Journal of Pediatric Rehabilitation Medicine: An Interdisciplinary Approach 3 (2010) 139–146
DOI 10.3233/PRM-2010-0116
IOS Press


Basic Science Review

# Potential role of cathepsin K in the pathophysiology of mucopolysaccharidoses

Susan Wilson and Dieter Brömme*
*From the Department of Oral Biological and Medical Sciences, Faculty of Dentistry, University of British Columbia, Vancouver, Canada*

Accepted 8 March 2010

**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 osteosclerot-

*Address for correspondence: Dieter Bromme, University of British Columbia, 2350 Health Sciences Mall, Life Sciences Institute, Rm 4558, Vancouver, V6T 1Z3, Canada. Tel.: +1 604 822 1787; Fax: +1 604 822 3562; E-mail: dbromme@interchange.ubc.ca.

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 Chondroitin sulfate | Activation; at higher concentration inhibition | Binding factor for osteoblasts [46] |
| MPS VI | Dermatan Sulfate | Inhibition | Promotes osteoclast differentiation [43] |
| MPS VII | Dermatan Sulfate Heparan Sulfate Chondroitin Sulfate | Inhibition | |
| MPS IX | Hyaluronic Acid | Inhibition | Inhibits osteoclast differentiation [7] |

ic 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 sufates 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 mucoplysaccharidoses 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

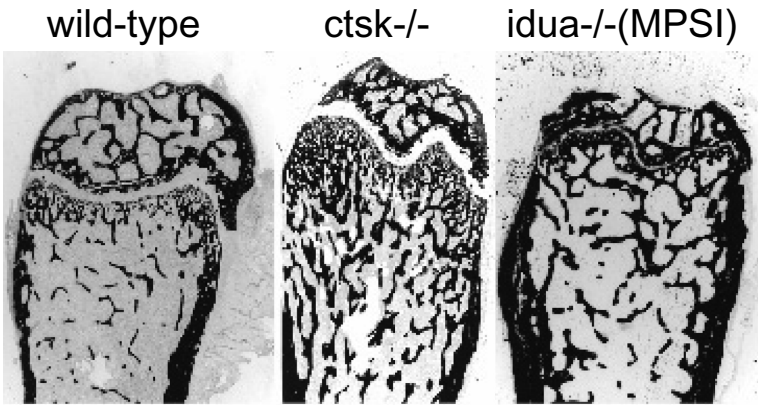


Fig. 1. Longitudinal sections of distal mice femura. 5$\mu$M methylmetacrylate sections (von Kossa staining) show thickened cortical bone and more trabecular bone area in the MPS I specimen (right panel). The MPS I femur also appears wider than the control wild-type specimen (left panel). The cathepsin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

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 $\alpha$-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 payed 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 wild-type 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,


Fig. 2. Neoepitope staining of cathepsin K catalyzed type II collagen cleavage in subepiphysial 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.

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 $\alpha$-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 colla-

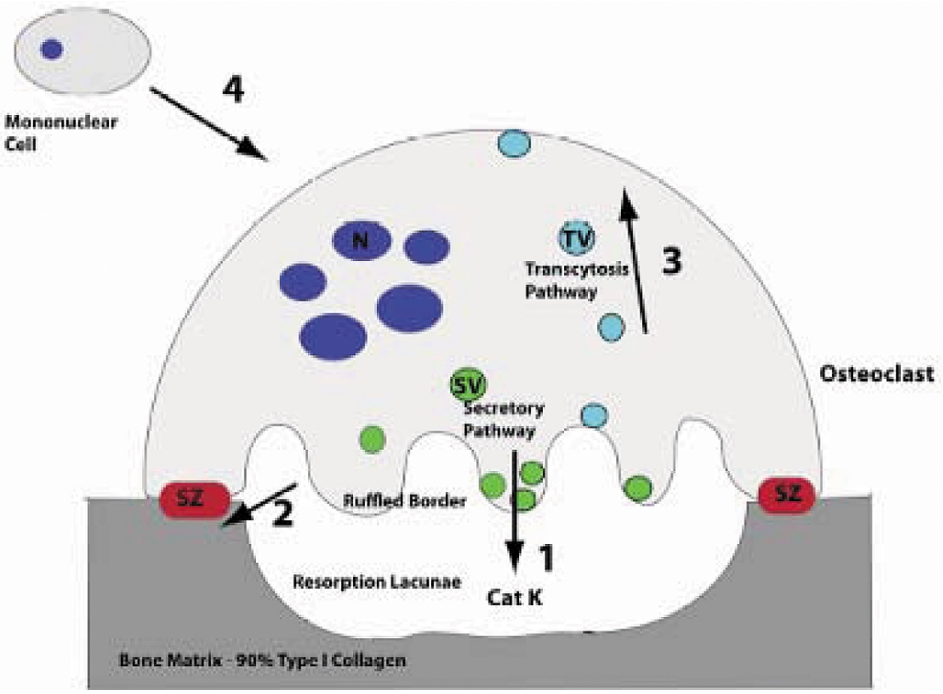


Fig. 3. Proposed mechanisms of GAG mediated osteoclast dysfunction. 1) Inhibition of cathepsin K in the resorption lacunae resulting in incomplete collagen degradation. 2) Inhibition of cathepsin K resulting in a lack of specific attachment factors for osteoclast sealing zone formation. 3) Vacuolation 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.

gen. 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

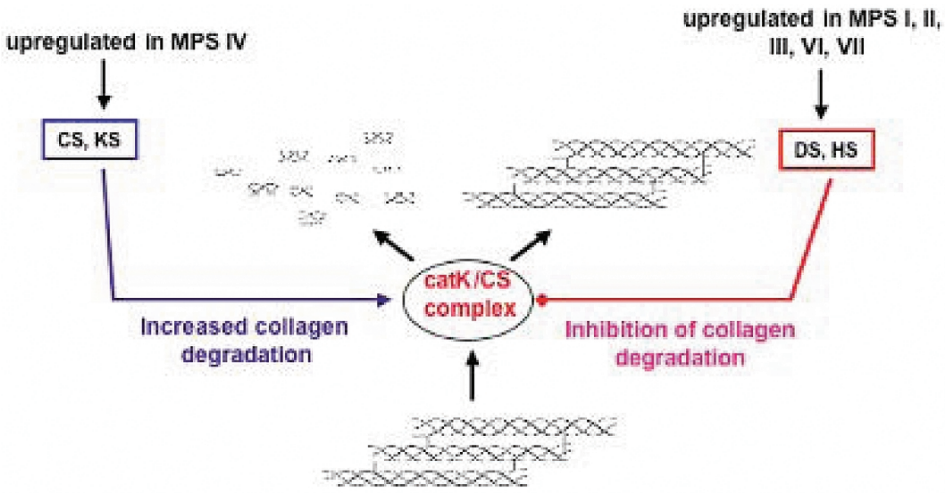


Fig. 4. Scheme of the potential interactions of MPS-related glycosaminoglycans with cathepsin K and their consequences to collagen degradation.

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.

## Acknowledgements

This work was supported by a National Institutes of Health grant DK072070 (D.B). We thank Dr. Schaffler, Mount Sinai School of Medicine in New York, NY, for helping with Fig. 1 (right panel). There is no conflict of interest.

## References

[1] F.A. Abraham, S. Yatziv, A. Russell and E. Auerbach, A family with two siblings affected by Morquio syndrome (MPS IV). Electrophysiological and psychophysical findings in the visual system, *Arch Ophthalmol* **91** (1974), 265–269.

[2] S. Abreu, J. Hayden, P. Berthold, I.M. Shapiro, S. Decker, D. Patterson and M. Haskins, Growth plate pathology in feline mucopolysaccharidosis VI, *Calcif Tissue Int* **57** (1995), 185–190.

[3] J.R. Bishop, M. Schuksz and J.D. Esko, Heparan sulphate proteoglycans fine-tune mammalian physiology, *Nature* **446** (2007), 1030–1037.

[4] A.L. Boskey, B.D. Gelb, E. Pourmand, V. Kudrashov, S.B. Doty, L. Spevak and M.B. Schaffler, Ablation of cathepsin k activity in the young mouse causes hypermineralization of long bone and growth plates, *Calcif Tissue Int* **84** (2009), 229–239.

[5] D. Bromme and F. Lecaille, Cathepsin K inhibitors for osteoporosis and potential off-target effects, *Expert Opin Investig Drugs* **18** (2009), 585–600.

[6] S. Byers, J.D. Nuttall, A.C. Crawley, J.J. Hopwood, K. Smith and N.L. Fazzalari, Effect of enzyme replacement therapy on bone formation in a feline model of mucopolysaccharidosis type VI, *Bone* **21** (1997), 425–431.

[7] E.J. Chang, H.J. Kim, J. Ha, J. Ryu, K.H. Park, U.H. Kim, Z.H. Lee, H.M. Kim, D.E. Fisher and H.H. Kim, Hyaluronan inhibits osteoclast differentiation via Toll-like receptor 4, *J Cell Sci* **120** (2007), 166–176.

[8] L.A. Clarke, C.S. Russell, S. Pownall, C.L. Warrington, A. Borowski, J.E. Dimmick, J. Toone and F.R. Jirik, Murine mucopolysaccharidosis type I: targeted disruption of the murine alpha-L-iduronidase gene, *Hum Mol Genet* **6** (1997), 503–511.

[9] L.A. Clarke, The mucopolysaccharidoses: a success of molecular medicine, *Expert Rev Mol Med* **10** (2008), e1.

[10] F.P. Coxon and A. Taylor, Vesicular trafficking in osteoclasts, *Semin Cell Dev Biol* **19** (2008), 424–433.

[11] V. Everts, D.C. Aronson and W. Beertsen, Phagocytosis of bone collagen by osteoclasts in two cases of pycnodysostosis, *Calcif Tissue Int* **37** (1985), 25–31.

[12] R.E. Field, J.A. Buchanan, M.G. Copplemans and P.M. Aichroth, Bone-marrow transplantation in Hurler's syndrome. Effect on skeletal development, *J Bone Joint Surg Br* **76** (1994), 975–981.

[13] N. Fratzl-Zelman, A. Valenta, P. Roschger, A. Nader, B.D. Gelb, P. Fratzl and K. Klaushofer, Decreased bone turnover and deterioration of bone structure in two cases of pycnodysostosis, *J Clin Endocrinol Metab* **89** (2004), 1538–1547.

[14] P. Garnero, O. Borel, I. Byrjalsen, M. Ferreras, F.H. Drake, M.S. McQueney, N.T. Foged, P.D. Delmas and J.M. Delaisse, The collagenolytic activity of cathepsin K is unique among

mammalian proteinases, *J Biol Chem* **273** (1998), 32347–32352.
[15] B.D. Gelb, G.P. Shi, H.A. Chapman and R.J. Desnick, Pycnodysostosis, a lysosomal disease caused by cathepsin K deficiency, *Science* **273** (1996), 1236–1238.
[16] W. Kafienah, D. Bromme, D.J. Buttle, L.J. Croucher and A.P. Hollander, Human cathepsin K cleaves native type I and II collagens at the N-terminal end of the triple helix, *Biochem J* **331** (1998), 727–732.
[17] T. Kalteis, T. Schubert, W.C. Caro, J. Schroder, C. Luring and J. Grifka, Arthroscopic and histologic findings in Morquio's syndrome, *Arthroscopy* **21** (2005), 233–237.
[18] H.M. Koura, A. El-Katoury, N.I. Abdallah, H.T. El-Bassyouni, D.F. Ayoub and R.I. Bassiouni, Bone Mineral Density in Egyptian Children with Mucopolysaccharidoses, *research Journal of Medicien and Medical ciences* **4** (2009), 100–1006.
[19] C. Le Gall, E. Bonnelye and P. Clezardin, Cathepsin K inhibitors as treatment of bone metastasis, *Curr Opin Support Palliat Care* **2** (2008), 218–222.
[20] F. Lecaille, D. Bromme and G. Lalmanach, Biochemical properties and regulation of cathepsin K activity, *Biochimie* **90** (2008), 208–226.
[21] F. Lecaille, J. Kaleta and D. Bromme, Human and parasitic papain-like cysteine proteases: their role in physiology and pathology and recent developments in inhibitor design, *Chem Rev* **102** (2002), 4459–4488.
[22] Z. Li, M. Kienetz, M.M. Cherney, M.N. James and D. Bromme, The crystal and molecular structures of a cathepsin K:chondroitin sulfate complex, *J Mol Biol* **383** (2008), 78–91.
[23] Z. Li, W.S. Hou, C.R. Escalante-Torres, B.D. Gelb and D. Bromme, Collagenase activity of cathepsin K depends on complex formation with chondroitin sulfate, *J Biol Chem* **277** (2002), 28669–28676.
[24] Z. Li, W.S. Hou and D. Bromme, Collagenolytic activity of cathepsin K is specifically modulated by cartilage-resident chondroitin sulfates, *Biochemistry* **39** (2000), 529–536.
[25] Z. Li, Y. Yasuda, W. Li, M. Bogyo, N. Katz, R.E. Gordon, G.B. Fields and D. Bromme, Regulation of collagenase activities of human cathepsins by glycosaminoglycans, *J Biol Chem* **279** (2004), 5470–5479.
[26] J. McClure, P.S. Smith, G. Sorby-Adams and J. Hopwood, The histological and ultrastructural features of the epiphyseal plate in Morquio type A syndrome (mucopolysaccharidosis type IVA), *Pathology* **18** (1986), 217–221.
[27] J.J. McGill, A.C. Inwood, D.J. Coman, M.L. Lipke, D. de Lore, S.J. Swiedler and J.J. Hopwood, Enzyme replacement therapy for mucopolysaccharidosis VI from 8 weeks of age – a sibling control study, *Clin Genet* (2009).
[28] J.A. Metcalf, Y. Zhang, M.J. Hilton, F. Long and K.P. Ponder, Mechanism of shortened bones in mucopolysaccharidosis VII, *Mol Genet Metab* (2009).
[29] M.A. Monroy, F.P. Ross, S.L. Teitelbaum and M.S. Sands, Abnormal osteoclast morphology and bone remodeling in a murine model of a lysosomal storage disease, *Bone* **30** (2002), 352–359.
[30] J.D. Nuttall, L.K. Brumfield, N.L. Fazzalari, J.J. Hopwood and S. Byers, Histomorphometric analysis of the tibial growth plate in a feline model of mucopolysaccharidosis type VI, *Calcif Tissue Int* **65** (1999), 47–52.
[31] G.M. Pastores, Musculoskeletal complications encountered in the lysosomal storage disorders, *Best Pract Res Clin Rheumatol* **22** (2008), 937–947.
[32] N. Perrimon and M. Bernfield, Cellular functions of proteoglycans – an overview, *Semin Cell Dev Biol* **12** (2001), 65–67.
[33] D. Rigante and P. Caradonna, Secondary skeletal involvement in Sanfilippo syndrome, *Qjm* **97** (2004), 205-209.
[34] D. Rigante, P.S. Buonuomo and P. Caradonna, Early-onset osteoporosis with high bone turnover in children with Morquio-Brailsford syndrome, *Rheumatol Int* **26** (2006), 1163–1164.
[35] P.J. Roughley, The structure and function of cartilage proteoglycans, *Eur Cell Mater* **12** (2006), 92–101.
[36] C. Russell, G. Hendson, G. Jevon, T. Matlock, J. Yu, M. Aklujkar, K.Y. Ng and L.A. Clarke, Murine MPS I: insights into the pathogenesis of Hurler syndrome, *Clin Genet* **53** (1998), 349–361.
[37] P. Saftig, O. Wehmeyer, E. Hunziker, S. Jones, A. Boyde, W. Rommerskirch and K. von Figura, Impaired osteoclastic bone resorption leads to osteopetrosis in cathepsin K-deficient mice, *Proc Natl Acad Sci USA* **95** (1998), 13453–13458.
[38] P.C. Schultheiss, S.A. Gardner, J.M. Owens, D.A. Wenger and M.A. Thrall, Mucopolysaccharidosis VII in a cat, *Vet Pathol* **37** (2000), 502–505.
[39] C.P. Silveri, F.S. Kaplan, M.D. Fallon, E. Bayever and C.S. August, Hurler syndrome with special reference to histologic abnormalities of the growth plate, *Clin Orthop Relat Res* (1991), 305–311.
[40] C.M. Simonaro, M. D'Angelo, M.E. Haskins and E.H. Schuchman, Joint and bone disease in mucopolysaccharidoses VI and VII: identification of new therapeutic targets and biomarkers using animal models, *Pediatr Res* **57** (2005), 701–707.
[41] C.M. Simonaro, M.E. Haskins and E.H. Schuchman, Articular chondrocytes from animals with a dermatan sulfate storage disease undergo a high rate of apoptosis and release nitric oxide and inflammatory cytokines: a possible mechanism underlying degenerative joint disease in the mucopolysaccharidoses, *Lab Invest* **81** (2001), 1319–1328.
[42] Z. Stark and R. Savarirayan, Osteopetrosis, *Orphanet J Rare Dis* **4** (2009), 5.
[43] S. Theoleyre, S. Kwan Tat, P. Vusio, F. Blanchard, J. Gallagher, S. Ricard-Blum, Y. Fortun, M. Padrines, F. Redini and D. Heymann, Characterization of osteoprotegerin binding to glycosaminoglycans by surface plasmon resonance: role in the interactions with receptor activator of nuclear factor kappaB ligand (RANKL) and RANK, *Biochem Biophys Res Commun* **347** (2006), 460–467.
[44] H.K. Vaananen and T. Laitala-Leinonen, Osteoclast lineage and function, *Arch Biochem Biophys* **473** (2008), 132–138.
[45] C. Vogler, E.H. Birkenmeier, W.S. Sly, B. Levy, C. Pegors, J.W. Kyle and W.G. Beamer, A murine model of mucopolysaccharidosis VII. Gross and microscopic findings in beta-glucuronidase-deficient mice, *Am J Pathol* **136** (1990), 207–217.
[46] M. Wendel, Y. Sommarin and D. Heinegard, Bone matrix proteins: isolation and characterization of a novel cell-binding keratan sulfate proteoglycan (osteoadherin) from bovine bone, *J Cell Biol* **141** (1998), 839–847.
[47] S.R. Wilson, C. Peters, P. Saftig and D. Bromme, Cathepsin K Activity-dependent Regulation of Osteoclast Actin Ring Formation and Bone Resorption, *J Biol Chem* **284** (2009), 2584–2592.
[48] S. Wilson, S. Hashamiyan, L. Clarke, P. Saftig, J. Mort, V.M. Dejica and D. Bromme, Glycosaminoglycan-mediated loss of cathepsin K collagenolytic activity in MPS I contributes to osteoclast and growth plate abnormalities, *Am J Pathol* **175** (2009), 2053–2062.
[49] Y. Yasuda, J. Kaleta and D. Bromme, The role of cathepsins in osteoporosis and arthritis: rationale for the design of new

therapeutics, *Adv Drug Deliv Rev* **57** (2005), 973–993.
[50] M.F. Young, Y. Bi, L. Ameye, T. Xu, S. Wadhwa, A. Heegaard, T. Kilts and X.D. Chen, Small leucine-rich proteoglycans in the aging skeleton, *J Musculoskelet Neuronal Interact* **6** (2006), 364–365.
[51] Q. Zhao, Y. Jia and Y. Xiao, Cathepsin K: a therapeutic target for bone diseases, *Biochem Biophys Res Commun* **380** (2009), 721–723.