Contribution of metabolic disease to bone fragility in MAGP1-deficient mice

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Abstract

Microfibril-associated glycoprotein-1 (MAGP1) is an extracellular matrix protein that interacts with fibrillin and is involved in regulating the bioavailability of signaling molecules such as TGFβ. Mice with germline MAGP1 deficiency (*Mfap2*$^{-/-}$) develop increased adiposity, hyperglycemia, insulin resistance, bone marrow adipose tissue expansion, reduced cancellous bone mass, cortical bone thinning and bone fragility. The goal of this study was to assess whether the *Mfap2*$^{-/-}$ bone phenotypes were due to loss of MAGP1 locally or secondary to a change in whole body physiology (metabolic dysfunction). To do this, mice with conditional deletion of MAGP1 in the limb skeleton were generated by crossing MAGP1-flox mice (*Mfap2*$^{lox/lox}$) with Prx1-Cre mice. *Mfap2*$^{Prx-/-}$ mice did not show any changes in peripheral adiposity, hyperglycemia or insulin sensitivity, but did have increased bone length and cancellous bone loss that was comparable to the germline *Mfap2*$^{-/-}$ knockout. Unlike the germline knockout, marrow adiposity, cortical bone thickness and bone strength in *Mfap2*$^{Prx-/-}$ mice were normal. These findings implicate systemic metabolic dysfunction in the development of bone fragility in germline *Mfap2*$^{-/-}$ mice. An unexpected finding of this study was the detection of MAGP1 protein in the *Mfap2*$^{Prx-/-}$ hematopoietic bone marrow, despite the absence of MAGP1 protein in osseous bone matrix and absent *Mfap2* transcript expression at both sites. This suggests MAGP1 from a secondary site may accumulate in the bone marrow, but not be incorporated into the bone matrix, during times of regional MAGP1 depletion.

Introduction

Microfibrils are a component of the ECM, created by the polymerization of fibrillin proteins, that not only provide structural support to tissues, but also are involved in growth factor signaling and interactions with cell-surface receptors (reviewed in [1,2]). Microfibril-associated glycoprotein-1 (MAGP1) is a protein that interacts with microfibrils to influence the bioavailability of signaling molecules (reviewed in [3]). MAGP1 is expressed in most tissues over time, but expression is highest during the neonatal period in interstitial and mesenchymal cells [4]. The C-terminal domain of MAGP1 facilitates binding and incorporation into the matrix, while the N-terminal domain binds and sequesters signaling molecules such as transforming growth factor beta (TGFβ) and bone morphogenetic proteins (BMPs) [5]. The ability of MAGP1 to bind both fibrillin-1 and TGFβ contributes to its role in regulating the signaling level of TGFβ [6–8].

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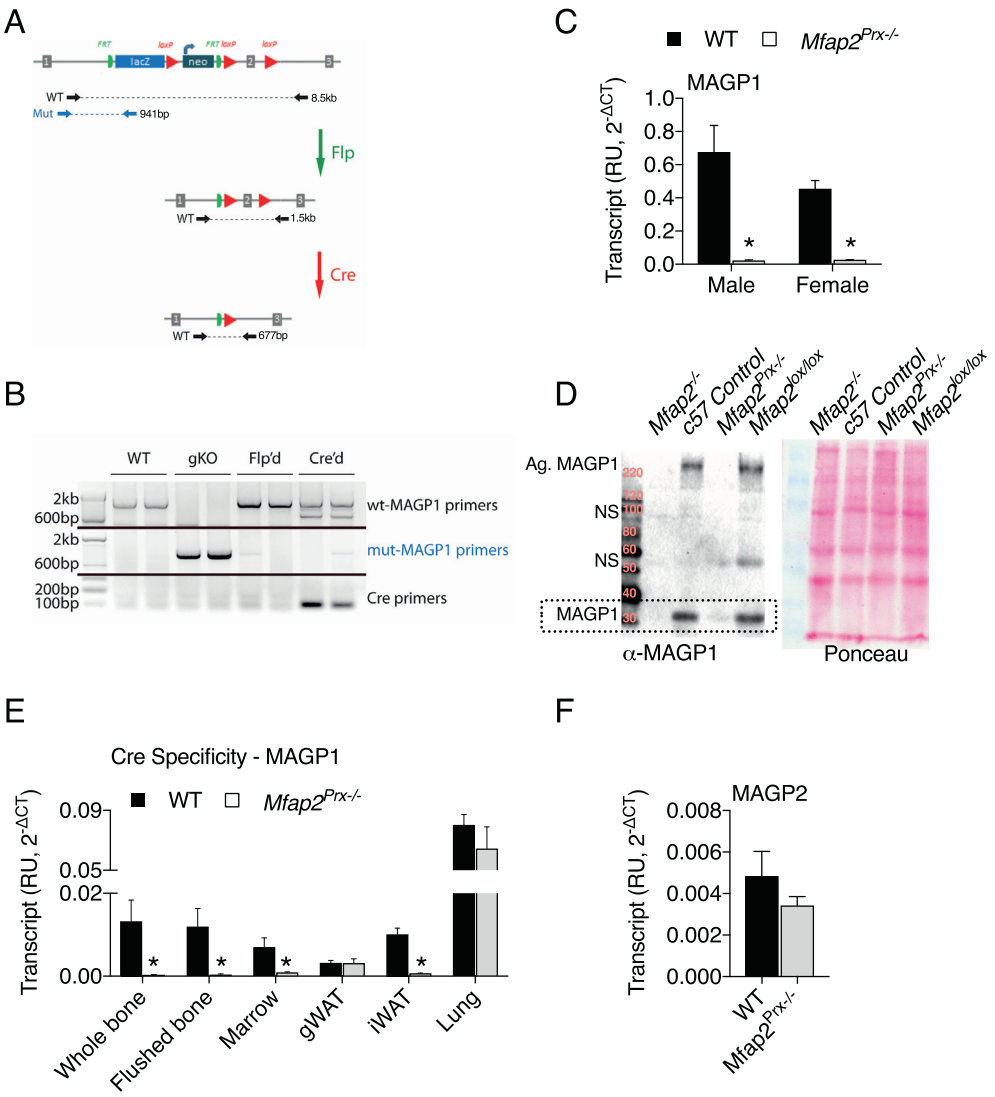

Fig. 1. *Mfap2*$^{lox/lox}$ construct design, genotyping and MAGP1 expression. 1A) Breeding strategy and schematic of flox'd *Mfap2* construct, showing Frt (green) flanking the neo cassette, and loxP sites (red) flanking exon 2 of *Mfap2*. The schematic also shows the construct following flipase (Flp)-mediated removal of the neo cassette (restoring MAGP1 expression), then the construct following Cre-mediated excision of exon 2 for conditional deletion of *Mfap2*. Genotyping required the use of three primer sets. "WT" primers are in the introns of the Mfap2 gene flanking exon 2 (outside the construct). "Mut" primers utilized a sequence found only in the *Mfap2*$^{lox/lox}$ cassette. 'Cre' primers are necessary for detection of the Prx1-Cre transgene. The location of the 'WT' and 'Mut' primers used for genotyping are indicated by black and blue arrows respectively. 1B) Genotyping strategy demonstrated on whole tibia samples. 1C) Prx1-Cre efficiency in male and female bones. RT-qPCR on RNA from whole femurs, using TaqMan primer-probes demonstrates efficient deletion of Mfap2 transcript by one copy of the Prx1-Cre transgene (N = 6). 1D). Western immunoblot of whole bone protein lysates to show loss of MAGP1 protein in the presence of the Prx1-Cre. Whole blot is shown for visualization of MAGP1 monomers (~35 kDa, boxed), non-specific bands (NS, ~55 kDa & 80 kDa), and aggregated MAGP1 (Ag. MAGP1, >220 kDa). Protein marker (kDa) is also shown. Ponceau stain was used prior to antibody incubation to confirm equal protein loading. 1E) MAGP1 transcript expression was measured using TaqMan primer-probe sets and TaqMan universal master mix as in 1C, across several tissue types demonstrating the specificity of the Prx1-Cre (N = 4). 1F) MAGP2 (*Mfap5*) expression in whole tibia was similarly assessed using TaqMan primer-probe sets (N = 4). Control (WT) mice are *Mfap2*$^{lox/lox}$ littermates. iWAT is inguinal white adipose tissue. gWAT is gonadal white adipose tissue. Student's *t*-test was used to make single comparison between control and Cre-positive samples, * = $p < 0.05$. Data shown as mean +/– SEM. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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