Mechanisms of bone and phosphorus metabolism regulation by cross-talk between ageing control factors SIRT6 and PAI-1

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INTRODUCTION: The mechanistic regulation of bone mass in aged animals is poorly understood. The expression of both FGF23 (a key factor in phosphorus metabolism) and sclerostin increases with aging. This may be one of the causes of age-related bone loss. However, we do not know much about either of these molecular mechanisms. We further found that defects in the longevity-associated factor SIRT6 induced an increase in the expression of the senescence-inducing factor PAI-1. In this study, we investigated the mechanisms of regulation of SOST and FGF23 by the longevity-associated factor SIRT6 and the senescence-inducing factor PAI-1 using mice lacking Sirt6 in Dmp-1-expressing cells (cKO mice) and the MLO-Y4 osteocyte-like cell line.

METHODS: By crossbreeding, we obtained osteoblast-specific SIRT6-deficient mice, i.e. Dmp1Cre::Sirt6f/f mice (cKO). The cKO and PAI-1-deficient mice (PAI-1KO) were intercrossed to obtain cKO::Pai-1-/- mice (dKO). PAI-1KO or WT mice were euthanized at 6 or 18 months of age and subjected to bone histological analysis. Micro-CT analysis was performed on the femur and lumbar spine. cKO mice were euthanized at 5 months of age and cortical bone was collected for qPCR and Histological and histomorphometric analysis. Micro-CT analysis was performed on the femur and lumbar spine. The mouse osteocyte-like MLO-Y4 cell line and gene knockout with the CRISPR/Cas9 system, senescence induction, Sirt6 overexpression assay and Chromatin immunoprecipitation assay were cultured. Human bone samples were harvested under informed consent from the femoral neck of patients with hip osteoarthritis or femoral hip fracture scheduled to undergo total hip arthroplasty or surgical fixation and were analyzed by qPCR.

RESULTS: cKO mice grew as well as the target group, and bone mass was reduced in both the lumbar spine and femur. Bone morphometry of the lumbar spine showed increased bone resorption and decreased bone formation because of increased osteoclasts. Cortical bone qPCR results showed increased expression of Sost, Fgf23, and Pai-1 in cKO, with a concomitant increase in senescence markers. In contrast, in dKO, the expression of these markers decreased to the same level as in the control group. Immunostaining results showed that the expression of sclerostin, FGF23, and PAI-1 in osteocytes was increased in cKO. Serum phosphorus concentration of cKO was significantly reduced compared to controls with increased FGF23, whereas phosphorus levels of dKO were comparable to controls. In osteocyte-like MLO-Y4 cells, SIRT6 knockdown or induction of senescence increased Pai-1, Sost, and Fgf23 expression. In contrast, when SIRT6 was overexpressed in MLO-Y4 cells, the expression of Fgf23 and Sost was suppressed. The Fgf23 promoter region contains a HIF-1α binding domain, and the ChIP assay showed that HIF-1α binding was promoted in both SIRT6 knockdown and senescence induction. Fgf23 expression was increased by HIF-1α activation. Conversely, increased Fgf23 expression by SIRT6 knockdown was blocked by HIF-1α inactivation. Furthermore, aged PAI-1-deficient mice were analyzed to determine the function of PAI-1 in aged bone. PAI-1-deficient mice at 2 years of age had greater bone mass and increased serum phosphorus levels compared to controls. Expression of Sost and Fgf23 in bone cortex was increased in PAI-1-deficient mice. Finally, to analyze the relationship between the expression of FGF23, SOST, and PAI-1 in human bone and age, q-PCR analysis was performed using bone samples from the femoral neck bone taken during hip arthroplasty. The results showed that the expression levels of FGF23, SOST, and PAI-1 were significantly correlated with age of donors.

DISCUSSION: The regulatory mechanisms of bone mass in aged animals are poorly understood. We aimed to investigate the role of SIRT6, a longevity-associated factor, in osteocytes. We found that Sost, Fgf23, expression was increased in SIRT6-deficient osteocytes, leading to a decrease in osteoblasts and an increase in osteoclasts, resulting in decreased bone mass. In addition, PAI-1, whose expression was promoted by SIRT6 deficiency, was actively involved in bone metabolism, indicating that PAI-1 deficiency suppresses age-related bone loss. Thus, we showed that induction of senescence by SIRT6 deletion was one of the causes of age-related changes in bone metabolism. Based on the results of this study, we proposed two signaling pathways downstream of SIRT6, a senescence-dependent and a senescence-independent pathway involved in the regulation of SOST and FGF23. SIRT6 directly binds HIF-1α and inhibits transcription of its target gene. SIRT6 inactivation results in HIF-1α binding to the Fgf23 consensus motif within its enhancer sequence in MLO-Y4 cells. SIRT6 inactivation enhances the expression of PAI-1, which induces cell senescence. Induction of senescence in MLO-Y4 cells also increases the binding of HIF-1α to the Fgf23 enhancer sequence. Sost expression also increases as a result of SIRT6 deletion through the senescence-dependent and -independent pathways.

SIGNIFICANCE/CLINICAL RELEVANCE: These results support the potential therapeutic application of a SIRT6 agonist, such as NMN or a PAI-1 inhibitor, against aging-related disruptions of bone metabolism.

IMAGES AND TABLES:

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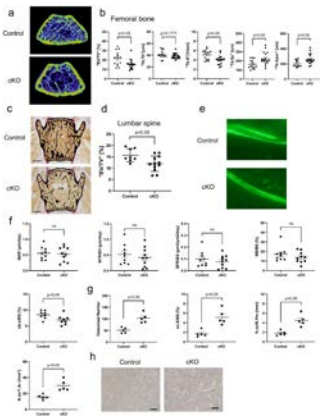

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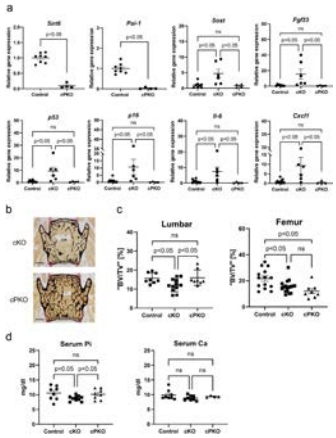

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