Novel role of TGFβ in osteocytes: Regulation of perilacunar remodeling and bone quality

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INTRODUCTION: Half of fractures occur in people with normal bone mass, highlighting the critical importance of bone quality. However, many questions remain about the cellular and molecular control of bone quality. As an essential regulator of bone homeostasis, transforming growth factor-beta (TGFβ) regulates both bone mass and bone quality. Though TGFβ acts on osteoblasts and osteoclasts to control bone mass, the cellular target of TGFβ in the control of bone quality is unknown. This gap in knowledge persists, partly due to our lack of understanding of the function of TGFβ in osteocytes, the most abundant bone cells and master regulators of bone remodeling. Osteocytes are also capable of remodeling the matrix surrounding their intricate lacuno-canalicular network (LCN) through perilacunar remodeling (PLR). We previously implicated PLR, specifically through matrix metalloproteinase 13 (MMP13), in the control of bone quality. Since TGFβ regulates MMP13 expression, we hypothesize that TGFβ regulates bone quality through osteocyte-mediated PLR. Here, we test this hypothesis to elucidate the unknown role of TGFβ in osteocytes and the cellular mechanisms controlling bone quality.

METHODS: Two in vivo murine models and in vitro studies were used to evaluate the role of TGFβ in PLR. Using our established model (1), 5-week old mice were administered either vehicle or a TβRII kinase inhibitor (SD208, 60mg/kg, twice daily) for 6 weeks to systematically knock down TGFβ signaling. MicroCT analysis of the bone phenotype and PLR outcomes were conducted in 11-week old mice. To define the osteocyte specific role of TGFβ in PLR, we developed a novel tissue-specific model with ablated TGFβ function only in DMP1-expressing osteocytes, DMP1-Cre +/TβRII 1−/−. MicroCT analysis and PLR outcomes were examined in 8-week old control (DMP1-Cre +/TβRII 1−/−) and TβRII 1−/−/+ (DMP1-Cre +/TβRII 1−/−) mice. In vivo PLR outcomes analyzed include LCN network through silver staining, PLR gene expression through qPCR, and immunohistochemistry. In vitro PLR outcomes were assayed in MLO-Y4 osteocyte-like cells, and include gene expression profiling and measurement of intracellular pH using carboxy-SNARF-AM dye. To examine the effect on bone quality of osteocyte specific TGFβ ablation, three-point bending tests were conducted on unnotched femora of 8-9-week old TβRII 1−/−/+ and control mice using a Bone Electroforce 3200 test frame with a displacement rate of 0.01 mm/s. Flexural strength tests measured yield strength and other standard outcomes. Sample size for in vitro and in vivo approaches was defined as N=3 and N=5-8 respectively. Statistical analysis was performed using ANOVA or Student’s t-test. All animal procedures were approved by the Institutional Animal Care and Use Committee (IACUC) at UCSF.

RESULTS: Using the same model in which we evaluated the effect of systemic TβRII-inhibition on osteoclasts and osteoblasts, we investigated the unknown role of TGFβ in osteocytes. After verifying the high bone mass phenotype, we found that systemic inhibition of TβRII drove a strong and coordinated repression of several key PLR enzymes, including MMP2, MMP13, MMP14, Cathepsin K (CatK) and Tartrate resistant acid phosphatase (TRAP) genes in bone (Fig. 1A). Immunohistochemistry of cortical femurs revealed a significant decrease in the number of MMP13, MMP14, CatK and TRAP-expressing osteocytes upon TβRII inhibition. Given that suppression of these genes has been linked to deregulated PLR by osteocytes, we speculated a unique regulatory role of TGFβ in PLR. Therefore, we evaluated hallmarks of PLR, including the osteocyte LCN, in TβRII-inhibitor and vehicle-treated mice. Impeded TGFβ signaling caused a qualitative decline in the integrity of the osteocyte lacuno-canalicular network in the cortical bone (Fig. 1C). As LCN connectivity is a crucial functional outcome of PLR, our results suggest that TGFβ signaling is an essential regulator of PLR, directly in osteocytes and/or indirectly through interactions with other cells.

To determine if the effect of TGFβ on PLR is osteocyte-intrinsic, we analyzed in vitro PLR outcomes in MLO-Y4 cells. TGFβ significantly induced the expression of PLR genes, including MMP13, MMP14 and CatK. TGFβ also caused a drop in intracellular pH, a functional measure of osteocyte PLR. This decrease in intracellular pH supports bone resorption by osteocytes engaged in PLR, just as it does for osteoclast-mediated bone resorption. Furthermore, we developed and characterized a novel in vivo model of osteocyte-specific ablated TGFβ signaling (TβRII 1−/−/+). Immunohistochemistry demonstrated near complete loss of TβRII in osteocytes, but intact TβRII in osteoblasts and other cell types. In addition to a high trabecular bone mass bone phenotype, TβRII 1−/−/+ bones express dramatically lower levels of MMP2, MMP13, MMP14 and CatK mRNA compared to the control mice (Fig. 1B). Immunohistochemical staining for MMP13 and MMP14 confirmed this finding. Moreover, degeneration in osteocyte LCN integrity and area was even more pronounced in the TβRII 1−/−/+ cortical bone than in mice treated systemically with TβRII inhibitors (Fig. 1D). Together, these findings indicate that TGFβ regulates PLR through osteocyte-intrinsic mechanisms. Analyses are underway to determine if loss of osteocyte-intrinsic TGFβ signaling is sufficient to impact bone quality. So far, flexural strength tests show that yield strength is significantly reduced by 27.5% in TβRII 1−/−/+ bone compared to controls. Together, our results identify a novel function for TGFβ in bone, such that osteocyte-intrinsic TGFβ regulates bone quality through PLR.

DISCUSSION: Here we report a novel function of TGFβ in osteocyte-mediated PLR and bone quality. Although we previously elucidated the critical function of TGFβ in controlling bone quality, the cellular mechanism was unknown (2). Using a combination of unique in vivo and in vitro tools, this study demonstrates that osteocyte-intrinsic TGFβ signaling orchestrates the activities of osteoblasts and osteoclasts, while it directly regulates local bone matrix remodeling through PLR. At the molecular level, we find that TGFβ partly relays its effects on PLR through MMP13, the absence of which also impairs LCN integrity and fracture resistance (3). In conclusion, our results highlight an osteocyte intrinsic-role for TGFβ signaling in dictating bone quality.

SIGNIFICANCE: Our findings provide novel mechanistic insight into the role of TGFβ signaling in osteocytes, the regulation of PLR, and the cellular mechanisms controlling bone quality. The discovery that TGFβ regulates bone quality in an osteocyte-dependent manner is critical to treating bone fragility in individuals with normal bone mass and preventing potentially adverse side effects from clinical use of TGFβ inhibitors for cancer and other conditions.


IMAGES AND TABLES:

Figure 1: Osteocyte-mediated PLR is regulated by TGFβ. TGFβ regulates expression of PLR genes and osteocyte LCN integrity. qPCR analysis shows fold changes in bone mRNA levels of MMP13, MMP14, CatK, TRAP in SD208 (A) and TβRII 1−/−/+ (B) in vivo models. Silver staining shows a TGFβ-dependent loss of osteocyte LCN integrity at 100x magnification in SD208 (C) and TβRII 1−/−/+ (D) models. *p<0.05, N=5 for all, Scale bar =20μm.

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