INCREASED PREVALENCE OF DISTAL-LESS HOMEOBOX 5 PROVIDES EVIDENCE FOR A ROLE OF CHONDROCYTE TRANSDIFFERENTIATION IN OSTEOARTHRITIS

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Introduction

Osteoarthritis (OA) is a debilitating joint disease comprising degradation of articular cartilage and underlying subchondral bone. Notwithstanding the prevalence of the condition, the etiology remains poorly understood. Focusing on specific gene mutations in familial OA[1], we noted an underlying osteogenic role in many mutated gene products[2-4], leading us to believe that transdifferentiation of chondrocytes to osteoblasts may be a driver of OA. In order to test this hypothesis, we assessed percentages of cells staining positive for distal-less homeobox 5 (DLX5), considered by many to be a master regulator of osteogenesis, in OA and control tissues.

Materials and Methods

Three groups of adult male C57BL6 mice between 15 and 16 weeks of age underwent surgical destabilization of the medial meniscus (DMM) and groups were allowed to to survive four, eight, and 12 weeks post-surgery. The contralateral knee was employed as a control for each group. These knees were stained using safranin-O and scored using modified Mankin and OARSI scoring to assess cartilage morphology; IHC-HP was carried out staining for DLX5 (Abcam AB109737 1:100) and cell counting was employed to quantify prevalence of DLX5. Tukey HSD for multiple comparisons was carried out using JMP (Version 17.0.0).

Results

12 weeks following surgery we observed statistically significant increases in modified Mankin and OARSI scores (Figure 1) between controls ($p = 0.04$ and $p = 0.016$); the four- and eight-week timepoints showed increases that were not statistically significant. Further, we found that statistically significant increases in percentages of cells staining positive for DLX5 (Figure 2) occurred at the four- week mark ($p = 2.0 \cdot 10^{-3}$). Surprisingly, we also found that a statistically significant increase in percentages of cells staining positive for DLX5 occurred between the four-week controls and the eight-week controls ($p < 1.0 \cdot 10^{-3}$. As biomarker changes tend to occur before cartilage degradation, we submit that the use of the contralateral knee as a control in the case of DMM may not be advisable.

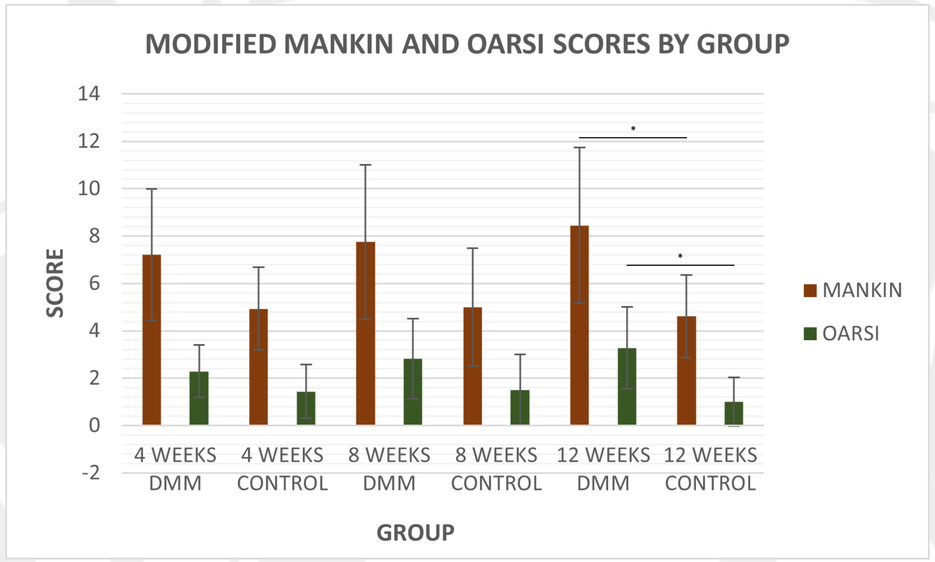

Figure 1—Modified Mankin and OARSI scores of OA and control tissues.

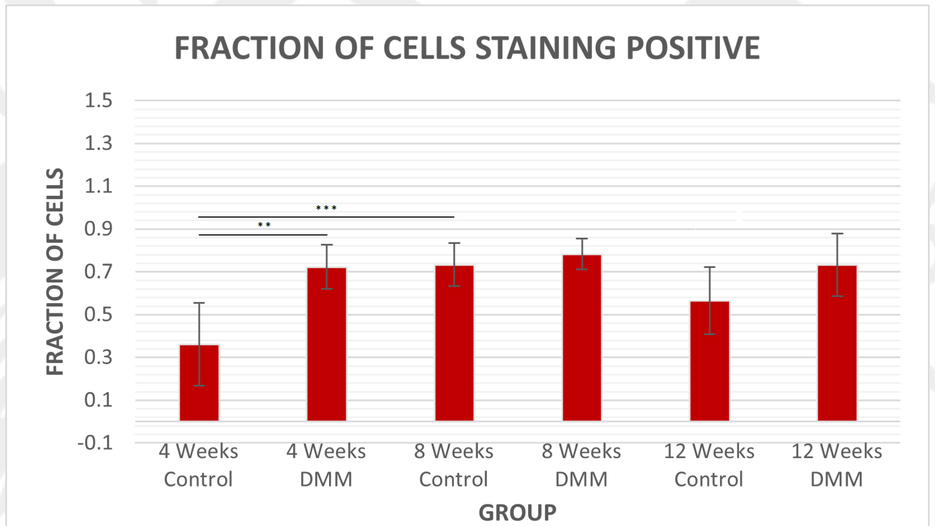

Figure 2—Fractions of cells staining positive for DLX5 in OA and control tissues. Quantities for each group were as follows 4 weeks control *n=6*, 4 weeks DMM *n*=7, 8 weeks control/DMMl *n*=8, 8 weeks control/DMM *n*=9

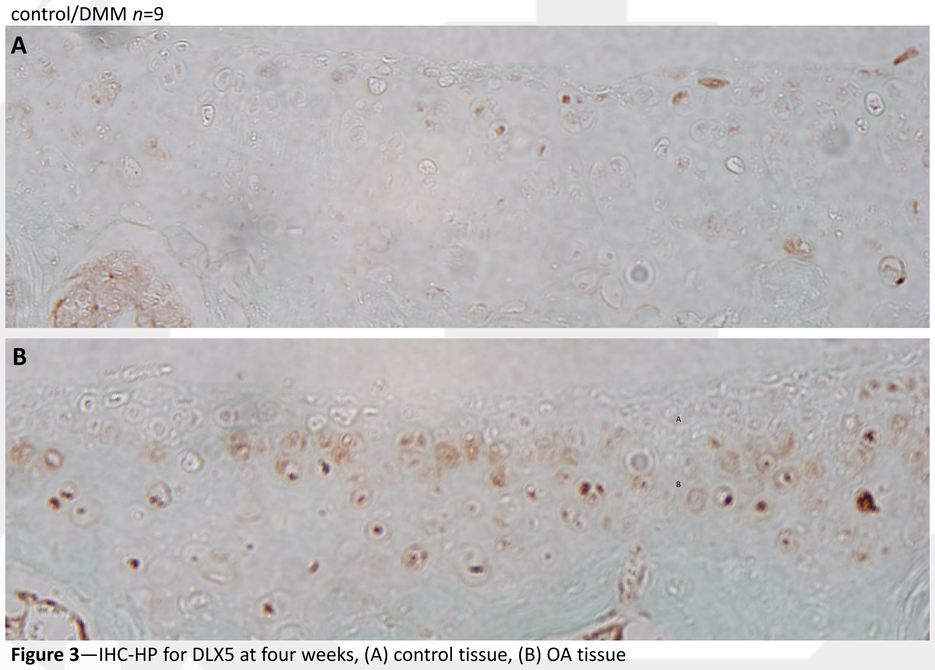

Figure 3—IHC-HP for DLX5 at four weeks, (A) control tissue, (B) OA tissue

Conclusion

We found that statistically significant increases in percentages of cells staining positive for DLX5 occurred as a result of DMM-induced OA. Given the role of DLX5 in osteogenesis, we submit this as preliminary evidence as a role for transdifferentiation in OA. We also found that as time passed increases in percentages of cells staining positive for DLX5 occurred in control knees, indicating that DMM produced systemic changes that impacted articular cartilage in the contralateral knee. Future research will utilize DLX5 knockout and overexpressor mice to further study this novel transdifferentiation angle and the role of DLX5 in OA pathogenesis.

Citations

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