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Therapeutic Potential of Anterior Cruciate Ligament (ACL) Derived Stem Cells Ex-Vivo Transduced With BMP2 for Rat ACL Reconstruction

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Summary:
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 in a rat model.

Abstract:
INTRODUCTION
We have recently reported that the ruptured and septum regions of the human anterior cruciate ligament (ACL) contain vascular stem cells capable of enhancing the healing of tendon grafts. On the other hand, BMP-2 injection was reported to be useful for direct tendon-to-bone enthesis. 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.

METHODS
ACL-derived CD34+ cells were isolated via Fluorescence Activated Cell Sorting (FACS) from the rupture sites of human ACLs. The cells were then virally-transduced with BMP2. A reproducible model of ACL reconstruction was created in nude rats using the graft wrapped with cell sheets.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)

We established four groups: CD34+BMP2 (100%), CD34+BMP2 (25%), CD34+ and No cells. The tendon graft was wrapped with the cell sheet.

RESULTS
BMP2-transduced CD34+ cells demonstrated a high osteogenic differentiation capacity and kept similar endothelial differentiation capacity and proliferation capacity when compared with CD34+ cells in vitro. In vivo biomechanical evaluation by failure load of tensile test demonstrated that biomechanical strength was significantly higher in the CD34+BMP2 (25% and 100%) and CD34+groups than in the no cell group at 4 weeks and was significantly greater in the CD34+BMP2 (25%) group than in all the other groups at 8 weeks. Immunohistochemical staining at week 2 revealed that Osteoblast density was greater in both the BMP2 (100%) group and BMP2 (25%) group compared with the other groups. Capillary density in the tendinous insertion sites was significantly greater in the CD34+BMP2 (100%), CD34+BMP2 (25%) and CD34+groups compared to the no cell group. Graft maturation was significantly accelerated in the CD34+BMP2 (25%) group and CD34 groups compared to the other groups. The micro-computed

tomography (μCT) analysis revealed that the bone tunnels healing was significantly smaller in the BMP2 (25%) group than the other groups.

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. 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. 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.