The Role of the CD44 Receptor in Post-Traumatic and Age-Related Osteoarthritis

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INTRODUCTION: Osteoarthritis (OA) is a debilitating joint disease and instigator of chronic disability that affects approximately half of the global population over the age of 65, incurring a substantial socioeconomic burden. The prevalence of the disease is expected to continue to increase as global life expectancies rise. Our laboratory is interested in investigating osteoactivin, also known as glycoprotein nonmetastatic melanoma protein B (Gpnmb) as a potential therapeutic in the treatment of OA. Unpublished data from our laboratory have shown that mice that ubiquitously overexpress Gpnmb experience less severe cartilage and subchondral bone changes pathological of OA follow the induction of post-traumatic OA via destabilization of the medial meniscus (DMM) surgery. Additionally, we have shown that intra-articular injection of recombinant Gpnmb (rGpnmb) prevented continued damage follow DMM surgery when injected 6 weeks post-surgery. CD44 is a known anti-inflammatory receptor of Gpnmb and CD44 signaling has been well reported in joint homeostasis and OA biology. Immunoprecipitation data from our laboratory showed that Gpnmb binds to CD44 in chondrocytes. We have also shown that when human cartilage explants or chondrocyte cultures were pre-treated with rGpnmb prior to the induction of inflammation via interleukin-1 beta (IL-1β) treatment, extracellular matrix degradation and expression of pro-inflammatory markers were decreased in comparison to IL-1β treatment alone. Overall, we seek to investigate the role of CD44 in post-traumatic and age-related OA and if the anti-inflammatory effect of Gpnmb is CD44-dependent in chondrocytes.

METHODS: This study was approved by the IACUC. For the post-traumatic OA model, male CD44$^{-/-}$ animals and their control C57/BL6 animals (Jackson Laboratories, B6) were randomized to sham operated or DMM operated groups (N=10-11 per group). Animals underwent surgery at 10 weeks of age on the right knee. All animals were sacrificed at 20 weeks of age. For the age-related OA model, male CD44$^{-/-}$ and B6 animals were aged to approximately 52 weeks and sacrificed (N=9-11). For both studies, right knees were assessed via general histological survey with thionin staining, and micro-computed topography (μCT) analysis. μCT scans were analyzed using CTan software. Further joint analysis and female animal assessment are currently underway. To determine if the anti-inflammatory effect of Gpnmb is CD44 dependent, CD44$^{-/-}$ mouse primary chondrocytes were isolated and treated with IL-1β (10ng/ml) for 24 hours, with or without rGpnmb treatment (50ng/ml). Expression of matrix metalloproteinase-3 (MMP-3), MMP-9, MMP-13 and interleukin 6 (IL-6) were assessed via qPCR.

RESULTS: For the post-traumatic OA model, cartilage changes suggest damage following DMM surgery for both CD44$^{-/-}$ and B6 animals, however, further investigation is required to delineate responses between phenotypes. There was, however, a phenotype in the subchondral bone of the CD44$^{-/-}$ versus B6 animals. μCT analysis showed significant tibial subchondral bone sclerosis for CD44$^{-/-}$ animals subjected to DMM surgery in comparison to CD44$^{-/-}$ animals that underwent sham surgery (Fig. 1A), with increased bone volume/tissue volume (BV/TV), trabecular (tb.) number, and tb. thickness and decreased tb. spacing. These changes were not seen in B6 DMM operated animals compared to B6 sham operated animals. For the age-related OA model, cartilage assessment is currently underway, however significant differences were noted in the subchondral bone of aged CD44$^{-/-}$ and B6 animals. μCT analysis of the tibial subchondral bone showed subchondral bone sclerosis with significant increases to BV/TV and tb. thickness of CD44$^{-/-}$ aged animals compared to B6 aged animals (Fig. 1B). Results of the primary CD44$^{-/-}$ chondrocyte culture showed significant elevation of MMP-3 (200-fold increase), MMP-9 (14-fold increase), MMP-13 (50-fold increase) and IL-6 (600-fold increase) expression following IL-1β treatment alone. Interestingly, data showed that rGpnmb treatment did not inhibit expression of MMP-3, MMP-9, MMP-13 or IL-6 in CD44$^{-/-}$ primary chondrocytes following IL-1β treatment (Fig. 1C), unlike our previous data showing rGpnmb treatment did inhibit expression of MMPs and IL-6 in B6 primary chondrocytes.

DISCUSSION: Overall, our *in vivo* data suggest that CD44$^{-/-}$ animals experience more severe subchondral bone sclerosis in both post-traumatic and age-related models of OA. Additionally, our *in vitro* results show that Gpnmb works via the CD44 receptor in chondrocytes to inhibit inflammation. Further studies will seek to investigate the effect of Gpnmb injection in both post-traumatic and age-related OA models in CD44$^{-/-}$ animals, as our laboratory has shown that intra-articular injection of rGpnmb significantly reduced OA progression in a post-traumatic model of OA in B6 animals. These results provide mechanistic insight and support the role of Gpnmb and its therapeutic effects in post-traumatic and age-related OA via the CD44 receptor.

SIGNIFICANCE: Our preliminary results suggest that the CD44 receptor plays a major role as an ant-inflammatory receptor in the progression of post-traumatic and age-related OA and propose it as a potential mechanism through which Gpnmb provides therapeutic effects.

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IMAGES: Fig. 1: The Contribution of CD44 in OA. Right knees of B6 or CD44$^{-/-}$ animals were subjected to sham surgery or DMM surgery. μCT of tibial subchondral bone underwent bone volume/tissue volume (BV/TV), trabecular (tb.) number, tb. spacing, and tb. thickness analysis (A). Aged B6 or CD44$^{-/-}$ animals underwent μCT analysis of tibial subchondral bone for BV/TV, tb. number, tb. spacing and tb. thickness (B). qPCR analysis of primary chondrocytes isolated from CD44$^{-/-}$ animals treated with IL-1β showed that Gpnmb failed to inhibit inflammation as shown via MMP-3 (Fig. 1C-A), MMP-9 (Fig. 1C-B), MMP-13 (Fig. 1C-C), and IL-6 (Fig. 1C-D). Experiments were run in duplicates. N=9-11. Data presented represent Mean + SEM. NS= nonsignificant. *p≤0.05 **p≤0.01 ***p≤0.001 ****p≤0.0001