The Therapeutic and Genetic Benefit of Gpnmb in Osteoarthritis

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INTRODUCTION: Osteoactivin, also known as glycoprotein nonmetastatic melanoma protein B (Gpnmb), is a transmembrane glycoprotein with well document skeletal function and known anti-inflammatory properties. Gpnmb's role in osteoarthritis (OA) and its effect on the cartilage and the subchondral bone remains undefined. Our laboratory has shown in the context of OA that Gpnmb expression is upregulated in damaged versus undamaged human knee cartilage. Additionally, *in vitro,* when human cartilage explants or chondrocyte cultures were pre-treated with recombinant Gpnmb (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 vivo,* our laboratory has shown when mice with an inactivating point mutation in the Gpnmb gene (DBA/2J mice) were subjected to either surgically induced post-traumatic (PT) OA, or age-related OA, DBA/2J mice had more severe cartilage degradation when compared to control mice. Our findings support the hypothesis that Gpnmb is playing a chondroprotective role *in vitro* and *in vivo.* Given these findings, we seek to investigate the potential genetic and therapeutic benefit of Gpnmb in OA utilizing a mouse model of PT OA. To do so, we employed two approaches, first assessing the effects of the induction of PT OA via destabilization of the medial meniscus (DMM) surgery on animals that ubiquitously overexpress Gpnmb ($Gpnmb^{TG}$) and second assessing the effect of intra-articular recombinant Gpnmb (rGpnmb) and Gpnmb related-peptide (pGpnmb) injection following the induction of PT OA via DMM. Previous studies have shown that $Gpnmb^{TG}$ animals overexpress Gpnmb 3-fold in bone. These studies will investigate Gpnmb as a potential future therapeutic in the treatment of OA.

METHODS: This study was approved by the IACUC. Genetic Study: Male $Gpnmb^{TG}$ and their control C57/BL6 animals (Taconic Biosciences, B6) were randomized to sham operated or DMM operated groups (N=8-10 per group). Animals underwent surgery at 10 weeks of age on the right knee. All animals were sacrificed at 20 weeks of age. Therapeutic Study: C57BL/6 male mice (Jackson Laboratories) underwent surgery of the right knee. Treatment groups included sham surgery, or DMM surgery with PBS injection, rGpnmb injection (120mM, Thermo Fisher Scientific) or pGpnmb injection (120mM, Anaspec) (N=8-10 per group). pGpnmb is a synthetic 18-amino acid peptide comprised of a portion of the C-terminus domain of Gpnmb. DMM or sham surgery occurred at 10 weeks of age, and injections were performed at 6 weeks post-DMM surgery. Animals were sacrificed at 14 weeks post-DMM surgery. For both studies, right knees were assessed via general histological survey with Safranin-O and fast green or thionin staining, micro-computed topography (μCT) analysis or histological BV/TV analysis, and tartrate resistant acid phosphatase (TRAP) staining for osteoclast (OC) assessment. μCT scans were analyzed using CTan software and histomorphometric analyses were performed using Osteomeasure software. Female animal assessment is currently underway.

RESULTS: Genetic Study: Following the induction of PT OA in $Gpnmb^{TG}$ and B6 mice, cartilage changes suggest damage following DMM surgery for both groups, however, $Gpnmb^{TG}$ animals experienced decreased cartilage damaged as assessed via OARSI scoring and fewer osteophytes (Fig. 1A). μCT analysis showed tibial subchondral bone sclerosis for B6 animals following DMM surgery compared to B6 animals following sham surgery, with increased bone volume/trabecular volume (BV/TV), decreased trabecular (tb.) spacing and increased tb. thickness (Fig. 1B). Interesting when comparing $Gpnmb^{TG}$ DMM operated versus sham operated animals, these subchondral bone changes did not occur. Subchondral OC number and OC surface/bone surface, as assessed via TRAP staining, showed an increase in B6 DMM operated animals in comparison to B6 sham operated animals, while $Gpnmb^{TG}$ sham and DMM operated animals showed no difference in OC number or OC surface/bone surface. Therapeutic Study: Intra-articular injection of rGpnmb or pGpnmb led to significantly less severe OA compared to those treated with PBS as shown via decreased OARSI and osteophyte scores (Fig. 1C). Animals treated with rGpnmb or pGpnmb had increased minimum cartilage thickness, and increased chondrocyte number compared to PBS treated animals. Histological BV/TV assessment showed increased subchondral BV/TV in PBS, rGpnmb and pGpnmb treated animals compared to sham treated animals, as Gpnmb is a known negative regulatory of osteoclastogenesis. Trabecular histological BV/TV analysis showed increased BV/TV in animals treated with pGpnmb in comparison to sham operated animals. Subchondral OC number and OC surface/bone surface trended upward with PBS treatment and did not for rGpnmb or pGpnmb treated animals.

DISCUSSION: Overall, our data suggest that Gpnmb overexpression leads to less severe OA progression follow the induction of PT OA via DMM surgery. $Gpnmb^{TG}$ animals experienced less severe cartilage degradation, fewer osteophytes and no subchondral bone sclerosis, with no changes to OC number, or OC surface/bone surface following DMM surgery. Future studies will seek to investigate the role of Gpnmb in OA via overexpression or knock down in a cell specific manner, as Gpnmb is expressed in various cells within the joint. Therapeutically, our results suggest that rGpnmb or pGpnmb prevent continued cartilage damage following joint injury and even thickened trabecular bone following pGpnmb treatment. This shows rGpnmb or pGpnmb's potential therapeutic use in the treatment of PT OA. To ensure continued bioavailability of rGpnmb and pGpnmb, thermoresponsive hydrogel preparation is being investigated to ensure sustained release following intra-articular injection. Future studies are underway to assess the potential therapeutic use of rGpnmb and pGpnmb at the time of injury. Further analysis will be performed to assess safety of this therapeutic. With no disease modifying treatment options for OA available, the chondroprotective effects of rGpnmb or pGpnmb shows positive potential for their use as therapeutics.

SIGNIFICANCE: Our preliminary results suggest a beneficial effect of Gpnmb overexpression and Gpnmb intra-articular injection to the cartilage and subchondral bone in OA. Our results indicate the potential use of Gpnmb in the treatment of OA and propose it as an alternative to current therapies.

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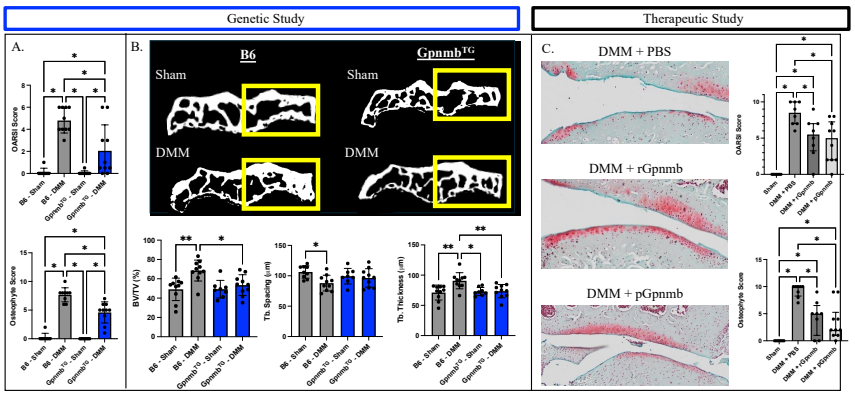

IMAGES: Fig. 1: The Contribution of Gpnmb in OA. Genetic Study: Right knee joints of B6 or $Gpnmb^{TG}$ animals subjected to sham surgery or DMM surgery underwent OARSI scoring, and osteophyte scoring of the tibia and femur (A). μCT of tibial subchondral bone underwent bone volume/tissue volume (BV/TV), trabecular (tb.) spacing, and tb. thickness analysis (B). Therapeutic Study: Right knee joints of animal that underwent sham surgery or DMM surgery with PBS, rGpnmb or pGpnmb treatment were subjected to Safranin-O and fast green staining (C). OARSI and osteophyte scoring of tibia and femur were performed (C). *discovery for ordinal analysis. * p≤0.05 **p≤0.01. N=8-10.