Promoting Endochondral Bone Repair using Osteoarthritic Articular Chondrocytes

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Introduction: The principle behind regenerative medicine is to develop a functional replacement for damaged or diseased tissues in a clinically relevant manner. In the case of bone regeneration, current bone graft technologies focus on promoting repair through direct osteogenesis. Clinical limitations associated with these therapies include poor vascularity in the graft and limited osseointegration between the graft and the regenerate. In previous work, we have demonstrated that stimulating endochondral bone repair using cartilage grafts in place of bone grafts can effectively generate a vascularized and well-integrated bone regenerate in a murine segmental defect model. To translate this into a patient based therapy a major challenge is developing a cartilage tissue source capable of filling large defects. Articular chondrocytes are believed resistant to the process of hypertrophic maturation seen in the growth plate. However, in vitro culture and osteoarthritis are both known to promote aberrant gene and protein expression. The purpose of this study was to understand what types of cartilage are capable of promoting endochondral bone repair. We hypothesized that we could modulate the phenotype of articular chondrocytes in vitro to promote bone formation in vivo.

Methods: All in vivo work was approved by our IACUC. 2 mm segmental defects were made in the mid-diaphysis of externally stabilized murine tibiae and filled with cartilage grafts taken from endochondral cartilage, bovine articular cartilage (bAC), or human osteoarthritic cartilage (hOA). Healing was examined histologically 4 weeks post-operatively using safranin-O and trichrome staining. Additionally, chondrocytes were isolated from bAC or hOA using standard matrix digestion techniques, expanded in monolayer to P5, and pelleted into 3D culture systems. Pellets were exposed to defined serum-free basal medium with the addition of BMP4 or TGFβ + BMP4 for short-term exposure (1 week) or continuously throughout culture. These pellets were assayed after in vitro culture for evidence of hypertrophy and then transplanted in vivo into subcutaneous pockets of immunocompromised mice to look for evidence of bone formation using standard histology and μCT. Quantitative RT-PCR was performed using Taqman Assays.

Results: While endochondral cartilage tissue grafts could effectively repair bone (Fig 1B), tissue explants from bAC (Fig 1C) or hOA (Fig 1D-G) were unable to promote bone regeneration. Within the segmental defect both cartilage explants retained strong safranin-o staining, remained avascular, and did not integrate with repair tissue (Fig 1F-G). Articular cartilage has always been considered privileged from the process of endochondral ossification, and these results support this view. We hypothesized that by isolating, expanding, and culturing chondrocytes in vitro with BMP4 or TGFβ + BMP4 we could sufficiently alter their phenotype in a manner that would allow them to undergo endochondral ossification. In the bovine system this was most effectively accomplished by treating the pellets made from expanded bACs with TGFβ + BMP4 for 1 week followed by basal media for 3 weeks (Fig 2A). After 4 weeks in subcutaneous pockets the pellets became highly mineralized and show evidence of vascularized bone formation (Fig 2B-D). With the hOA tissue, extensive vascular invasion and the beginning of endochondral bone formation was observed after subcutaneous implantation in pellets treated continuously with TGFβ + BMP4 for 3 weeks (Fig 3D-L). Short-term exposure to growth factors produced small, non-mineralized fibrous pellets after subcutaneous implantation (Fig 3A-C), perhaps because these pellets have a less hypertrophic phenotype (Fig 3M, less ColX & MMP13), or because sustained growth factor treatment is required to produce metabolically active cells and short-term pellets had limited viability when implanted.

Discussion: Taken together, these data suggest that articular chondrocytes expanded in vitro can be phenotypically modulated to take on a more endochondral phenotype and subsequently promote vascular invasion and mineralization in vivo. The mechanism by which these pellets promote bone formation subcutaneously is not clear at this point, but interestingly they are very effective at promoting vascularization, which offers a clear therapeutic advantage to traditional bone grafts. The expanded cells behave significantly differently than the transplanted cartilage tissue from which they were derived. Specifically, cartilage explants transplanted into a segmental bone defect underwent little to no remodeling, did not vascularize, nor did they permit integration with the surrounding tissue: this data is in line other cartilage transplant procedures such as osteochondral allografts that demonstrate little to no healing response.

Significance: Using cartilage to repair bone through endochondral ossification is an underappreciated therapy that we believe offers significant advantages over traditional treatments that promote intramembranous ossification. We found that chondrocytes from osteoarthritic patients could be prepared in a way that promoted vascularization, mineralization and early bone formation. These results suggest that a commonly discarded surgical tissue could be transformed into a novel therapy.

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FIGURE 1: In Vivo Endochondral Bone Regeneration in Segmental Tibia Defect. 4 weeks healing: (A) Empty segmental defect model, trichrome stain. (B) Endochondral cartilage transplanted from a fracture callus, trichrome stain. (C) Bovine articular cartilage, safranin-o stain. (D-F) Human osteoarthritic cartilage. (D-F) safranin-O, (E,G) trichrome.
FIGURE 2: Bovine articular cartilage pellets undergo endochondral ossification in subcutaneous pockets. (A) Safranin-O staining of healthy hAC following 4 weeks of in vitro culture with short term TGFβ + BMP4 exposure. (B-D) Histology of pellets following in vitro culture plus 4 weeks subcutaneous implantation: (B) safranin-O, (C) Masson’s trichrome, (D) Von Kossa, (E) µCτ.

FIG 3: Human OA cartilage pellets (A-C) Pellets exposed to short term growth factor in vitro after subcutaneous implantation appeared small and fibrous by histology, scale bar = 200µm. (A) Safranin-O, (B) Trichrome stain, (C) Von Kossa. (D-L) Pellets exposed to continuous growth factor in vitro after subcutaneous implantation appeared promoted vascular invasion and mineralization, scale bar = 200µm (D,E) or 100 µm (G-L): (D,G-H) Safranin-O, (E, J-L) Trichrome stain, (F) µCτ. (M) RT-PCR after in vitro culture.

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