Characterization of Human Meniscus-Derived Vascular Stem Cells; Comparison of Periphery and Inner cells

Aki Osawa †‡ *MD, PhD, Tomoyuki Matsumoto †‡ MD, PhD, Seiji Kubo †‡ MD, PhD, Burhan Gharibeh † PhD, Alison Logar †, Arvydas Usas † MD, Hisashi Kurosawa. *MD, PhD, Christopher D. Harner MD and Johnny Huard PhD.

† Stem Cell Research Center, Children’s Hospital of Pittsburgh, and the Department of Orthopaedic Surgery, University of Pittsburgh, Pittsburgh, ‡ Department of Orthopaedic Surgery, Juntendo University, School of Medicine, Tokyo Japan

Introduction: In this study, we have investigated the differences of cell characterization between the vascularized peripheral region of meniscus and the avascularized inner region of the meniscus. A previous report has demonstrated the presence of CD34 positive and CD31 negative cells in the outer vascular portion and the superficial regions of the meniscus. Other investigators have also attempted to isolate and characterize cells populations in human meniscus based on their marker profile. On the other hand, our laboratory has demonstrated that the origin of muscle stem cells is associated with the walls of the blood vessels in skeletal muscle. We have investigated if the highly vascularized peripheral region of the meniscus has a richer supply of vascular stem cells when compared to the avascular zone of the meniscus. Moreover, we investigated if CD34 and CD146 positive cells derived from meniscus can differentiate into chondrocytes, osteocytes, and adipocytes, which is consistent with our theory that the more vascularized area of the meniscus contains more multipotent cells.

MATERIALS AND METHODS Sample: Twelve human lateral menisci were harvested from subjects undergoing total knee arthroplasty (61±7.7, years old, six male and six female), following Institutional Review Board (IRB) approved protocols. Cell isolation: Menisci were separated into 2 regions, the peripheral one third and the inner two thirds. Each tissue was minced and then digested with collagenase in DMEM, 5% type II collagenase. Immunohistochemical staining: Menisci were snap-frozen and were immunostained for CD31 (endothelial cell marker), CD34 (stem cell marker), and CD146 (pericyte marker), coupled with smooth muscle actin (SMA) to detect various cells around the arterioles. Characterization of meniscus-derived cells: Meniscus-derived cells were characterized by flow cytometry for CD34, CD146, and CD31 expression. The meniscus cells were cultured in osteogenic medium supplemented with BMP4 (100ng/ml) and TGF-β3 (10ng/ml). Pellets were assessed and stained for Oil Red O. PC34 and Pl34 were positive for Oil Red O. The number of Oil Red O positive cells in P34 and I34 were 50.40 ± 12.84, and 31.20 ± 16.21, respectively (P < 0.05 for peripheral vs. inner, Fig.2c). The mRNA expression of COLII and osteocalcin was detected from pellets of AP34 and AI34 (Fig.3b). Adipogenesis: P34 and I34 were positive for Oil Red O. The number of Oil Red O positive cells in P34 and I34 were 50.40 ± 12.84, and 31.20 ± 16.21, respectively (P < 0.05 for peripheral vs. inner, Fig.2c). The mRNA expression of PPARγ and LPL was detected from pellets of AP34 and AI34 as same levels (Fig. 2d).

DISCUSSION Our data showed that CD34 positive cells were found primarily in the peripheral region of meniscus than the inner region. Additionally, sorted CD34 positive cells by FACS from the peripheral and inner region showed the loss of CD34 expression during expansion. Interestingly, instead of decreased expression of CD34, the expression of CD146 was increased. Our data suggested cells which were originally positive for CD34 switched to their surface markers to express CD146. Moreover, CD34 positive cells from the peripheral vascularized region and the inner avascularized region of human meniscus underwent multilineage differentiation: chondrogenic, osteogenic, and adipogenic differentiation. However, CD34 positive cells from the peripheral-vascularized region have more multilineage differentiation potency compared to the inner-vascularized region. These findings may explain for the fact that meniscal tears in the peripheral region can heal, because the peripheral one third of the meniscus receives a richer blood supply. These findings suggest that meniscal vascular cells have stem cells characteristics and are found predominantly in the peripheral region, which may explain the low success rate of healing in the inner region. Our study showed that meniscus-derived CD34 positive and CD146 positive cells, which were found more prevalent in the peripheral vascular region than in the inner avascular region, exhibited potential for multilineage differentiation suggesting that these populations exhibit stem cell characteristics and may contribute to meniscal regeneration. The cells isolated from the peripheral vascular region have a greater multilineage differentiation potential than cells isolated from the inner region. The present findings provide important clinical insight for cell-based therapy aimed at enhancing meniscal repair and regeneration following injury.


ACKNOWLEDGEMENTS The authors are grateful for technical and scientific advice provided by James Cummings, Jessica Tebbets, Bo Zheng, Guangheng Li, Karin Corsi, Laurie Meszaros and Lauren Drowley (SCRC); Sarah Henry and Kimberly Francis (Orthopaedic Surgery, University of Pittsburgh). Funding was provided by the Henry Mankin endowed chair at the University of Pittsburgh and the Donaldson chair at Children’s Hospital of Pittsburgh.