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Potential role of cathepsin K in the pathophysiology of mucopolysaccharidoses

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Abstract

Cathepsin K, a papain-like cysteine protease, is highly expressed in osteoclasts and plays a critical role in bone resorption. Dysfunction of the enzyme leads to various skeletal abnormalities. The recent knowledge that the collagenolytic activity of cathepsin K depends on interactions with bone and cartilage-resident glycosaminoglycans (GAGs) may shed some light on diseases such as mucopolysaccharidoses (MPSs). MPSs are a group of lysosomal storage diseases characterized by the accumulation of GAGs in tissues including bone. Typical pathological features of these diseases include skeletal abnormalities such as dysostosis multiplex, short stature, and multiple irregularities in bone development. We describe how further investigation of the cathepsin K/GAG complexes could provide valuable insights into the bone pathology associated with MPS diseases. In this review, we discuss the inhibition of osteoclast function through altered activity of cathepsin K by GAGs and offer insight into a mechanism for the bone pathology seen in MPS patients.

Keywords

Cathepsin K; chondroitin sulfate; cartilage; collagen; glycosaminoglycan; mucopolysaccharidoses; osteoclast

1. Cathepsin K in bone resorption

Cathepsin K is a member of the cysteine protease class and plays a vital role in bone resorption [20,21]. The enzyme is highly expressed in osteoclasts. These cells are critical for bone remodeling as they are capable of dissolving the organic matrix and digesting it in a tightly controlled manner (for review see [44]). During osteoclast-mediated bone resorption mature osteoclasts form specific interactions with the bone matrix which trigger the polarization of the osteoclast. The osteoclast forms several specialized membranes including the ruffled border at the osteoclast/bone interface. The ruffled border is formed by the fusion of cathepsin K containing vesicles with the membrane, which leads to the release of cathepsin K into the so-called resorption lacuna where the collagen degradation occurs. Proton pumps and a Cl^- channel act to acidify the resorption lacuna ensuring it is at the required acidic pH for cathepsin K activity. Acidification also acts to dissolve the mineral component of the matrix allowing the organic component to become exposed to cathepsin K degradation. In addition, osteoclasts are also employed in the removal of cartilage during endochondral ossification [4]. Cathepsin K is highly effective at degrading type I collagen

(major component of long bone), and type II collagen (major component of cartilage) [14, 16]. Whereas collagenases of the matrix metalloproteinase family degrade collagen into characteristic 1/4 and 3/4 fragments, cathepsin K completely degrades the entire triple helix.

The importance of cathepsin K in bone and cartilage resorption is best demonstrated by the deficiency of cathepsin K protein in pycnodysostosis. Pycnodysostosis is a rare autosomal recessive disorder characterized by a decrease in bone resorption. This results in a short stature, increased bone mass, and an osteosclerotic phenotype [13,15,42]. Cathepsin K-deficient osteoclasts are unable to degrade triple-helical collagen as demonstrated by the intracellular accumulation of collagen fibrils and shallow resorption pits [11,37]. The overexpression of cathepsin K leads to excessive bone loss and is thought to result in a condition known as osteopenia. This makes cathepsin K a much sought after therapeutic target for diseases such as osteoporosis, arthritis, and metastatic bone disease, which are plagued by excessive bone and cartilage degradation [5, 19,51].

2. Cathepsin K regulation by glycosaminoglycans and mucopolysaccharidoses

GAGs are a diverse family of polysaccharides found in all tissues. They are bound to core proteins to form proteoglycans. The most abundant proteoglycan in cartilage is aggrecan with chondroitin and keratan sulfates being the dominant GAG chains [35]. Small leucine-rich proteins such as decorin and biglycan are found in bone and cartilage and depending on their tissue location and the age of the tissue, they contain chondroitin and/or dermatan sulfates as glycosaminoglycan chains [35,50]. Typical roles for GAGs include interactions with growth factors and cytokines, specific protein binding such as with collagens, and cell-surface receptor binding [3,9,32]. Li et al. first demonstrated that chondroitin 4-sulfate (C4S) was necessary for the collagenolytic activity of cathepsin K [24]. This effect was specific for cathepsin K with having no effect on other collagenases such as cathepsin L or MMP-1. Without C4S, cathepsin K has only a minimal if any collagenolytic activity against type I and II collagens [24]. It was later demonstrated that C4S and cathepsin K form an oligomeric complex, which acts as a collagenase. This complex was confirmed by dynamic light scattering and ultracentrifugation and revealed a molecular mass of more than 250 kDa with cathepsin K alone having a mass of 23.5 kDa [23]. The interaction is brought about by positive charges on the cathepsin K protein interacting with negative charges on the chondroitin sulfate (CS). Crystallography revealed a structure for this interaction [22]. The active site of cathepsin K does not appear to be involved in the complex thereby retaining all of its catalytic ability. It is speculated that the complex enables the enzyme to unwind the triple helical structure of collagen. However, Li et al. demonstrated the ability of other GAGs such as dermatan (DS) and heparan sulfates (HS) to compete with chondroitin sulfate and that they may form alternative, but collagenolytically inactive complexes with cathepsin K [25]. The concentration of GAGs also appears to be important, as higher concentrations of CS have been shown to inhibit the collagenolytic activity of cathepsin K (unpublished data, DB). Therefore conditions such as MPS VII, where there is an accumulation of CS besides DS and HS, the combined accumulation of GAGs may cause the inhibition of cathepsin K resulting in thickened and shortened bones as well as irregular growth plates [38,45]. This implies, that the *in vivo* activity of cathepsin K is highly regulated by *in situ* concentrations of GAGs. Thus, excess of GAGs as seen in mucopolysaccharidoses may inhibit the bone and cartilage resorbing activity of cathepsin K during bone development and growth (Table 1).

3. Growth plate pathology in MPS I and cathepsin K activity

MPS diseases are a group of lysosomal storage disorders, and arise due to the diminished activity of the enzymes responsible for the degradation of various GAGs. There are several

different types of these diseases with symptoms ranging from mild to severe depending on the extent of enzyme inactivity. The resulting accumulation of glycosaminoglycans in tissues leads to many pathological conditions including mental retardation, short stature, dysostosis multiplex, corneal clouding, hepatosplenomegaly, and cardiac involvement [9]. The skeletal phenotype can be particularly strong and is hard to treat as existing therapies such as enzyme replacement therapy and bone marrow transplantation have limited effects on skeletal conditions [12,31]. Although, this problem might be overcome by very early treatment initiation prior to the onset of the skeletal phenotype [27].

Bone remodeling is essential for skeletal growth and development. Typical skeletal manifestations of the MPS diseases involve disorganization of the growth plate and shortened bones, suggesting problems with endochondral ossification. Endochondral ossification is the process by which new long bone is formed and occurs at the growth plate. There are many important factors in endochondral ossification and the activity of a range of bone cells are essential to the correct formation of bone. Initially a cartilage scaffold is formed by chondrocytes, but before this can be replaced with a new bone matrix by osteoblasts, osteoclasts are required to breakdown the cartilage. Cathepsin K is vital in the role of osteoclasts as bone remodeling mediators [49]. In conditions where cathepsin K activity is limited, as with pycnodysostosis, a disorganized growth plate is apparent. Cathepsin K deficient mice have a disorganized growth plate as well and there is also a build up of cartilage compared to wild-type mice [13]. We have previously investigated a murine MPS I model, in which the α -L-iduronidase gene is disrupted resulting in an accumulation of dermatan (DS) and heparan (HS) sulfates [8]. Studies in our laboratory on murine MPS I bone morphology and other studies have described disorganized growth plates with an increased presence of cartilage similar to that of cathepsin K-deficient specimens (Fig. 1) [28,36]. Disorganized growth plates and a build up of abnormal cartilage are known for MPS VII [28,38], feline MPS VI [2], MPS IVA [26], MPS IVA [17], and human MPS I [39]. Although MPSVI specimen have an abnormal endochondral ossification zone they also display osteopenia including the thinning of the trabeculae [30]. This is thought to be due to other factors involved with endochondral ossification such as early chondrocyte death and increased expression and activity of MMP-2 and MMP-9 as well as decreased activity of osteoblasts [6,30,40].

As the development of the growth plate area is critically influenced by the collagenolytic activity of cathepsin K, we compared this area in wild-type and MPS I mice. We paid particular attention to the hypertrophic zone where there is reported to be a retention of cartilage [36]. Immunofluorescent staining for cathepsin K of the subepiphyseal growth plate area of the long bones and vertebrae revealed that in both wildtype and MPS I murine long bones cathepsin K was expressed. Interestingly, the levels of cathepsin K staining were even higher in MPS I bones [48]. However, despite the increase in cathepsin K expression, cartilage degradation was decreased. An antibody against the cathepsin K cleavage site on type II collagen revealed dramatically less cathepsin K mediated type II collagen cleavage in MPS I mice when compared with the wild-type growth plate (Fig. 2) [48]. Although we found cathepsin K to be present at the growth plate, the reduction in its collagenolytic activity and an increase in cartilage suggests it is largely nonfunctional. Further studies were able to demonstrate a strong co-localization of cathepsin K and HS in this area in MPS I bone [48] thus suggesting the increased likelihood of their interaction.

As we have shown that high concentrations of GAGs can adversely affect the collagenolytic activity of cathepsin K ([25] DB, unpublished data), we analyzed the potential of recombinant cathepsin K to degrade bone powder from wild-type and MPS I mice. Due to the α -L-iduronidase deficiency, MPS I bones have increased lysosomal concentrations of DS

and HS. As expected, we observed a significant reduction in collagen degradation using MPS1 bones [48].

Although there are some similarities between the phenotypes of long bones from cathepsin K deficient and MPS I mice (thickened bone and growth plate abnormalities, see Fig. 1), there are other pathological features of MPS bone not apparent in cathepsin K-deficient bone. These are likely to be due to other factors associated with MPS disease. The high concentration of GAGs has the potential to interfere with numerous cell types and biological processes. Previous work in this area has suggested that the increased presence of GAGs creates an inflammatory response resulting in chondrocyte apoptosis and the release of matrix metalloproteinases in bone and joints which could also contribute to growth plate abnormalities [40,41].

Osteoporosis and osteomalacia may also be seen at different stages in various MPS diseases. It has been suggested that other factors may contribute to these conditions including nutritional deficiencies such as lack of calcium intake, vitamin D deficiency, as well as lack of exercise due to immobility, sex hormone deficiencies, low body weight, lower limb disuse and treatment with anti-epileptic drugs [33]. For instance osteoporosis and osteomalacia can be found in MPS III in which HS accumulates predominantly neurologically and not in the skeleton [33]. Interestingly, MPS IVA, B are particularly characterized by an osteoporotic phenotype [1,18,34]. As MPS IV accumulate rather cathepsin K activity promoting GAGs (keratan sulfate and chondroitin-6-sulfate), it is tempting to speculate that this may lead to more collagenolytically active complexes (Table 1). Initial inhibition of the cathepsin K activity by a disease-mediated accumulation of these GAGs might be offset by an increase of cathepsin K protein expression as we have observed for MPS I [48].

4. Osteoclast function in MPS I and cathepsin K activity

Few *in vitro* studies have been published on the activity of osteoclasts in MPS bone. It has previously been shown that in murine MPS VII many osteoclasts are detached from the bone matrix and fail to form the ruffled border necessary for bone resorption activity [29]. Other studies on osteoclast differentiation have revealed a role for GAGs in both the promotion and inhibition of osteoclastogenesis (see Table 1 for more details) [7,43]. Studies performed in our laboratory have shown the ability of cathepsin K to regulate osteoclast activity [47]. Cathepsin K may uncover attachment factors in type I collagen necessary for initiating actin ring formation and resulting in osteoclast activity. At the same time the proteolytic release of bioactive RGD peptides may serve to inhibit osteoclast resorption in a negative feedback pathway. Therefore the inhibition of cathepsin K in a culture of murine wild-type osteoclasts resulted in a decrease in the percentage of osteoclasts displaying an actin ring, which signified a decrease in actively resorbing osteoclasts [47].

Our studies suggest that many MPS I osteoclasts failed to form actin rings when plated on type I collagen. Their actin molecules were arranged in clumps throughout the cell or displayed disrupted rings. This is contrary to wild-type osteoclast cultures, which predominantly display complete actin rings over 24h cultures. In bone resorption assays, MPS I osteoclasts plated onto dentine revealed a similar number of osteoclasts compared to wild-type cultures. However MPS I osteoclasts created significantly fewer resorption pits of smaller area [48].

It must be noted that lysosomal dysfunction may also contribute to MPS I osteoclast inactivity [10]. MPS I murine bone cells such as osteocytes have been reported as showing lysosomal vacuolation [36] as have murine MPS VII osteoclasts [29]. When murine wild-type osteoclasts were plated onto type I collagen containing concentrations of DS similar to

estimates in MPS diseases this was sufficient to reduce the number of osteoclasts with actin rings [48]. This suggests that a high concentration of GAGs is able inhibiting osteoclast activity either through cathepsin K inhibition or lysosomal network dysfunction.

Studies on murine cathepsin K knockout osteoclasts plated on type I collagen revealed a low number of osteoclasts with actin rings, similar to MPS I osteoclasts or wild-type osteoclasts in the presence of cathepsin inhibitors. This low occurrence of actin rings was reversed by plating the cells on type I collagen which has been predigested by cathepsin K, thereby uncovering the attachment factors for the deficient osteoclasts. When MPS I osteoclasts were plated on to cathepsin K-predigested type I collagen this was able to rescue the numbers of osteoclasts displaying actin rings to a range similar to wild-type cells. Suggesting cathepsin K inhibition is indeed an important factor in murine MPS I osteoclast function. Figure 3 summarizes the proposed pathways in osteoclast differentiation and activation which could be affected by high GAG concentrations.

5. Conclusion

In conclusion, cathepsin K is a major bone and cartilage degrading protease that likely requires optimal concentrations of C4S for its collagenolytic activity. This activity is inhibited by high concentrations of GAGs such as DS, HS, and also C4S. As these GAGs accumulate in all MPS-affected tissues, they could lead to the inactivation of the collagenolytic activity of cathepsin K, which in turn may contribute to the bone pathology observed in MPSs. Mucopolysaccharidoses which primarily accumulate collagenolytic activity-enhancing GAGs such as KS and C6S (MPS IV) might promote under certain conditions an excessive cathepsin K activity leading to an osteoporotic phenotype. Figure 4 summarizes the potential effects of MPS-related GAGs on the collagenolytic activity of cathepsin K. In order to prevent the abnormal endochondral ossification and avoid the skeletal deformities associated with MPS disease, future treatments aimed at the disruption of unwanted GAG/cathepsin K interactions could be beneficial.

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Fig. 1. Longitudinal sections of distal mice femura. 5 μ M methylmetacrylate sections (von Kossa staining) show thickened cortical bone and more trabecular bone area in the MPS I specimen (right panel). The MPS I femur also appears wider than the control wild-type specimen (left panel). The cathepsin K-deficient bone also reveals significantly more trabecular bone and increased thickness of the cortical bone (middle panel).

Fig. 2.

Neoepitope staining of cathepsin K catalyzed type II collagen cleavage in subepiphyseal growth plate area of wild-type, *ctsk*^{-/-} and *idua*^{-/-} tibia (red or yellow for neoepitopes; green is enhanced background using the green filter to visualize the joint structure). Whereas the *ctsk*^{-/-} sample does not reveal any collagen epitope release and the wild-type sample displays abundant collagen degradation, the MPS I sample only shows minor type II cleavage by cathepsin K.

Fig. 3.

Proposed mechanisms of GAG mediated osteoclast dysfunction. 1) Inhibition of cathepsin K in the resorption lacunae resulting in incomplete collagen degradation. 2) Inhibition of cathepsin K resulting in a lack of specific attachment factors for osteoclast sealing zone formation. 3) Vacuolation of lysosomes impeding trafficking of collagen fragments from the resorption lacunae to the apical membrane and possible inhibition of cathepsin K prevent complete degradation of collagen in lysosomes. 4) Promotion of osteoclast differentiation from mononuclear cells. N, nucleus; SZ, sealing zone; SV, secretory vesicle; TV, transcytotic vesicle; Cat K, cathepsin K.

Fig. 4.
Scheme of the potential interactions of MPS-related glycosaminoglycans with cathepsin K and their consequences to collagen degradation.

Table 1

Known and proposed effects of GAGs on osteoclast mediated bone resorption

Name	Accumulated GAG substrate	Proposed effect of high GAG conc on Cat K collagenolytic activity	Known effect of GAGs on bone cells
MPS I	Dermatan Sulfate	Inhibition	Promotes osteoclast differentiation [43]
MPS II	Heparan Sulfate		
MPS VI			
MPS III A-D	Heparan Sulfate	Inhibition	Promotes osteoclast differentiation [43]
MPS IV A/B	Keratan Sulfate	Activation; at higher concentration inhibition	Binding factor for osteoblasts [46]
	Chondroitin sulfate		
MPS VI	Dermatan Sulfate	Inhibition	Promotes osteoclast differentiation [43]
MPS VII	Dermatan Sulfate	Inhibition	
	Heparan Sulfate		
	Chondroitin Sulfate		
MPS IX	Hyaluronic Acid	Inhibition	Inhibits osteoclast differentiation [7]