Discovery of a novel articular cartilage skeletal stem cell

Guang-Ting Cong, Seoyeon Bok, Alisha Yallowitz[1], Michelle Cung, Ren Xu[2], Shawon Debnath, Jun Sun, Lingling Hu, Matthew B Greenblatt[2]
[1]Weill Cornell Medicine, [2]Weill Cornell Medical College

INTRODUCTION:

The mature articular cartilage does not exhibit regenerative capacity. Additionally, identity of the true endogenous articular cartilage stem cell is unknown, and this significantly constrains the cartilage repair paradigm. In this context, despite second- and third-generation improvements in chondral repair techniques, they still retain significant rates of failurewith formation of sub-par fibrocartilage. The orthopaedic surgery field has been implementing cellular techniques in the absence of a refined understanding of reparative mechanisms, in spite of paradoxical findings in the role of the mature articular chondrocyte in chondral repair[5]. Building upon our recent investigations into skeletal stem cells (SSCs)[1,3,4], we have identified a novel skeletal stem cell giving rise to articular chondrocytes (termed acSSCs). Our studies demonstrate that acSSCs possess the ability to generate articular cartilage both in transplantation settings and during normal joint development, representing significant potential for cartilage repair.

METHODS:

Regeneration in the rest of the skeleton is driven by activation of skeletal stem cells to produce mature cell types mediating new bone formation[3]. These SSCs express a cell surface marker complement[2]fundamentally different from that of mesenchymal stromal cells. Embarking in a new direction building upon our expertise in the discovery of skeletal stem cell subsets[3], we utilized two adipose tissue cell surface markers, CD24 (a cell adhesion molecule) and CD29 (an integrin) that were found to be upregulated in a specific subset of SSCs based on bulk RNA expression analysis. Using similar cell surface markers as previously described[3], a CD24+CD29+ fraction of cells expressing SSC markers were found to display stemness and self-renewal capacity via gold-standard serial transplantation studies in mice. These cells were found to be non-overlapping with all previously studied populations.

RESULTS:

Only these candidate articular cartilage SSCs and not other subsets appear to give rise to the articular cartilage following syngeneic transplantation, contributing to all layers of the articular cartilage from deep to the superficial zones. Graft-derived cartilage was found to be persistent, and acSSCs also demonstrate multipotency giving rise to the epiphyseal osteoblasts, adipocytes, and physeal chondrocytes (Fig 1). This represents the first demonstration of a cell population that can directly give rise to morphologically normal hyaline articular cartilage after isolation and syngeneic orthotopic transplantation. Flow cytometric data, alongside label retention and lineage tracing data formulated a novel differentiation sequence for the acSSC as a (at minimum) tripotent articular cartilage stem cell (Fig 2). In preclinical models of OA induced by destabilization of the medial meniscus (DMM), acSSC grafts not only improved histological features of cartilage degeneration (Fig 3) but also enhanced functional outcomes as assessed by gait analysis (Fig 4).

DISCUSSION AND CONCLUSION:

These findings represent a significant advancement in our understanding of articular cartilage generation, regeneration and offer potential targets for therapeutic intervention. By identifying druggable targets that regulate articular cartilage lineage commitment or are selectively present in acSSCs, our research opens the door to novel biologic and small molecule therapies for OA and other musculoskeletal degenerative diseases. Ultimately, these insights may lead to the development of effective treatments that address the critical impediment to articular cartilage regeneration in adults.

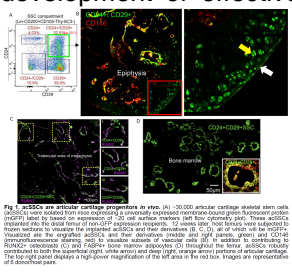

Fig 1. acSSCs are articular cartilage progenitors *in vivo*. (A) ~30,000 articular cartilage skeletal stem cells (acSSCs) were isolated from mice expressing a universally expressed membrane-bound green fluorescent protein (mTdTomato) label by based on expression of ~20 cell surface markers (left flow cytometry plot). These acSSCs implanted into the distal femur of non-GFP expression recipients. 12 weeks later, host femurs were subjected to histological analysis. Visualized are the engrafted acSSCs and their derivatives (middle and right panels, green) and CD144 (immunofluorescence staining, red) to visualize subsets of vascular cells (B). In addition to contributing to RUNX2+ osteoblasts (C) and FABP4+ bone marrow adipocytes (D) throughout the femur, acSSCs robustly contributed to both the superficial (right, white arrow) and deep (right, orange arrow) portions of articular cartilage. (E) Schematic displays a high-power magnification of the left area in the red box. Images are representative of 6 donor/host pairs.

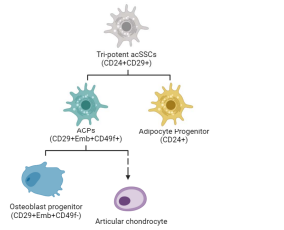

Fig 2. acSSCs appear at minimum tripotent. They differentiate into an articular chondrocyte progenitor (ACP) prior to differentiating into osteoblasts and articular chondrocytes. They also separately, robustly differentiate into adipocyte progenitors. All differentiation sequences appear identifiable by specific cell surface marker immunophenotypes.

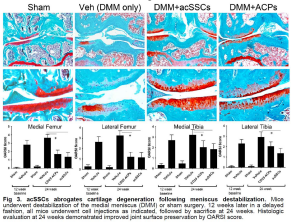

Fig 3. acSSCs abrogates cartilage degeneration following meniscus destabilization. Mice underwent destabilization of the medial meniscus (DMM) or sham surgery. 12 weeks later in a delayed fashion, all mice underwent cell injections as indicated, followed by sacrifice at 24 weeks. Histologic evaluation at 24 weeks demonstrated improved joint surface preservation by OARSI score.

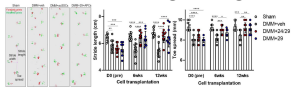

Fig 4. acSSC injection improves gait dysfunction in established OA. Mice underwent destabilization of the medial meniscus (DMM) surgery or sham. After 12 weeks, mice underwent gait analysis baseline (D0) followed by femoral acSSC injection. After an additional 6 or 12 weeks, gait analysis was again repeated to demonstrate superior recovery in the CD29+ and CD24+CD29+ cohorts.