Decreased Trabecular Bone Mass in Col22a1-Deficient Mice

Subjects: Physiology

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The bone matrix is constantly remodeled by the coordinated activities of bone-forming osteoblasts and bone-resorbing osteoclasts. Whereas type I collagen is the most abundant bone matrix protein, there are several other proteins present, some of them specifically produced by osteoblasts. In a genome-wide expression screening for osteoblast differentiation markers we have previously identified two collagen-encoding genes with unknown function in bone remodeling. Here we show that one of them, Col22a1, is predominantly expressed in bone, cultured osteoblasts, but not in osteoclasts. Based on this specific expression pattern we generated a Col22a1-deficient mouse model, which was analyzed for skeletal defects by µCT, undecalcified histology and bone-specific histomorphometry. We observed that Col22a1-deficient mice display trabecular osteopenia, accompanied by significantly increased osteoclast numbers per bone surface. In contrast, cortical bone parameters, osteoblastogenesis or bone formation were unaffected by the absence of Col22a1. Likewise, primary osteoblasts from Col22a1-deficient mice did not display a cell-autonomous defect, and they did not show altered expression of Rankl or Opg, two key regulators of osteoclastogenesis. Taken together, we provide the first evidence for a physiological function of Col22a1 in bone remodeling, although the molecular mechanisms explaining the indirect influence of Col22a1 deficiency on osteoclasts remain to be identified.

Keywords: bone remodeling ; Col22a1 ; osteoclasts ; osteoblasts

1. Introduction

The skeleton is a highly complex tissue, which needs to be constantly remodeled in order to provide life-long stability. This remodeling process is mediated by two fundamentally different cell types, i.e., bone-forming osteoblasts and bone-resorbing osteoclasts [1][2]. Whereas multinucleated osteoclasts are generated by fusion of hematopoietic progenitor cells in a process regulated by the cytokine Rankl and its antagonist Opg, osteoblasts derive from mesenchymal progenitor cells to accumulate in cellular groups producing an extracellular matrix that subsequently mineralizes [3][4]. In this process hydroxyapatite, i.e., crystalline calcium phosphate, is incorporated into a scaffold of collagen fibrils, which mostly consist of type I collagen, the most abundant protein of the bone matrix [5]. The importance of proper synthesis, processing and folding of type I collagen for skeletal integrity is underscored by the fact that mutations in the genes encoding either the type I collagen subunits or enzymes required for its posttranslational modification, cause osteogenesis imperfecta, a severe skeletal disorder with poor bone quality and high fracture risk [6]. Furthermore, type I collagen is also a main constituent of dentin, produced by odontoblasts, which explains why osteogenesis imperfecta can be associated with dental defects [7].

Besides type I collagen there are many other less abundant bone matrix proteins, some of which are specifically produced by osteoblasts and/or odontoblasts. The majority of these proteins have been analyzed for a potential role in regulating bone matrix integrity and turnover, which also included the generation and analysis of mouse deficiency models [8]. Here it was found that specific proteins are required to ensure the unique material properties of the bone matrix, whereas others additionally served initially unexpected functions [9][10][11]. One example for the latter is Dmp1, an extracellular matrix protein specifically produced by terminally differentiated osteoblasts and odontoblasts. It was found that Dmp1-deficient mice display severe defects of bone matrix mineralization, yet this phenotype was primarily explained by hypophosphatemia, as a consequence of increased bone-specific production of the phosphaturic hormone Fgf23 [12][13]. A similar pathology is caused by deficiency of another gene with specific expression in terminally differentiated osteoblasts and odontoblasts, i.e., *Phex*, encoding a transmembrane endopeptidase [14]. Moreover, the identification of inactivating *DMP1/PHEX* or activating *FGF23* mutations in patients with hypophosphatemic rickets has underscored the human relevance of these genes, all of them displaying a skeleton-specific expression pattern [15].

Therefore, based on the hypothesis that osteoblast-specific expression potentially predicts a physiological function in the skeleton, we have previously performed a screen for novel osteoblast differentiation markers [16]. More specifically, we applied genome-wide expression analysis to identify genes with increasing expression during the course of primary osteoblast differentiation. For the hundred genes with the strongest induction, we applied RT-PCR to compare the expression in bone (femur, calvaria) and seven non-skeletal tissues. We hereby confirmed the bone-specific expression of various established genes, including *Dmp1* and *Phex*, but we also identified other genes with a similar expression pattern. While we have previously analyzed the putative function of one of these genes, i.e., *Panx3*, the present study is focused on *Col22a1*, one of two collagen-encoding genes that were identified in our screening approach [16]. Type XXII collagen (COLXXII), the protein encoded by *Col22a1*, was originally identified as a novel tissue junction component, particularly at the myotendinous junction of heart and skeletal muscle [17]. It belongs to the FACIT (fibril-associated collagens with interrupted triple helix) family of collagens, which do not form fibrils [18]. Importantly, however, the physiological functions of COLXXII are essentially unknown, which is also explained by the lack of phenotypic data in a corresponding mouse deficiency model.

Here we show that *Col22a1* is predominantly expressed by osteoblasts, but not by osteoclasts. We further generated a *Col22a1*-deficient mouse model, where we identified a low bone mass phenotype, restricted to the trabecular bone compartment. Histomorphometric quantification revealed that this osteopenia was associated with increased numbers of osteoclasts, whereas bone formation and matrix mineralization were unaffected. Taken together, our findings provide the first evidence for a specific role of *Col22a1* in regulating skeletal turnover.

2. Current Insights

Bone remodeling is mediated by bone-resorbing osteoclasts and bone-forming osteoblasts, two entirely different cell populations. While osteoid, the organic component of the bone matrix, consists mostly of type I collagen that provides the scaffold for the mineral phase consisting of calcium phosphate crystals, there are numerous other proteins that contribute to the composition of the bone matrix and are essential for its proper function [8]. In a previous study we have analyzed the transcriptome of differentiating osteoblasts and observed many well-established osteoblastogenesis markers among the genes with the strongest induction during differentiation, thereby demonstrating the validity of our approach. In this screening process, we also identified several genes with a hitherto unknown function in skeletal turnover, despite predominant expression in bone tissue. While the main objective of our previous study was related to *Panx3* [16], our current investigation is focused on the role of *Col22a1*, encoding type XXII collagen (COLXXII). *Col22a1* was one of two collagen transcripts identified among the hundred most strongly induced genes during osteoblast differentiation. In the current study we were able to show that *Col22a1*, unlike *Col13a1*, is predominantly expressed in bone tissue and differentiated osteoblasts, which led us to investigate its putative role in the skeleton.

COLXXII has originally been identified as a novel tissue junction component, as it is mostly localized in the transition zones of tissues, such as the myotendinous junction of heart and skeletal muscle, the articular cartilage/synovial fluid junction, and the junction of hair follicles and the dermis [17]. Targeting of *Col22a1* in Zebrafish morpholino models has led to the observation that these animals display muscular dystrophy most likely due to a severely disrupted myotendinous junction that could even lead to muscle detachment [19]. In a different Zebrafish study, genetic disruption of the *Col22a1* gene has led to an increased risk of intracranial hemorrhages due to increased vascular permeability [20]. The latter was attributed to a disruption of the proper structure of the basal lamina. These findings were supported by the association of genetic variants of human *COL22A1* with a higher risk of aneurysms [20][21]. Importantly, however, since there is no published analysis on the phenotype of a *Col22a1*-deficient mouse model, the physiological role of COL XXII is still not well-defined.

Based on the observed *Col22a1* expression pattern, our primary focus was to generate such a mouse model in order to study their skeletal phenotype. Importantly, we did not observe any obvious morphological alterations or extraskeletal phenotypes, such as aneurysms. However, we did observe significantly less *Col22a1*-deficient mice born than would be expected from the Mendelian ratio. Nonetheless, it remains purely speculative at this point, whether this discrepancy can be attributed to one of the previously proposed functions of COLXXII. With respect to the skeleton, it was also obvious that *Col22a1* deficiency did not impair developmental processes, i.e., desmal or endochondral ossification, or skeletal growth. Moreover, we did not detect enrichment of non-mineralized bone, i.e., osteoid, unlike it is the case in mice carrying type I collagen mutations, or in mice lacking Dmp1 [13][22][23]. Finally, there was no indication of impaired osteoblast differentiation and function in association with *Col22a1* deficiency. On the other hand, trabecular osteopenia in vertebral bodies, albeit moderate, was observed in all analyzed groups, where wildtype and heterozygous littermates were used as controls.

Of note, we also observed a significant reduction of trabecular bone mass in the distal femoral metaphysis, yet this difference was only found in the 24-week-old mice. Although this may indicate that the axial skeleton is more affected than the appendicular skeleton in *Col22a1*-deficient mice, there is also another possible explanation for this apparent discrepancy. In fact, since there are far less trabecular bone structures in long bones when compared to the spine, a reduction of trabecular bone mass is generally more robust, when spine sections are analyzed. On the other hand, assessing bone parameters in femora by µCT is advantageous for quantification of cortical parameters in the midshaft region. Here we observed that cortical bone mass and most cortical bone mechanical properties, as determined by a three point bending test, remained unaffected by the loss of *Col22a1*. This indicates that COLXXII is predominantly relevant in the trabecular bone compartment. The only effect on cortical bone that we were able to determine was reduced bone matrix stiffness. It is conceivable to speculate that this phenotype is caused by reduced type I collagen crosslinking via COLXXII, although such a function has not yet been described for COLXXII [24]. Indeed, COLXXII belongs to the FACIT subfamily of collagens, which does not form fibrils but copolymerize into superstructures with fibril-forming collagens and mediate interactions with the environment [25].

When investigating the underlying cause for the reduced trabecular bone mass, we specifically observed increased osteoclast parameters. Since *Col22a1* was found barely expressed in osteoclasts, these results were fully unexpected and suggest that they are indirectly mediated. Since osteoblast lineage cells are major producers of Rankl and Opg, which in turn regulate osteoclastogenesis [26], we particularly analyzed the possibility, that *Col22a1*-deficient osteoblasts produce more Rankl and/or less Opg. By comparing primary osteoblasts isolated from wildtype and *Col22a1*-deficient littermate mice, we did not observe a cell-autonomous osteogenesis defect, and there was no differential expression of the genes encoding Rankl or Opg. Moreover, the serum concentrations of these two factors were not different between wildtype and *Col22a1*-deficient littermate mice. Interestingly, it has been shown that *Col22a1* colocalizes with integrins such as α2β1 and α11β1 in a pattern that is reminiscent of integrin signaling [24]. Moreover, an involvement of *Col22a1* in integrin-mediated mechanosensing and negative regulation of chondrocyte hypertrophy has been hypothesized [24][27]. Therefore, it is conceivable that an altered bone matrix composition lacking COLXXII influences osteoclast differentiation or attachment via integrin signaling [28][29][30]. Further research is required to adequately test these hypotheses and to fully elucidate the underlying mechanism.

Apart from the fact that our current data do not provide a molecular mechanism explaining the increased trabecular osteoclast numbers in *Col22a1*-deficient mice, there are other limitations of our study. At the age of 24 weeks, we only analyzed male mice. Considering the data obtained in 6-week-old animals, it is likely that female mice might display a similar phenotype. However, this has not been verified. Furthermore, since we only analyzed a global knockout model, we cannot exclude that the observed phenotype might be caused by effects originating from other cell types. Therefore, even if we found *Col22a1* to be predominantly expressed by osteoblasts, we truly believe that models with cell-specific deletion of *Col22a1* are required to fully investigate the effect of COLXXII on the skeleton.

3. Conclusions

In conclusion, we present the first data on the skeletal phenotype of a Col22a1-deficient mouse model. Our data demonstrate that Col22a1-deficiency specifically causes trabecular osteopenia with an increased osteoclast number, which is not explained by a shifted Rankl/Opg ratio.

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