX1

- 50% of hBMSCs-PDX1 treated mice maintained severe hyperglycemia
- 50% showed reduction of hyperglycemia but again developed hyperglycemia after 2-3 weeks
Left:
- „Temporary reversed“ (F) and „unrescued“ (G) mice showed reduction of insulin expression in the pancreatic islets

Right:
- Engraftment of human cells in mouse pancreas
hBMSCs without genetic modification did not ameliorate diabetic phenotypes
- survival rate similar to STZ-induced diabetic mice
- alteration of pancreatic islet morphology, inversion in the insulin/glucagon ratio, poor engraftment of hBMSCs in the pancreas
A: Only mice treated successfully with hBMSCs-VEGF showed significantly higher levels of mouse insulin compared with other groups.

B: Levels of human insulin were detectable in the therapy-groups → de novo differentiation of hBMSCs into β-cells.
Results 4.2
Endogenous vs. Transplant-derived β-cell differentiation

C: Levels of total serum insulin were higher in the therapy-groups.
D: Number of β-cells higher in therapy-groups (correlation with total insulin levels).
Results 5.1
Mechanisms of endogenous β-cell recovery in hBMSCs-VEGF treated mice

Decreasing expression of genes related with insulin receptor signaling pathway in pancreases of diabetic mice

Up-regulation of genes involved in the insulin/IGF signaling pathway in pancreases of hBMSCs-VEGF treated mice
AKT and downstream proteins required for β-cell proliferation, differentiation and survival are highly expressed in hBMSCs-VEGF treated mice.
Results 5.3
Mechanisms of endogenous β-cell recovery in hBMSCs-VEGF treated mice

- P27kip1 (cell cycle inhibitor protein negatively regulated through PI-3K/AKT) was upregulated in diabetic mice and downregulated in hBMSCs-VEGF mice
- c-CASP3 was highly increased in diabetic mice
Discussion

- hBMSCs alone were not able to reverse hyperglycemia
- Recovery from diabetes following hBMSCs-VEGF injection
  - Engraftment of hBMSCs-VEGF in the pancreas of diabetic mice
  - Differentiation of hBMSCs-VEGF into blood vessels and ß-cells
  - Detectable levels of human insulin \(\rightarrow\) chimerism
  - Higher levels of mouse insulin \(\rightarrow\) endogenous ß-cell regeneration
- Only transient recovery from diabetes following hBMSCs-PDX1 injection
- Upregulation of insulin receptor associated genes in hBMSCs-VEGF mice
- Upregulation of genes involved in the PI-3K/AKT pathway
  - Inhibition of apoptosis
  - ß-cell differentiation and proliferation through activation of PDX1 and inhibition of P27Kip1
  - Modulation of intra-islet angiogenesis \(\rightarrow\) VEGF expression