Identification and Characterization of Adult Mouse Meniscus Stem/Progenitor Cells
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INTRODUCTION: The most common knee injury is damage to the meniscus, a fibrocartilaginous cushion with a pivotal role in protecting the articular cartilage from damage during movement. Although treatment of acute meniscal injuries has evolved dramatically in recent years, surgical procedures aimed at repairing or replacing damaged menisci are often unsuccessful. In fact, most surgical repair of meniscal tears cannot reliably prevent the progression of degenerative changes and clinical symptoms that presage the development of knee osteoarthritis (OA). Attempts to enhance meniscal healing with addition of fibrin clots or growth factors have shown some promise, consistent with the idea that the intrinsic healing potential of the meniscus might be improved by activation of endogenous meniscal stem cells. However, progress in this area has been limited by a lack of information about the origin of meniscal progenitors and the signaling pathways controlling their proliferation and differentiation. A greater understanding of the basic biology of meniscus-derived stem cells will be necessary for their application in cell based repair and tissue engineering strategies. To this end, we isolated and characterized meniscal stem progenitor cells (MSPCs) from adult mouse meniscus. We chose to analyze murine cells because our data could be used in future studies on the regulatory mechanisms underlying meniscal regeneration and the mouse is an ideal system for genetic manipulation. Mouse MSPCs exhibit the general features of tissue-specific stem cells isolated from other musculoskeletal tissues, including clonogenicity, multi-potency and expression of several common cell surface markers. In addition, adult mouse MSPCs express significant levels of genes first identified in embryonic mouse meniscus that may be important for meniscal formation. We also show that markers associated with MSPCs localize in distinct regions of the adult mouse meniscus hypothesized to harbor cells capable of responding to meniscal injury.

METHODS: This study was approved by the Harvard Medical School IACUC. MSPCs were isolated from C57Bl/6 mouse meniscus grown in explant culture. These cells were characterized for stem cell properties using colony-forming assays and for their ability to differentiate in osteogenic, adipogenic and chondrogenic media. Flow cytometry was used to detect the presence of surface antigens related to stem cells on MSPC, and qRT-PCR was used to examine the gene expression profile of MSPCs. The major proteins associated with MSPCs were localized in the adult mouse knee using immunohistochemistry (IHC).

RESULTS: Based on explant culture procedures for human meniscus and cartilage, a protocol was developed for isolating meniscal progenitor cells from adult mouse meniscus. After 5-7 days, cells began to grow out of the explanted menisci (Figure 1A). In culture, these meniscus-derived cells grew clonally and exhibited a spindle-shaped morphology (Figure 1B). Cells grew out of both the lateral and medial menisci of mice of all ages tested (8wk, 6 mo, 1yr) and grew well in monolayer. Mouse MSPCs showed universal stem cell-like characteristics including clonogenicity and multi-potentiality (Figure 1C-F). FACs analysis revealed MSPCs expressed the mesenchymal stem cell markers CD44, Sca-1, CD90 and CD73, and when cultured in monolayer had elevated levels of biglycan and collagen type I, important extracellular matrix components of adult meniscus. MSPCs also expressed robust levels of the meniscus signature gene lysyl oxidase (Lox), an enzyme responsible for collagen cross-links in skeletal and connective tissue, as well as Igf-1, the major signaling pathway enriched in the developing meniscus. To verify data obtained from FACs and qPCR analyses with MSPC behavior in vivo, the spatial localization of a select group of MSPC expressed factors was examined using IHC. CD44, biglycan, Lox and IGF-1 were all detected in the outer periphery of the meniscus in the fibro-chondrocytes of the superficial zone of 8 wk old mice (Figure 2B-E). This superficial zone is thought to contain endogenous progenitor cells with regenerative capabilities. In addition, positive staining was seen for CD44, biglycan, Lox and IGF-1 in the fibroblast like cells of the outer vascular zone. This region is rich in collagen type I and has a higher capacity for healing and repair.

DISCUSSION: We believe that identification of MSPCs provides a powerful tool for enhancing cell-based strategies focused on meniscal regeneration, as there is increasing evidence that tissue-resident stem cells are critical for organ homeostasis and effective wound healing. Although MSCs and synovium-derived stem cells have been tested in meniscal injury models, endogenous stem/progenitor cells may be best suited for repair of the meniscus, a tissue with distinct composition, architecture and function in the knee joint.

SIGNIFICANCE: A greater understanding of the basic biology of meniscal stem/progenitor cells is needed to enhance treatments for meniscal pathologies. Analysis of meniscus-derived stem cells in animal models such as the mouse will allow for detailed studies of their behavior during injury and repair and of the regulatory pathways that guide these processes, critical steps in identifying therapeutic targets for the regeneration of diseased or injured meniscal tissue.