Anterior Cruciate Ligament (ACL) Derived Stem Cells Transduced Ex-vivo with BMP2 Accelerates Tendon-Bone Healing in ACL Reconstruction

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Introduction: We have recently reported that the ruptured and septum regions of the human ACL contain vascular stem cells capable of enhancing the healing of tendon grafts (1, 2). Moreover, we also revealed that ACL-derived CD34+ cell sheet transplantation, as a graft wrap, is more efficient than the direct intracapsular injection of the cells for enhancing recovery time after ACL reconstruction (3). On the other hand, BMP-2 like other bone morphogenetic proteins plays an important role in the development of bone and cartilage (4). It was reported that recombinant human (rh) BMP-2 injection was useful for direct tendon-to-bone enthesis (5). It is important that both angiogenesis and osteogenesis work in an integrated manner for proper tendon-bone healing to occur. However, the combination of cell and gene therapy and angiogenesis and osteogenesis for ACL reconstruction have not yet been well investigated, which is the purpose of the current study. Here we investigate whether ACL-derived stem cells engineer to express BMP2 can be used to improve ACL healing.

Methods: All animal procedures were approved by the University of Pittsburgh’s Institutional Animal Care and Use Committee. The use of human stem cells was approved by the University of Pittsburgh’s Institutional Review Board.

1) Cell sheet construction and animal model of ACL reconstruction:
CD34+ cells were isolated by fluorescence-activated cell sorting (1). ACL-derived CD34+ cells were transduced with a lenti-viral vector encoding for BMP2. Cell sheets were constructed using temperature-responsive culture plates seeded with 5x105 BMP2 virally transduced cells or non-transduced cells (6). A reproducible model of ACL reconstruction was created in nude rats (NIH-Whn NIHNRNU-M, 10 wk old females) using the graft wrapped with cell sheets (7).

2) Experimental Groups:
We established four groups. 1) ACL-derived CD34+ cells-Lenti-hBMP2 100% (BMP2(100) group), 2) ACL-derived CD34+ cells-Lenti-hBMP2 (25%) mixed with ACL-derived CD34+ cells (75%) (BMP2(25) group), 3) ACL-derived CD34+ cells only (CD34 group), 4) PBS alone (No cell group). (n=18 in each group)

Results: (1) In vitro characteristics of the BMP2-transduced CD34+ cells:
BMP2-transduced CD34+ cells demonstrated a high osteogenic differentiation capacity and kept similar endothelial differentiation capacity and proliferation capacity when compared with CD34+ cells.
(2) BMP2-transduced CD34+ cell transplantation leads to functional tendon-bone in ACL reconstruction:
Biomechanical evaluation by failure load of tensile test demonstrated that biomechanical strength was
significantly higher in the BMP2 (25% and 100%) and control groups than in the other groups at 4 weeks and was significantly greater in the BMP2 (25%) group than in all the other groups at 8 weeks. (Fig.1)

(3) CD34+ cells exhibited potent therapeutic potentials for tendon-bone healing:
Masson’s trichrome staining was used to assess the tendon-bone healing at week 2. Quantitative analysis demonstrated that the area of oblique collagen fibers, similar to Sharpey’s fibers at week 2, was significantly greater in the BMP2 (25%) and CD34 group than in the other groups. (Fig.2)

Immunohistochemical staining for αSMA demonstrated the number of αSMA positive cells was significantly greater in the BMP2 (25%) group and CD34 group compared with the other groups. Bone tunnel healing was also assessed radiographically 8 weeks after surgery. The micro-computed tomography (μCT) analysis revealed that the bone tunnels were significantly smaller in the BMP2 (25%) group than the other groups. (Fig.3)

(4) Enhancement of intrinsic vascularization and osteogenesis:
Vascularity in the tendinous insertion sites was assessed by immunohistochemical staining with rat isolectin B4 at week 2. Capillary density was significantly greater in the CD34 groups than in the no cell group. Osteoblast staining with rat OC at week 2 also revealed that Osteoblast density was greater in both the BMP2(100%) and BMP2(25%) groups compared with the other groups. (Fig.4)

(4) Human cell-derived vasculogenesis:
Differentiated human endothelial cells were identified by immunostaining with hCD31. The results showed that the animals receiving BMP2 transduced CD34+ cells (both the 25% and 100% groups) and the CD34+ cell only group contained hCD34+ cells.

Discussion: We demonstrated that the ACL-derived CD34+ cells transduced with BMP2 (25%) exhibited a therapeutic effect on rat ACL reconstruction, promoting osteogenesis at the bone-tendon junctions and increasing the biomechanical strength 4 and 8 weeks after ACL reconstruction. These results indicate the importance of neoangiogenesis and osteogenesis after ACL reconstruction. On the other hand, excessive over expression of BMP2 showed no significant difference compared to the control groups. This result is consistent with other reports which showed that the over-expression of BMP2 induces deleterious side effects in vivo. (8) In conclusion, transplanted ACL-derived CD34+ cells with BMP2 (25%) increased neoangiogenesis and osteogenesis and contributed to better bone-tendon healing and biomechanical strength.

Significance: ACL-derived CD34+ cells can be obtained from the rupture site of the injured tendon and preserved for future use. Cell sheet technology with ACL-derived CD34+ cells expressing an appropriate level of BMP2 could readily be exploited for ACL reconstruction, leading to enhanced graft maturation and biomechanical strength.
Figure 4

(a) CD34+BMP2 (100%)  CD34+
   CD34+BMP2 (25%)  No Cell

Red: Osteocalcin, Blue: DAPI, Bar: 100μm

(b) Osteoblast Density (mm²)

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<th>Condition</th>
<th>Osteoblast Density</th>
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<tbody>
<tr>
<td>CD34+ BMP2 (100%)</td>
<td>**</td>
</tr>
<tr>
<td>CD34+ BMP2 (25%)</td>
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<tr>
<td>CD34+ No Cell</td>
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