

Human BMP4 mRNA Encapsulated in Lipid Nanoparticle Improved Age-related Osteoarthritis After Intra-articular Injection

Xueqin Gao^{1*}, Zuokui Xiao¹, Keisuke Nakayama¹, Matthieu Huard¹, Shintaro Mukohara¹, Aryn Cummins¹, Britney Force¹, Linda H Li², Chiara Mancino², John P Cooke², Francesca Tarabali², Marc J Philippon, Johnny Huard^{1*}

¹ Linda and Mitch Hart Center for Regenerative and Personalized Medicine, Steadman Philippon Research Institute, Vail, CO, 81657;

²Center for Musculoskeletal Regeneration, Houston Methodist Academic Institute; ³The Steadman Clinic, Vail Colorado USA. *Corresponding authors.

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Introduction. Age-related osteoarthritis (OA) affects tens of millions of patients which often cause disability and currently no disease modification treatment. At the end stage of OA, patients often need joint especially knee replacement. Hence, developing of novel therapy to treat osteoarthritis is in critical need. Previously, we have shown retro-human bone morphogenetic protein 4 (BMP4) transduced with muscle derived stem cells improved cartilage repair in a rat model of MIA induced OA¹. BMP4 promoted human MSCs chondrogenic differentiation dramatically and cocervate delivered various BMPs including BMP4 promote cartilage repair in an osteochondral defect model². BMP4 also reverse aged muscle derived stem cells proliferation decline and bone regeneration³. mRNA encapsuled in lipid nanoparticle mediated target gene delivery has become feasible since the successful use of COVID19 mRNA vaccine to control pandemic and save life. BMP2 mRNA has been shown to regenerate critical size long bone defect equivalent to clinical dose of BMP2 protein via endochondral bone formation⁴. More recent study demonstrated BMAC clot in conjugated with TGFβ1 mRNA promoted osteochondral defect healing via decreased fibrosis⁵. But no study has investigated human BMP4 mRNA for age-related osteoarthritis which prompted the current investigation.

Methods. 1. Human BMP4 (hBMP4) mRNA preparation and encapsulation. Human BMP4 clone was purchased from OriGENE and subcloned for in vitro transcription and subsequently encapsuled in lipid nanoparticle according to previous protocol⁶ and quality verified. **2. In vivo study.** This animal study was approved by IACUC of Colorado State University (Protocol#4839). C57BL6J mice at 19-month-old including both males and females were divided into 3 groups: Group 1 (N=8), intra-articular injection of mRNA freezing medium (CTL); group 2 (N=7), intra-articular injection of 2.5μg lipid nanoparticle encapsuled hBMP4 mRNA; group 3 (N=8), intra-articular injection of 5μg lipid nanoparticle encapsuled hBMP4 mRNA. Intra-articular injections were performed in the left knee at baseline in 15.8μl volume, then every three weeks for total of 3 times and mice were sacrificed 2 weeks after last injections and the knee was harvested and fixed in formalin. **3. Pain measurement:** Pain threshold was measured using Von Frey device at baseline and then 4 and 8 weeks after injection. **4. Micro-CT scan and analysis:** The entire knee was scanned with Vivo-CT 80 using 15 μm voxel size. The micro-CT was analyzed using built-in software and 3D images of the entire knee were reconstructed to reveal heterotopic bone (HO) formation and general morphology of knee. **5. Histology:** After micro-CT scan, the tissues were decalcified with 10% EDTA plus 1% NaOH for 4 weeks, and paraffin embedded and sectioned in 5μm thickness. Alcian blue and Safranin O staining were performed to reveal the cartilage matrix. Herovici's staining and H&E staining were also performed and cartilage repair was evaluated using OARSI histology score⁷. Immunohistochemistry (IHC) staining of type 2 collagen (COL2) was performed using Chondrex primary antibody (#7048) following same protocol as previously described². **6. Serum cartilage damage marker hyaluronic acid (HA)** was measured using ELISA. **7. Statistical analysis** was performed using Graphpad Prism 10 using ANOVA followed by Tukey's multiple comparison. P<0.05 was deemed statistically significant.

Results. 1. HBMP4 mRNA final concentration was 316 μg/ml after encapsulation. 2. Micro-CT results: Micro-CT 3D images revealed mild HO formation (red arrows) in each group. HBMP4 mRNA at 2.5 and 5μg doses did not increase rate of HO formation (Fig.1A). **3. Pain threshold** was not different between groups at baseline but increased from 4 weeks to 8 weeks in 2.5 and 5μg hBMP4 mRNA groups (Fig.1B). **4. Serum HA** was not different between groups (Fig.1C). **5. Histology results:** Safranin O staining showed best and worst cartilage OA repair in all groups. CTL group has 3 severe cartilage damage and worst repair has no cartilage. Both 2.5 and 5μg hBMP4 mRNA has only 1 mouse has severe damage (Fig.2A-C). Alcian blue stained cartilage matrix in blue and revealed complete loss in the worst repair of CTL group (Fig.2D-F). OARSI histology score of lateral femur condyle was significantly lower (better repair) in the 2 doses of hBMP4 mRNA groups than CTL group, but no difference between these two groups (Fig.2G). OARSI histology score of medial tibia plateau cartilage was also significantly lower in two hBMP4 mRNA dose groups compared to CTL group with no difference between two groups (Fig.2H).

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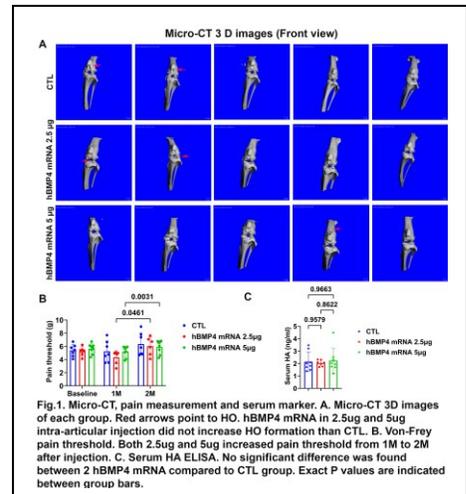


Fig.1. Micro-CT, pain measurement and serum marker. A. Micro-CT 3D images of each group. Red arrows point to HO. hBMP4 mRNA in 2.5μg and 5μg intra-articular injection did not increase HO formation than CTL. B. Von-Frey pain threshold. Both 2.5μg and 5μg increased pain threshold from 1M to 2M after injection. C. Serum HA ELISA. No significant difference was found between 2 hBMP4 mRNA compared to CTL group. Exact P values are indicated between group bars.

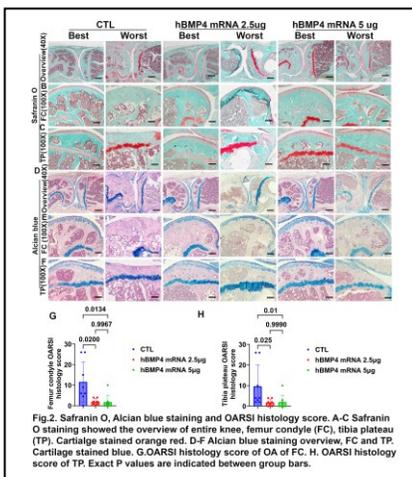


Fig.2. Safranin O, Alcian blue staining and OARSI histology score. A-C Safranin O staining showed the overview of entire knee, femur condyle (FC), tibia plateau (TP). Cartilage stained orange red. D-F Alcian blue staining overview, FC and TP. Cartilage stained blue. G. OARSI histology score of OA of FC. H. OARSI histology score of TP. Exact P values are indicated between group bars.

(Fig.3A-C). Herovici's staining revealed mixed blue and red in best cartilage repair of each group and only pink COL1 was present in the worst repair of CTL group while two hBMP4 mRNA groups showed partial cartilage loss (Fig.3D-E). H&E staining revealed typical morphology in best cartilage repair and only bone in the worst repair of CTL group and partial cartilage cells loss in worst repair of the two hBMP4 mRNA groups (Fig.3G-H).

Discussion. This study revealed hBMP4 mRNA intra-articular injection in aged mice's knee improved cartilage repair by increasing OARSI histology score of both femur condyle and tibia plateau articular cartilage. The hBMP4 mRNA did not increase chance of HO formation compared to CTL injection group. hBMP4 mRNA also improved pain after injection at 8 weeks compared to 4 weeks. **In conclusion, hBMP4 mRNA is beneficial to age-related OA. Significance/clinical relevance.** Human BMP4 mRNA delivered with lipid nanoparticle may represent a novel therapy for treatment of age-related OA.

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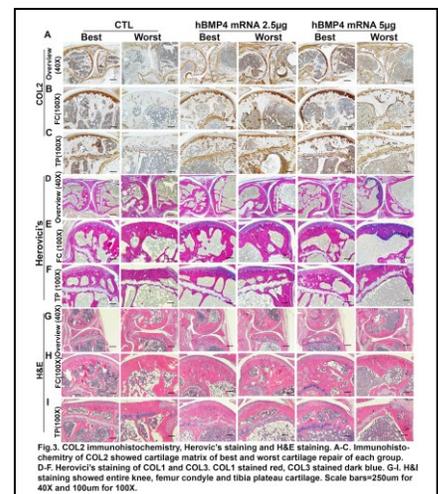


Fig.3. COL2 immunohistochemistry, Herovici's staining and H&E staining. A-C. Immunohistochemistry of COL2 showed cartilage matrix of best and worst cartilage repair of each group. D-F. Herovici's staining of COL1 and COL3. COL1 stained red, COL3 stained dark blue. G-H. H&E staining showed entire knee, femur condyle and tibia plateau cartilage. Scale bars=200μm for 40x and 100μm for 100x.