A Rare Mutation in the TNFRSF11B Gene Suggests a Novel Role for Osteoprotegerin in Chondrogenesis

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INTRODUCTION: Early onset (EO) osteoarthritis (OA) presents as a polyarticular disease in patients <50 years of age, resulting in increased musculoskeletal burden for subjects at an early age. One such case was discovered by Ramos et al[1]. in a Dutch family where a single nucleotide polymorphism in the TNFRSF11B gene (c.1205A=>T; p.Stop402Leu) adds 19 amino acids to the C-terminus of osteoprotegerin (OPG). The resulting condition is familial generalized OA with, in most patients, chondrocalcinosis. In screening for an OA gene therapy trial[2] we encountered a subject with EO-OA who had undergone multiple joint replacement surgeries before the age of 45. Upon performing whole genome sequencing, we found this subject to be heterozygous for this TNFRSF11B mutation but surprisingly, they did not present with signs of chondrocalcinosis[3], as reported in the literature[1, 4]. Although a mutation in OPG suggests disturbances in bone metabolism, we formulated an alternative hypothesis based upon defective chondrogenesis.

METHODS: This study was approved by the IRB (#22-007616). Mesenchymal stromal cells (MSCs) were isolated from surgical tissue waste of the subject (45yo F). Bone marrow derived MSCs (n=2F & n=1M donors; Lonza) were used as wild-type (wt) controls. PCR genotyping to confirm the TNFRSF11B mutation was performed on gDNA from culture expanded cells using custom-made probes. MSCs were expanded under standard conditions, followed by osteogenic and chondrogenic differentiation assays for 3 weeks. Mineralization was evaluated via Alizarin red staining. Chondrogenic differentiation was evaluated on pellets via colorimetric GAG assay, histology and compression testing (Cellscale Microtester G2). OPG production was measured in the conditioned medium of chondrogenic pellets using a wild-type OPG ELISA kit (R&D Systems). A custom-made lentivirus (LV.mtOPG: VectorBuilder) encoding the mutated (mt) OPG sequence and mCherry driven by the CBh promoter was used to overexpress mtOPG in wtMSCs during chondrogenic pellet differentiation. A lentivirus encoding eGFP (LV.eGFP) was used as a transduction control. Recombinant (r) OPG was added to the culture medium of chondrogenic pellets (0-200 ng/mL) as a control for mtOPG overexpression. All experiments were performed in triplicate wells. Statistical analysis was performed in GraphPad Prism using one-way ANOVA with Tukey post-hoc comparison; the elastic modulus values were log10-transformed before analysis.

RESULTS: MSC genotyping confirmed the presence of the mutation in the mtMSCs and its absence in wtMSCs (Fig. 1A). While osteogenic differentiation of mtMSCs was confirmed by strong alizarin red staining (Fig. 1B), chondrogenic differentiation failed, as evidenced by small pellets with poor GAG staining in histology (Fig. 1C) and confirmed by the colorimetric assay (Fig. 1D). OPG concentration in the conditioned medium of mtMSCs pellets was significantly higher and remained elevated, while those of wtMSCs decreased after 1 week in culture (Fig. 1E). To study the effect of mtOPG in wtMSCs, these were transduced at 10 TU/cell before chondrogenic differentiation with either LV.mtOPG or LV.eGFP, resulting in strong transgene expression, as evidenced by in vitro fluorescence (Fig. 2A). Pellets formed by wtMSCs transduced with LV.mtOPG resembled those of mtMSCs in pellet size, low GAG production (Fig. 2B-C), and presented with a lower degree of stiffness in the compression testing, whereas pellets formed by wtMSCs transduced with LV.eGFP had stiffnesses comparable to those formed by wtMSCs (Fig. 2D). wtMSCs pellets exposed to high levels of rOPG resulted in chondrogenic pellets similar in size and GAG staining (Fig. 2E), as well as GAG concentration (Fig. 2F).

DISCUSSION: This is the first study to analyze chondrogenic differentiation of MSCs derived from a patient with the 1205A=>T mutation in the TNFRSF11B gene. The few prior studies that have been performed on this mutation have focused on the presence of crystal deposits[5], which the subject for our study lacks. The results of this study represent OPG as a novel modulator of the formation of cartilage, whose study has the potential to open new therapeutic avenues for EO-OA. While promising, further research in chondrocytes will assess the role of OPG in cartilage formation and determine its potential relevance to the OA population at large.

SIGNIFICANCE/CLINICAL RELEVANCE: OA and EO-OA are debilitating conditions whose etiopathophysiology is largely unknown. The finding of OPG as a potential mediator in the pathophysiology of OA provides a novel target for future studies.

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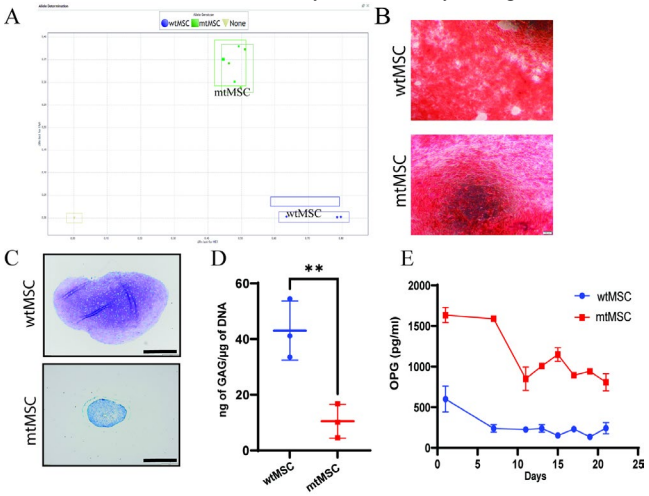

Figure 1. Characterization of mt- and wt-MSCs differentiation potential. (A) Genotyping for the TNFRSF11B mutation. **(B)** Alizarin red staining in monolayer culture. **(C)** Toluidine blue staining of pellets. Scale bar =500µm. **(D)** GAG concentration of pellets. **(E)** OPG concentration in conditioned medium of pellets.

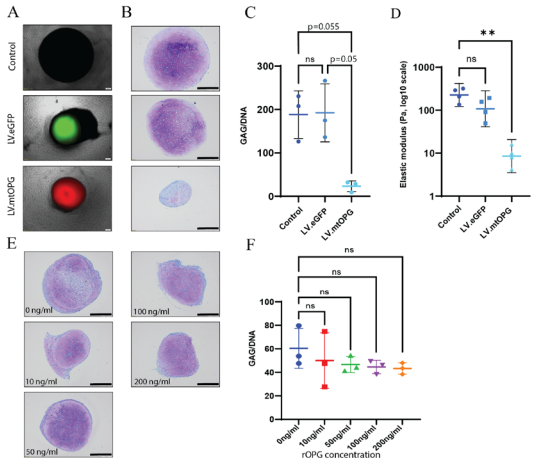

Figure 2. mtOPG overexpression is deleterious to chondrogenic pellet formation of wtMSCs. (A) In vitro fluorescence of transduced wtMSCs pellets. **(B)** Toluidine blue staining of transduced pellets. Scale bar=500µm. **(C)** GAG concentration of transduced pellets. **(D)** Compression testing of pellets. **(E)** Toluidine blue staining of rOPG treated pellets. **(F)** GAG concentration of rOPG treated pellets.