MT1-MMP Mediates Plasticity and Divergence of the Osteoblast and Adipocyte Lineages Through Cleavage of DLK1

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Introduction: Pericellular matrix remodeling is necessary for skeletal development, bone maintenance and fracture repair. Numerous proteases have been implicated in the processing and degradation of extracellular matrix (ECM) molecules. Among these, type 1 membrane-type matrix metalloproteinase (MT1-MMP/MMP14) has been shown to participate in both bone formation and resorption in a cell-specific manner. While the matrix-degrading function of MT1-MMP is well described, little is known about its role in processing non-ECM substrates, and what implications this may have in skeletal biology. Here, we investigate the role of MT1-MMP specifically in mature murine bone cells using Cre-recombinase expressed under the control of the osteocalcin (Ocn) promoter*, and identify Delta-like 1 homolog Drosophila (DLK1), a previously described negative regulator of adipocyte maturation, as a novel substrate of MT1-MMP that governs divergence and plasticity of the adipocyte and osteoblast lineages.

Methods: All experimental protocols involving animals were reviewed and approved by the NIDCR Animal Care and Use Committee. Mice with global (MT1-/-) or conditional deletion of MT1-MMP in osteocalcin-expressing mature osteoblasts (Ocn-Cre*; MT1F/F) were generated and maintained on an outbred background line for examination of skeletal phenotype, and used as a source of MT1-MMP deficient primary calvarial osteoblasts and bone marrow stromal cells (BMSCs). Similarly, primary cultures of osteoblasts and BMSCs were generated from mice with global DLK1 deficiency (DLK1-/-). The skeletal phenotypes of knock-out mice, relative to wild-type littermate controls, were characterized by gross examination, whole-mount histochemistry, conventional radiography, quantitative μCT and dynamic histomorphometry of craniofacial and long bones. Ectopic ossicles were generated by subcutaneous implantation of rhBMP2-infused GelFoamTM sponges to facilitate examination of osteoprogenitor recruitment and development of a bone/marrow organ. Adipogenic differentiation was studied in vitro, using primary cultures of osteoblasts and BMSCs from MT1-/-, Ocn-Cre;MT1F/F, and DLK1-/- mice by oil-red-O staining following exposure to an induction cocktail containing insulin, dexamethasone, isobutylmethykanthine and indomethacin. Cleavage and shedding of DLK1 was examined by lentiviral transduction of 293T cells with murine MT1-MMP, or a catalytically inactive variant (E240A), and HA-tagged DLK1, followed by immunoprecipitation and western-blotting.

Results: Whereas global deletion of MT1-MMP results in severe skeletal dysmorphism (runting, osteopenia and multiple fractures) and a drastically reduced life span, early skeletal development in Ocn-Cre;MT1F/F mice was overtly normal. However, a severe skeletal phenotype emerged as these mice aged. Relative to wild-type littermates, mature Ocn-Cre;MT1F/F mice displayed systemic progressive
osteopenia and developed spontaneous fractures. Histomorphometric analysis of femora from Ocn-Cre;MT1F/F mice by μCT imaging revealed significantly reduced cortical and trabecular volume and mineral density, reduced cortical thickness, reduced trabecular number, and increased trabecular spacing, relative to wild-type littermates. Dual-fluorophore labeling demonstrated a significant reduction of bone apposition in Ocn-Cre;MT1F/F mice although TRAP staining demonstrated no enhancement of osteoclast number or resorptive activity. These results indicate that the osteopenic phenotype observed in Ocn-Cre;MT1F/F mice results from a defect in the activity of mature osteoblasts, rather than by an enhancement of osteoclastic resorption. Histologic analysis of spontaneous fractures in Ocn-Cre;MT1F/F mice revealed a strikingly ineffective repair process. These fractures displayed only a thin bone rim of apparent membranous origin surrounding the fracture site, and little evidence of endochondral bone formation. Within this imperfect union, there was a conspicuous absence of hematopoietic marrow and an abundance of adipocytes. Similar to the spontaneous fractures, rhBMP2-induced ectopic ossicles recovered from Ocn-Cre;MT1F/F mice displayed reduced bone deposition, replaced by a rather dystrophic fibrosis, adipocytic infiltration, and failure to generate a proper hematopoietic organ. Interestingly, deletion of DLK1 in mice produced a similarly mild skeletal phenotype notable for reduced bone density and increased marrow adiposity, resembling the tissue-specific knockout of MT1-MMP in mature osteoblasts. Therefore, we considered the possibility that MT1-MMP may interact with DLK1 to regulate adipogenic conversion of the osteoblast lineage. To test this, we compared adipogenic differentiation capacity, as evidenced by accumulation of oil-red-O binding lipid droplets in osteoblasts and BMSCs derived from wild-type, MT1-MMP/- or DLK1/- mice. While adipogenic differentiation, was readily inducible in BMSCs from all strains tested, BMSCs from MT1-MMP/- or DLK1/- mice were frequently observed to undergo spontaneous adipocytic differentiation upon confluency. In contrast, calvarial osteoblast cultures from wild-type mice were largely resistant to adipogenic induction, while those from MT1-MMP/- or DLK1/- mice were readily susceptible. Spontaneous adipocytic differentiation of non-induced osteoblasts was rarely observed in cells from any donor origin. Co-expression of MT1-MMP and DLK1 in 293T cells revealed that MT1-MMP cleaved membrane-bound DLK1, releasing a N-terminally tagged peptide corresponding to the extracellular domain of DLK1 that is reported to function as an ‘anti-adipokine.’

**Discussion:** Skeletal development is dependent on coordinated lineage commitment and differentiation of multipotent skeletal progenitor cells. However, the mechanisms by which post-natal skeletal homeostasis is achieved remains incompletely understood. The conventional view holds that a population of quiescent, multi-potent progenitor cells may be activated as needed to maintain the osteoblast/osteocyte lineage. In contrast, recent evidence suggests that plasticity of mature osteoblastic cells may allow for limited de-differentiation and numeric expansion followed by commitment to specific lineages in response to local physiologic demands. Our data demonstrate that MT1-MMP is a key mediator of mature osteoblast activity and differentiation where, in the absence of this protease, multipotent cells default to an adipocytic fate due to the failure to release the anti-adipokine, DLK1. Ongoing studies will focus on identification of other substrates of MT1-MMP, and determine the signaling pathways that are affected by the absence of this pericellular protease.

**Significance:** This study illustrates a novel function of MT1-MMP as a determinant of osteoblast fate and identifies DLK1 as an MT1-MMP substrate that suppresses the divergent differentiation of adipocytes from the skeletal lineage stem cell pool. These findings may be relevant to the mechanistic
understanding of skeletal stem cell activation in skeletal development, maintenance and fracture healing, and may have relevance in the management of the adverse skeletal effects associated with thiazolidinedione medications.

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