ISAKOS

International Society of Arthroscopy, Knee Surgery and Orthopaedic Sports Medicine

9th Biennial ISAKOS Congress • May 12-16, 2013 • Toronto, Canada

Paper #250

Effect of Angiogenesis on the Regenerative Capacity of ACL-derived CD34+ Cells in ACL Reconstruction

Koji Takayama, MD, PhD, USA
Satoshi Terada, MD, USA
Yutaka Mifune, MD, PhD, JAPAN
Tomoyuki Matsumoto, MD, PhD, JAPAN
Ryosuke Kuroda, MD, JAPAN
Masahiro Kurosaka, MD, JAPAN
Freddie H. Fu, MD, USA
Johnny Huard, PhD, USA

University of Pittsburgh
Pittsburgh, PA, USA

Summary:
This study indicates the transplanted ACL-derived CD34+ cells with VEGF (25%) increased neo-angiogenesis, bone-tendon healing and biomechanical strength. On the other hand, blocking angiogenesis with sFLT1 significantly decreased biomechanical strength and excessive over expression of VEGF showed no significant difference compared to the control groups. Further studies will provide novel insights

Abstract:
Introduction:
We have recently reported that the ruptured and septum regions of the human ACL contain numerous vascular-derived stem cells (1) and these cells contribute to tendon-bone healing (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 prompt recovery after ACL reconstruction; however, the mechanisms of tendon graft healing after ACL reconstruction are still unclear. The purpose of this study was to investigate the effect of angiogenesis on ACL reconstruction. Our hypothesis is that the transplantation of VEGF expressing ACL-derived stem cells will improve ACL repair after reconstruction while blocking angiogenesis (using the VEGF antagonist, sFLT-1) will reduce the repair process.

Methods:
Procedures described were approved by the University of Pittsburgh Institutional Animal Care and Use Committee. The use of human stem cells was approved by the University of Pittsburgh's Institutional Review Board. ACL-derived cells were obtained from human ACL rupture sites. CD34+ cells were isolated by fluorescence-activated cell sorting (1). ACL-derived CD34+ cells were transduced with lenti-viral vectors encoding for VEGF, sFLT1 or GFP. We established five groups. 1) ACL-derived CD34+ cells-Lenti-hVEGF (VEGF(100) group), 2) ACL-derived CD34+ cells-Lenti-hVEGF (25%) mixed with ACL-derived CD34+ cells-Lenti-GFP (75%) (VEGF (25) group), 3) ACL-derived CD34+ cells-Lenti-GFP (Control group) 4) ACL-derived CD34+ cells-Lenti-hsFLT1 (sFLT1 group) 5) PBS alone (No cell group). Cell sheets were constructed using temperature-responsive culture plates with and without 5x105 virally transduced cells (4). ACL reconstruction was performed using the graft wrapped with cell sheets in immno-deficient rats (5). Histological evaluation was performed using Masson's trichrome staining and immunohistochemical staining for isolectin B4 and hCD31 at 2 weeks (n=4). Biomechanical testing was performed at 4 and 8 weeks (n=6).

Results:
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 VEGF (25%) group than in the other groups. Capillary density was determined using isolectin B4 at week 2 and

was significantly greater in the VEGF (100%) group than in the other groups. The numbers of human-derived endothelial cells were significantly greater in the VEGF (100% and 25%) and control group than in the other groups. Failure load of tensile test demonstrated that biomechanical strength was significantly higher in the VEGF (25%) group than in the other groups at 4 week and in the VEGF (100% and 25%) and control group than in the other groups at 8 weeks.

Discussion:
We demonstrated that the ACL-derived CD34+ cells transduced with VEGF (25%) accelerated the healing of the bone-tendon junction and increased biomechanical strength 4 weeks after rat ACL reconstruction. Blocking angiogenesis with sFLT1 significantly decreased biomechanical strength at 4 and 8 weeks. These results indicate the importance of neoangiogenesis after ACL reconstruction. On the other hand, excessive over expression of VEGF showed no significant difference compared to the control groups. This result is consistent with other reports which showed that the over-expression of VEGF induces deleterious side effects in vivo (4,5) Interestingly, VEGF released from the transplanted ACL-derived stem cells could promote not only human but also rat cell differentiation toward an endothelial lineage, demonstrating a paracrine mechanism requiring secreted factors. In conclusion, transplanted ACL-derived CD34+ cells with VEGF (25%) increased neoangiogenesis and contributed to bone-tendon healing and biomechanical strength.

Acknowledgements:
The authors are grateful for the technical advice provided by Jessica Tebbets and for the editorial assistance of James Cummins in preparing this abstract
References: (1) Matsumoto T. et al.Stem Cell Dev. 2012. (2) Mifune Y. et al.Cell Transplant 2012. (3) Kawamura S, et al. J Orthop Res. 2005 (4) Lee RJ et al. Circulation 2000. (5) Ju, Y. J, et al. AJSM 2006