# Role of Cathepsin K in the Turnover of the Dermal Extracellular Matrix during Scar Formation

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Cathepsins are a group of cystein proteinases that are involved in various aspects of extracellular matrix turnover. The collagenolytic activity of cathepsin K plays a pivotal role in bone resorption and lung matrix homeostasis, but so far has not been described in skin. To study the role of cathepsin K in the turnover of the cutaneous extracellular matrix, we studied the expression of cathepsin K in human skin and in cultured primary neonatal skin fibroblasts. Normal skin exhibited only low levels or no expression of cathepsin K. In contrast, dermal fibroblasts in surgical scars showed strong cytoplasmic cathepsin K expression. Cathepsin K expression was most prominent in young scars and declined with time. Cultured neonatal primary fibroblasts showed strong cathepsin K staining in the perinuclear endosomal compartment, consistent with intracellular degradation of internalized collagen in lysosomes. Cathepsin K was also found to be strongly expressed in keloids and dermatofibromas, but not in sclerotic areas of morphea. Our data suggest that cathepsin K may play an important role in the homeostasis of the dermal extracellular matrix and the dynamic equilibrium between matrix synthesis and proteolytic degradation, by counteracting deposition of matrix proteins during scar formation with its matrix-degrading activity.

## INTRODUCTION

Cathepsins are a group of papain-cystein proteases with collagenolytic and elastinolytic activities that are involved in various aspects of extracellular matrix turnover (Everts et al., 1996; Chapman et al., 1997). Several cathepsins, including cathepsins L and G, have been described to be expressed in skin or skin-derived cells (Breuckmann et al., 2002; Tobin et al., 2002; Potts et al., 2004; Zhao et al., 2005, and others), but so far not cathepsin K.

Cathepsin K is well established to play a pivotal role in bone resorption (Drake et al., 1996; Garnero et al., 1996; Yamaza et al., 1998; Xia et al., 1999; Goto et al., 2003). Its collagenolytic properties are unique in that it cleaves type I and type III collagen at the end and at multiple sites within the native triple helix. In addition, it is the most potent human elastase yet described. It is a lysosomal enzyme that has an acidic pH optimum of 5.5 and limited stability at neutral pH (Bossard et al., 1996). It is synthesized in mature osteoclasts as an inactive proenzyme. It is located in lysosomes, cleaved by autoproteolysis to produce the active form, and then secreted into the bone resorption lacunae (McQueney et al., 1997; Dodds et al., 2001; Rieman et al., 2001). It contributes to bone resorption by degrading collagen fibrils in the acidic bone resorption zones and by degrading fragmented collagens fibrils in endolysosomes after phagocytosis. The role of cathepsin K in bone resorption is further underlined by findings that cathepsin K mutations lead to pycnodysostosis, an autosomal-recessive osteochondrodysplasia characterized by defective bone matrix degradation (Gelb et al., 1996), and that cathepsin K-knockout mice develop osteopetrosis (abnormally dense bone) owing to defective bone resorption (Saftig et al., 1998).

Cathepsin K-knockout mice are also more prone to develop bleomycin-induced lung fibrosis (Bühling et al., 2004), indicating that cathepsin K also plays an important role in the homeostasis of the extracellular matrix in the lung. This is further supported by reports that in a mouse model of silica-induced lung fibrosis cathepsin K is strongly upregulated in fibroblasts of fibrotic lungs (van den Brûle et al., 2005).

In addition, cathepsin K has also been shown to be expressed in synovial fibroblasts from joints of patients with rheumatoid arthritis, where it is thought to contribute to cartilage and subcortical bone degradation (Hou et al., 2001).

In order to study a possible role of cathepsin K in the homeostasis of the cutaneous extracellular matrix, we studied expression of cathepsin K in normal skin, surgical scars of various age (time since surgical procedure ranging from 1 month to 3 years), keloids, dermatofibromas, sclerotic plaques of morphea, and in primary human neonatal fibroblasts cultured on glass slides using immune-histochemistry. In addition, Western blot analysis was used to evaluate cathepsin K expression in cultured primary dermal fibroblasts.

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## RESULTS

Western blot analysis and immune-histochemistry clearly demonstrate expression of cathepsin K in cultured human fibroblasts (Figure 1). Comparison of exponentially growing cells with cells grown to confluence shows stronger cathepsin K expression in the confluent cells in Western blots, affecting both the procathepsin K and the active cathepsin K (Figure 1a). Within cells, cathepsin K staining is found in the perinuclear endosomal compartment (Figure 1b).

Within apparent normal skin, peripheral to the scar, there is only weak or no expression of cathepsin K in dermal fibroblasts (Figure 2). In stark contrast to the normal skin, dermal fibroblasts of surgical scars show strong cathepsin K staining (Figure 2). The most prominent staining is observed in young scars, and a less intensive staining in older scars, at least up to 18 months after the surgical procedure. A 3-year-old scar did not show any cathepsin K expression (data not shown). Epidermal keratinocytes overlying some, but not all young surgical scars, also demonstrated intracellular cathepsin K expression (Figure 2). Such staining of epidermal keratinocytes was not observed in any area outside the surgical scars in the scar specimens.

Strong cytoplasmic cathepsin K expression was also observed in keloids and a dermatofibroma (Figure 3). In contrast, no cathepsin K expression was observed in the sclerotic areas of morphea (Figure 4). When a particular sample shows no staining at all, a false-negative staining cannot be ruled out completely, even if other samples that were processed in parallel do show positive staining. However, in some of the morphea specimens, a weak staining of fibroblasts was seen focally in the normal dermis overlying the fibrotic areas (Figure 4b). This serves as a positive internal control for the otherwise negative cathepsin K staining in this sample, as it demonstrates adequate staining technique.

## DISCUSSION

The homeostasis of the extracellular matrix depends on a dynamic equilibrium between synthesis and degradation of extracellular matrix proteins. It is tightly controlled under physiologic conditions, and its disruption may lead to either fibrosing or atrophic conditions. During wound healing and scar formation, this equilibrium is initially shifted to a profibrotic state in order to accommodate the required synthesis of new extracellular matrix proteins. This shift, however, also requires counteraction for remodeling of the scar and for rebalancing the equilibrium to a normal steady state after completion of wound healing.

Our data introduce cathepsin K as a new antifibrotic player in the homeostasis of extracellular matrix proteins in the skin. Although in the steady state of normal skin, cathepsin K is not or only weakly expressed, its proteolytic activities appear to be required for rebalancing the homeostasis during scar formation, as it is highly expressed in dermal fibroblasts of scars. Presumably, this counteracts excessive matrix deposition, facilitates remodeling, and prevents formation of hypertrophic scars. This model corresponds to the suggested roles of cathepsin K in fibroblasts of other organs: in lung fibroblasts, where it counteracts damage-induced fibrosis (Bühling *et al.*, 2004; van den Brûle *et al.*, 2005); in synovial fibroblasts, where it mediates cartilage and bone resorption in pathologic states (Hou *et al.*, 2001); and in fibroblasts of ligaments, where it is upregulated after subfailure (sprain) injury.

Consistent with its well-known character of a lysosomal protein, our data show that cathepsin K is localized in the perinuclear endosomal compartment also in dermal fibroblasts. Fibroblasts are known to internalize extracellular collagen after binding of collagen to collagen receptors (e.g., $\alpha_2\beta_1$ integrins) (Lee *et al.*, 1996), and inhibition of cathepsin K has been shown to result in intralysosomal collagen accumulation in synovial fibroblasts (Hou *et al.*, 2001). As cathepsin K is active only at low pH and normally confined to the lysosomal network (Bossard *et al.*, 1996; McQueney *et al.*, 1997; Dodds *et al.*, 2001; Rieman *et al.*, 2001), we assume that in skin fibroblasts its activity is also limited to lysosomes and that it cleaves dermal collagen after binding of collagen to the fibroblasts' collagen receptors and internalization of collagen through phagocytosis.

Pro-cathepsin K, 40 kDa

Active cathepsin K, 28 kDa

Actin
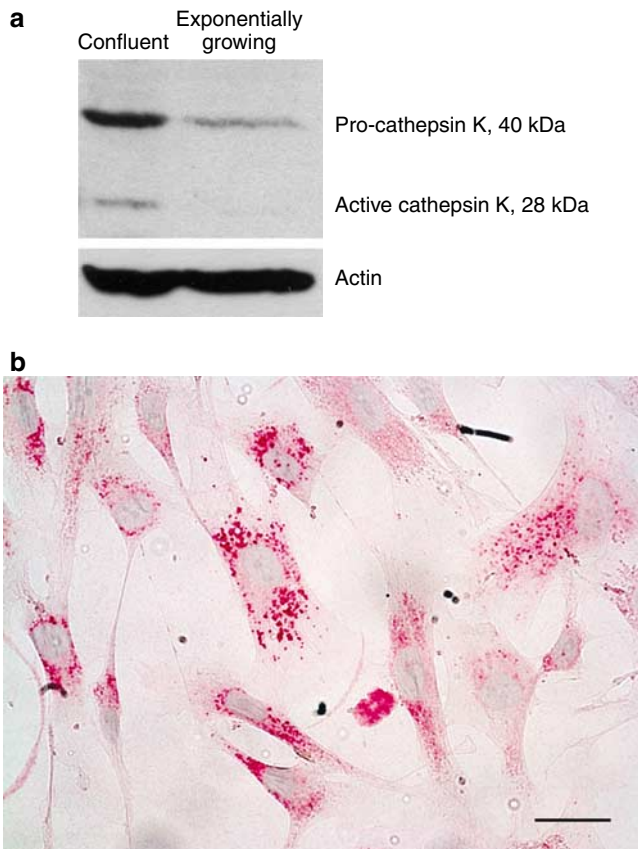
**Figure 1**. **Expression of cathepsin K in surgical scars.** Cathepsin K is expressed in cultured human neonatal dermal fibroblasts. (**a**) Western blot and (**b**) immune-histochemistry of cells grown on glass slides, showing localization of cathepsin K to the perinuclear endosomal compartment. Bar = 20 μm.
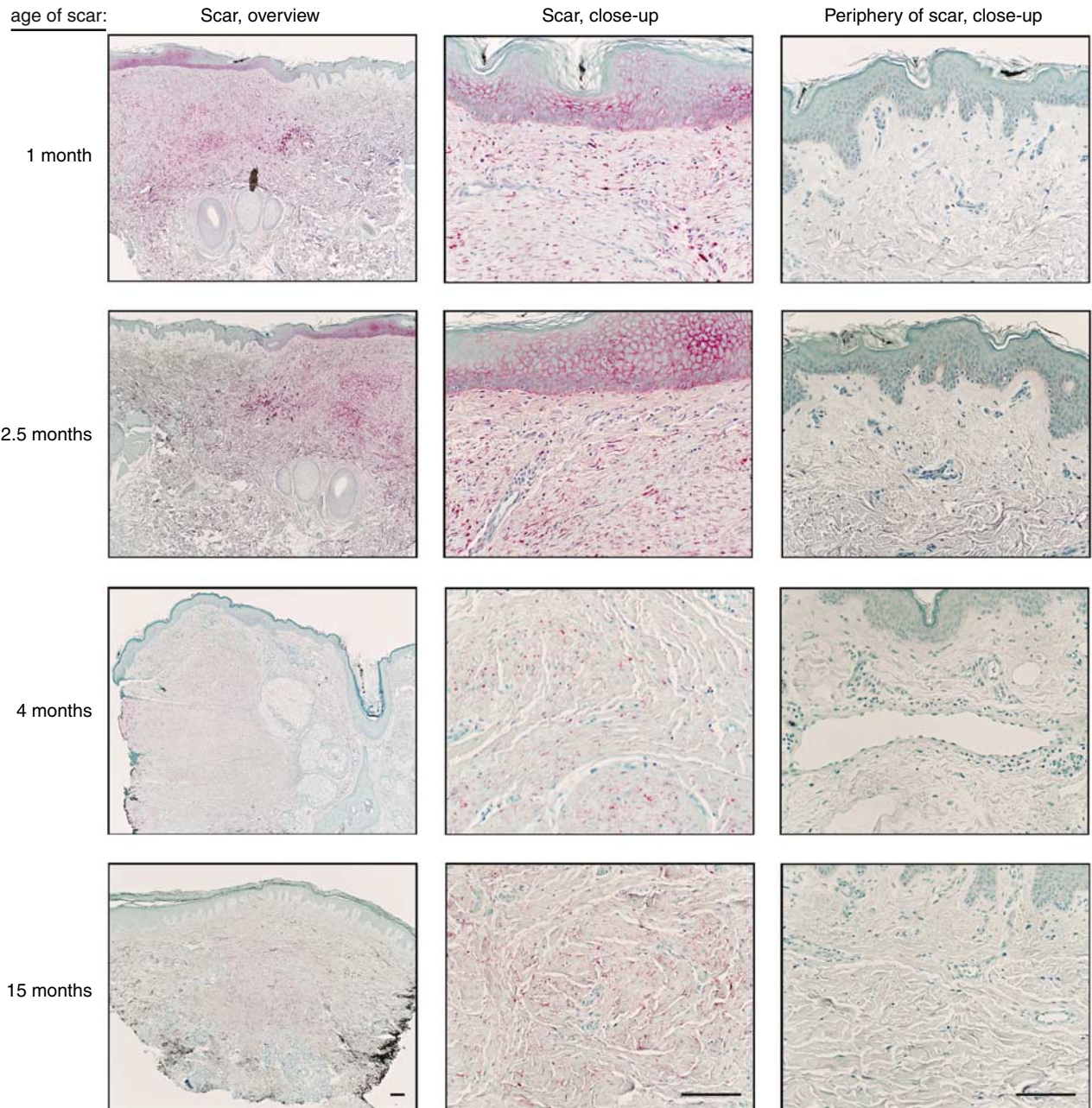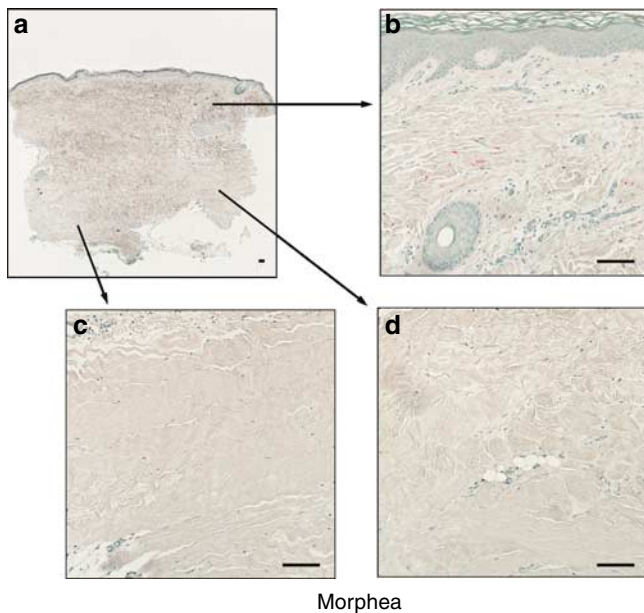
**Figure 2**. **Expression of cathepsin K in surgical scars.** Cathepsin K expression is more prominent in younger scars (1–2.5 months after surgical procedure) than in older scars (4–15 months after surgical procedure). Shown is a representative selection of the 43 scars examined. Bars = 100 $\mu$m for each column of panels.

In bone resorption, osteoclasts generate an acidic microenvironment in the bone resorption lacunae that allows secreted active cathepsin K to exert its proteolytic activity also in the extracellular space. Little is known about extracellular functions of cathepsin K in other cell types. Monocyte-derived macrophages have been shown to be capable to secrete cathepsin K, to acidify the pericellular milieu, and thus to maintain the enzyme in its active form (Punturieri *et al.*, 2000). It is perceivable that in acute wounds with their acidic milieu created, for example, by inflammatory neutrophil infiltrates, secreted cathepsin K could be active in the extracellular space. In the scars that we examined, however, in the absence of an inflammatory infiltrate, it is highly unlikely that the extracellular milieu is sufficiently acidic to allow cathepsin K to be active in the extracellular space. We therefore believe that the role of cathepsin K in the remodeling of cutaneous scars is primarily mediated through the above-mentioned intracellular action. This would be in contrast to the known role of secreted metalloproteinases in wound healing and their activity in the extracellular space (Everts *et al.*, 1996; Mott and Werb, 2004).
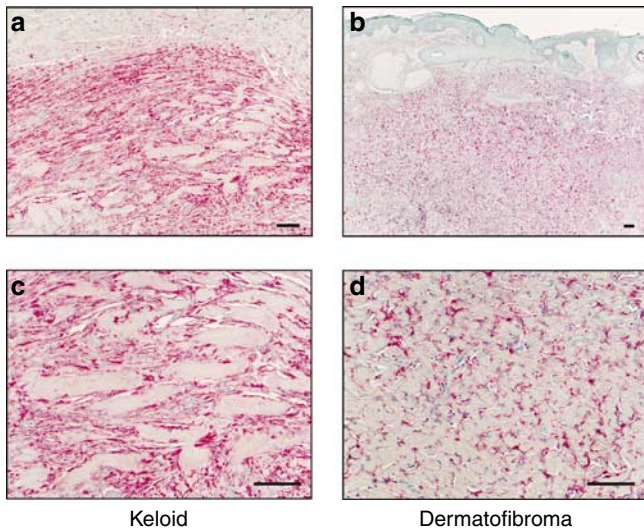
**Figure 3. Strong expression of cathepsin K in a keloid and a dermatofibroma.** Panels **a** and **c** show a keloid, panels **b** and **d** a dermatofibroma. All three keloids examined showed a similar strong cathepsin K expression. Bars = 100 $\mu$m.



**Figure 4. No expression of cathepsin K in the sclerotic areas of morphea.** Overview in **a**, close-up of sclerotic areas in **c** and **d**, whereas fibroblasts in the overlying normal dermis (**b**) show weak expression of cathepsin K (positive internal control). None of the five specimens of morphea examined showed any cathepsin K reactivity in the sclerotic areas. Bars = 100 $\mu$m.

Our finding that older scars demonstrate lower cathepsin K expression than younger scars could be related to different proliferative activities of dermal fibroblasts in young and older scars. However, our finding that proliferating neonatal fibroblasts express less cathepsin K than fibroblasts grown to confluence makes that explanation unlikely. Rather, it appears that cathepsin K expression is related to a higher

cell density. This in turn suggests an autocrine mechanism involving a cathepsin K-inducing mediator. A cell density-dependent cathepsin K expression might also explain why the fibroblasts in the cell-rich keloids and in dermatofibromas strongly express cathepsin K, whereas the few scattered fibroblasts in the sclerotic areas of morphea do not. Therefore, we do not want to affirm that the excessive deposition of extracellular matrix proteins in morphea is related to an insufficient cathepsin K expression of dermal fibroblasts after skin trauma, although this remains a possibility.

Cathepsin K expression by keratinocytes was limited to the epidermis overlying some young scars. The epidermis overlying young scars is most commonly flat, as rete ridges have not yet been formed. As keratinocyte migration is known to be greatly dependent on contact with the various extracellular matrix proteins (O'Toole, 2001), it is perceivable that the cathepsin K expression of keratinocytes in these areas, possibly in concert with keratinocyte-derived matrix metalloproteinases, enables keratinocytes to grow into the dermis for the formation of rete ridges.

Any attempts to use the antifibrotic properties of cathepsin K for treatment of skin disease would need to take into account that its activity is limited to acidic milieus. An induction of cathepsin K expression might be an avenue to use its properties for treatment of fibrotic skin diseases or for avoiding overly fibrotic scaring. Based on our data, this would be particularly promising in fibroblast-rich fibrosing conditions that do not express sufficient amounts of cathepsin K and less promising in skin fibroses that are characterized by low cellularity, such as morphea.

Inhibition of cathepsin K has been suggested to be a very promising strategy for the treatment of osteoporosis or rheumatoid arthritis (Chapman *et al.*, 1997; Grabowskal *et al.*, 2005; Yasuda *et al.*, 2005). Such an inhibition of cathepsin, however, has been warned to possibly increase the risk of lung fibrosis (Bühling *et al.*, 2004). Based on our data, we would like to extend that warning to the skin, suggesting that systemic inhibition of cathepsin K might make skin more prone to excessive matrix protein deposition in response to trauma.

## MATERIALS AND METHODS

Primary human fibroblasts were derived from dermal explants of neonatal foreskin as described previously (Stanulis-Praeger and Gilchrest, 1989) and cultured in DMEM (Gibco/BRL, Rockland, MA) supplemented with 10% calf serum at 37°C and 5% $CO_2$. For cathepsin K immunostaining, cells were grown on glass slides. For Western blot analysis, cells were grown in regular tissue culture dishes and protein was harvested either from exponentially growing cells or from cells that were growth arrested by confluence for 4 days.

Western blot analysis was performed according to standard procedures using a 10% polyacrylamide gel, a rabbit polyclonal cathepsin K antibody (ABcam, Cambridge, MA) at a dilution of 1:200 that detects both procathepsin K and the active cathepsin K, an enhanced chemiluminescent rabbit IgG horseradish peroxidase-linked secondary whole antibody (Amersham Biosciences; Piscataway, NJ) at a dilution of 1:2,000, and the Western lightning

chemiluminescence reagent (Perkin-Elmer, Wellesley, MA) for autoradiographic detection. For loading control, actin was detected using a goat polyclonal actin horseradish peroxidase-conjugated antibody (Santa Cruz Biotechnology; Santa Cruz, CA) at a dilution of 1:1,000.

Formalin-fixed paraffin-embedded sections of surgical scars ($n = 43$) of various age (time since surgery ranging from 1 month to 3 years), keloids ($n = 3$), sclerotic plaques of morphea ($n = 5$), and a dermatofibroma, and of fixed fibroblasts grown on glass slides were stained for cathepsin K using a monoclonal anti-cathepsin K antibody (Novocastra, Newcastle upon Tyne, UK) at a dilution of 1:40, for 32 minutes on an automated immune-histochemistry system (Ventana Benchmark LT; Ventana Medical Systems, Tucson, AZ), using the Enhance V-Red detection (alkaline phosphatase red) kit (Ventana Medical Systems, Tucson, AZ) with a biotinylated secondary antibody and following the manufacturer's protocol. The slides were treated with ChemMate blocking solution A and B (Ventana Biomedical Systems, Tucson, AZ), counterstaining with hematoxylin and postcounter staining with blueing reagent (Ventana Medical Systems, Tucson, AZ). The age of tissue donors ranged from 27 to 60 years. The study was performed with institutional approval and followed the Declaration of Helsinki Principles. It was exempt from patient consent, as no patient identifiers were used.

**REFERENCES**

Bossard MJ, Tomaszek TA, Thompson SK, Amegadzie BY, Hanning CR, Jones C et al. (1996) Proteolytic activity of human osteoclasts cathepsin K. *J Biol Chem* 271:12517–24

Breuckmann F, von Kobyletzki G, Avermaete A, Kreuter A, Altmeyer P, Gamblicher T (2002) Modulation of cathepsin G expression in severe atopic dermatitis following medium-dose UVA1 phototherapy. *BMC Dermatol* 2:12

Bühling F, Röcken C, Brasch F, Hartig R, Yasuda Y, Saftig P et al. (2004) Pivotal role of cathepsin K in lung fibrosis. *Am J Pathol* 164:2203–16

Chapman HA, Riese RJ, Shi GP (1997) Emerging roles for cysteine proteases in human biology. *Ann Rev Physiol* 59:63–88

Dodds RA, James IE, Rieman D, Ahern R, Hwang SM, Connor JR et al. (2001) Human osteoclast cathepsin K is processed intracellularly prior to attachment and bone resorption. *J Bone Min Res* 16:478–86

Drake FH, Dodds RA, James IE, Connor JR, Debouck C, Richardson S et al. (1996) Cathepsin K, but not cathepsins B, L, or S, is abundantly expressed in human osteoclasts. *J Biol Chem* 271:12511–6

Everts V, van der Zee E, Creemers L, Beertsen W (1996) Phagocytosis and intracellular digestion of collagen, its role in turnover and remodelling. *Histochem J* 28:229–45

Garnero P, Borel O, Byrjalsen I, Ferreras M, Drake FH, McQueney MS et al. (1996) The collagenolytic activity of cathepsin K is unique among mammalian proteases. *J Biol Chem* 273:32347–52

Gelb BD, Shi GP, Chapman HA, Desnick RJ (1996) Pycnodysostosis, a lysosomal disease caused by cathepsin K deficiency. *Science* 273:1236–8

Goto T, Yamaza T, Tanaka T (2003) Cathepsins in the osteoclast. *J Electron Microsc* 52:551–8

Grabowskal U, Chambers TJ, Shiv S (2005) Recent advances in cathepsin K inhibitor design. *Curr Opin Drug Discov Develop* 8:619–30

Hou WS, Li Z, Gordon RE, Chan K, Klein MJ, Levy R et al. (2001) Cathepsin K is a critical protease in synovial fibroblast-mediated collagen degradation. *Am J Pathol* 159:2167–77

Lee W, Sodek J, McCulloch CA (1996) Role of integrins in regulation of collagen phagocytosis by human fibroblasts. *J Cell Physiol* 168:695–704

McQueney MS, Amegadzie BY, D'Alessio K, Hanning CR, McLaughlin MM, MCNulty D et al. (1997) Autocatalytic activation of human cathepsin K. *J Biol Chem* 272:13955–60

Mott JD, Werb Z (2004) Regulation of matrix biology by matrix metalloproteinases. *Curr Opin Cell Biol* 16:558–64

O'Toole EA (2001) Extracellular matrix and keratinocyte migration. *Exp Dermatol* 26:525–30

Potts W, Bowyer J, Jones H, Tucker D, Freemont AJ, Millest A et al. (2004) Cathepsin L-deficient mice exhibit abnormal skin and bone development and show increased resistance to osteoporosis following ovariectomy. *Int J Exp Pathol* 85:85–96

Punturieri A, Filippov S, Allen E, Caras I, Murray R, Reddy V et al. (2000) Regulation of elastinolytic cystein proteinase activity in normal and cathepsin K-deficient human macrophages. *J Exp Med* 192:789–99

Rieman DJ, McClung HA, Dodds RA, Hwang SM, Lark MW, Holmes S et al. (2001) Biosynthesis and processing of cathepsin K in cultured human osteoclasts. *Bone* 28:282–9

Saftig P, Hunziker E, Wehmeyer O, Jones S, Boyde A, Rommerskirch W et al. (1998) Impaired osteoclastic bone resorption leads to osteopetrosis in cathepsin-K-deficient mice. *Proc Natl Acad Sci USA* 95:13453–8

Stanulis-Praeger BM, Gilchrest BA (1989) Effect of donor age and prior sun exposure on growth inhibition of cultured human dermal fibroblasts by all trans-retinoic acid. *J Cell Physiol* 139:116–24

Tobin DJ, Foitzik K, Reinheckel T, Mecklenburg L, Botchkarev VA, Peters C et al. (2002) The lysosomal protease cathepsin L is an important regulator of keratinocyte and melanocyte differentiation during hair follicle morphogenesis and cycling. *Am J Pathol* 160:1807–21

van den Brûle S, Misson P, Bühling F, Lison D, Huaux F (2005) Overexpression of cathepsin K during silica-induced lung fibrosis and control by TGF-beta. *Resp Res* 6:84

Xia L, Kilb J, Wex H, Li Z, Lipyanski A, Breuil V et al. (1999) Localization of rat cathepsin K in osteoclasts and resorption pits: inhibition of bone resorption and cathepsin K-activity by peptidyl vinyl sulfones. *Biol Chem* 380:679–87

Yamaza T, Goto T, Kamiya T, Kobayashi Y, Sakai H, Tanaka T (1998) Study of immunoelectron microscopic localization of cathepsin K in osteoclasts and other bone cells in the mouse femur. *Bone* 23:499–509

Yasuda Y, Kaleta Y, Bromme D (2005) The role of cathepsins in osteoporosis and arthritis: rationale for the design of new therapeutics. *Adv Drug Deliv Rev* 57:973–93

Zhao W, Oskeritzian CA, Pozez AL, Schwartz LB (2005) Cytokine production by skin-derived mast cells: endogenous proteases are responsible for degradation of cytokines. *J Immunol* 175:2635–42