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Review

Cathepsin L in metastatic bone disease: therapeutic implications

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Abstract

Cathepsin L is a lysosomal cysteine proteinase primarily devoted to the metabolic turnover of intracellular proteins. However, accumulating evidence suggests that this endopeptidase might also be implicated in the regulation of other important biological functions, including bone resorption in normal and pathological conditions. These findings support the concept that cathepsin L, in concert with other proteolytic enzymes involved in bone remodeling processes, could contribute to facilitate bone metastasis formation. In support of this hypothesis, recent studies indicate that cathepsin L can foster this process by triggering multiple mechanisms which, in part, differ from those of the major cysteine proteinase of osteoclasts, namely cathepsin K. Therefore, cathepsin L can be regarded as an additional target in the treatment of patients with metastatic bone disease. This review discusses the clinical and therapeutic implications related to these findings.

Keywords: bone metastasis; cancer; cathepsin K; cathepsin L; cysteine proteinases; proteinase inhibitors.

Introduction

Bone metastasis is a frequent complication of certain solid tumors (Mundy, 2002). Patients with metastatic bone disease are at high risk of skeletal related events (SREs) such as pathological fracture, nerve compression syndrome, debilitating bone pain and hypercalcemia (Mundy, 2002). Current clinical treatments of this pathological condition are merely palliative and do not provide a life-prolonging benefit to metastatic patients (Mundy, 2002; Lipton, 2007). Consequently, in recent years, consistent efforts have been directed to the discovery of new molecules which could be effective to inhibit SREs and to prevent bone metastasis formation (Mundy, 2002; Lipton, 2007). These studies have identified, as possible molecular targets, several proteolytic enzymes including some cysteine proteinases, which appear to be implicated in the regulation of normal and pathological bone turnover (Maciewicz et al., 1990; Brage et al., 2005; Everts et al., 2006; Solau-Gervais et al., 2007; Charni-Ben Tabassi et al., 2008; Wilson and Singh, 2008). Among cysteine proteinases, cathepsin K, an endopeptidase predominantly expressed in osteoclasts, is currently thought to play a major role in pathological conditions associated with an altered bone resorption including cancer induced osteolysis (Kiviranta et al., 2005; Le Gall et al., 2008; Wilson and Singh, 2008). Therefore, many studies in the field of lysosomal cysteine proteinases and bone metastases, undertaken in the past decade, have focused their attention mainly on this enzyme, perhaps underscoring the potential involvement of other proteinases of this family and, in particular, that of cathepsin L, a lysosomal endopeptidase which participates in tissue degradation and extracellular matrix remodeling and which makes a significant contribution to the degradation of bone matrix (Maciewicz et al., 1990; Hill et al., 1994; Li et al., 2004; Brage et al., 2005; Kiviranta et al., 2005; Everts et al., 2006; Charni-Ben Tabassi et al., 2008; Lion et al., 2009; Ogawa et al., 2009). By contrast, the hypothesis that cathepsin L can contribute to bone metastasis formation is supported by many experimental and clinical observations, which highlight that the expression levels of this proteinase are deregulated in bone diseases with an elevated bone turnover (Hill et al., 1994; Park et al., 1996; Söderström et al., 2001; Lang et al., 2004; Lindeman et al., 2004; Schedel et al., 2004; Kido et al., 2007; Solau-Gervais et al., 2007; Husmann et al., 2008; Wilson and Singh, 2008) and that the administration of selective cathepsin L antagonists can inhibit and prevent cancer induced bone resorption in mice (Hill et al., 1994; Katunuma et al., 2002a,b; Long and Chagnovich, 2009) (Figure 1●●Figure 1 was not cited in the original paper. Please cite accordingly●●).

Cathepsin L structure and function

Cathepsin L (EC 3.4.22.15) is a lysosomal cysteine endopeptidase synthesized as a 39-kDa inactive precursor form which is normally activated in the acidic environment of lysosomes (Chauhan et al., 1993; Rawlings et al., 2010). The active form of this enzyme consists of a heavy chain of approximately 21 kDa and a light chain of approximately 5 kDa (Chauhan et al., 1993; Rawlings et al., 2010). In humans, the gene encoding for cathepsin L (*CTSL*) maps to chromosome 9q21–q22 and contains eight exons and seven introns (Chauhan et al., 1993; Rawlings et al., 2010). This locus is adjacent to another gene coding for a cathepsin L-like protein, namely cathepsin L2 (also known as cathepsin V) which share a 80% protein sequence identity with cathep-

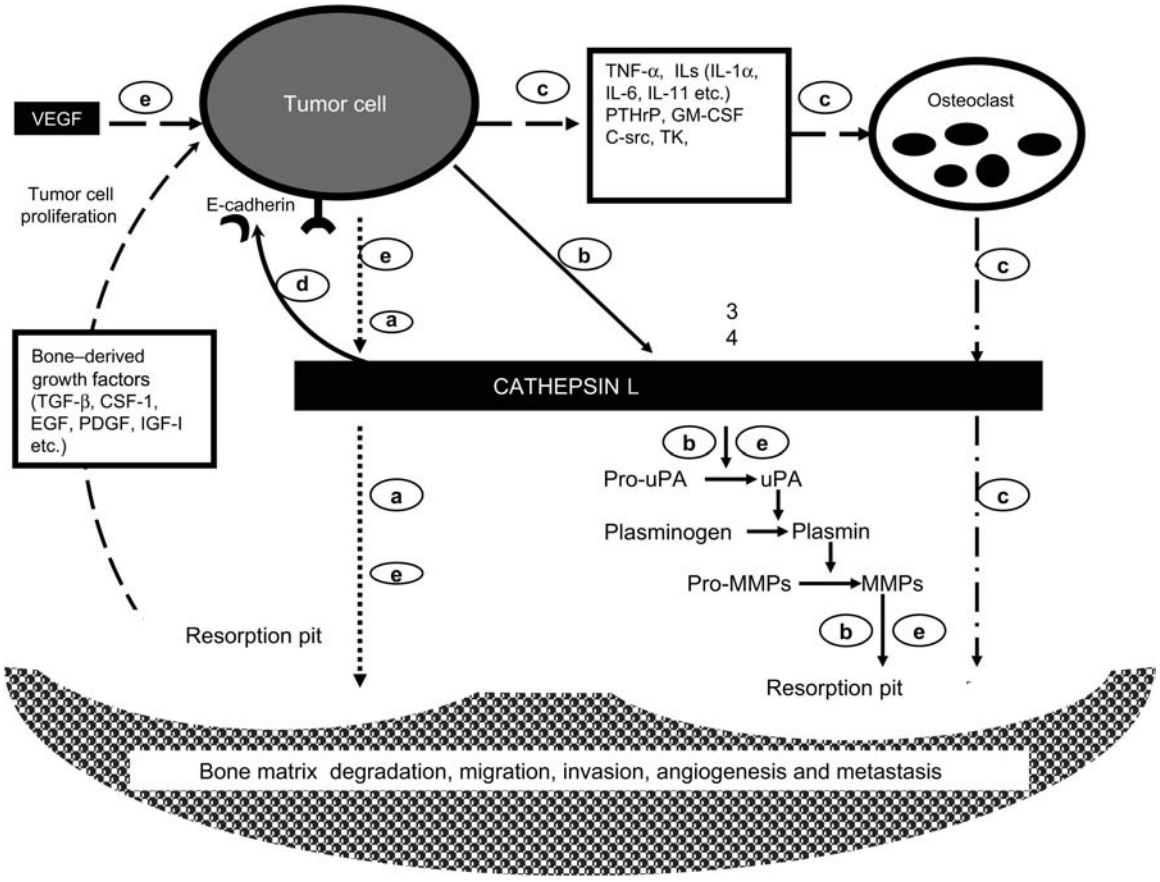

Figure 1 Schematic representation of the possible, multiple mechanisms by which cathepsin L could facilitate bone metastasis formation. (A) Cathepsin L secreted by cancer cells can directly degrade bone extracellular matrix thus promoting tumor cells invasion and metastasis. (B) Additionally, the enzyme can indirectly promote this process by triggering the activation latent precursor forms of other enzymes involved in the metastatic cascade such as matrix metalloproteinases (MMPs) and urokinase type plasminogen activator (uPA). (C) Following the upregulation of cathepsin L by proinflammatory cytokines which promote bone pit formation, the enzyme can contribute to degrade bone matrix by the mechanisms described above. (D) Similar to other proteinases such as cathepsin B and MMPs, cathepsin L can degrade E-cadherin, an adhesion molecule which is downregulated in metastatic tumor cells, thus enhancing their migratory and invasive ability. (E) Finally, cathepsin L might also facilitate bone metastasis formation by promoting tumor angiogenesis by multiple mechanisms: (i) activation of latent forms of proteolytic enzymes promoting tumor neovascularization such as uPA and MMPs and (ii) upregulation VEGF in tumor cells. See text for details and related references.

sin L and a 75% amino acid sequence identity with mouse cathepsin L (Brömme et al., 1999). However, despite their high amino acid sequence homology, these enzymes differ in their tissue distribution and biological functions. In fact, whereas cathepsin L is expressed in most eukaryotic cells, cathepsin V expression is restricted to a limited number of tissues and cells, namely corneal epithelium, epidermis, testes, thymus and activated macrophages (Chauhan et al., 1993; Brömme et al., 1999; Bernard et al., 2003; Yasuda et al., 2004). Furthermore, cathepsin L is mainly involved in the metabolic turnover of intracellular and secreted regulatory proteins (Chauhan et al., 1993; Collette et al., 2004; Rawlings et al., 2010), whereas the biological effects of cathepsin V appear to be confined to some specific immunological functions and to the regulation of epidermis homeostasis (Hagemann et al., 2004; Yasuda et al., 2004). The intracellular activity of these proteinases is regulated by endogenous inhibitors of the cystastins superfamily (Turk et al., 2008). However, the biological functions of cathepsin L are not merely confined to the breakdown of intracellular proteins but also comprise other important processes such as apoptosis and tissue homeostasis (Hagemann et al., 2004; Potts et al., 2004; Stoka et al., 2007; Luft, 2009; Dennemärker et al., 2010; Lankelma et al., 2010), immune response (Hsing and Rudensky, 2005; Zavasnik-Bergant and Turk, 2007), activation of latent forms of other proteolytic enzymes (Everts et al., 2006; Laurent-Matha et al., 2006; Abboud-Jarrous et al., 2008) and hormone production and processing (Yasothornsrikul et al., 2003; Funkelstein et al., 2008). The active role of cathepsin L in some of these processes is further confirmed by *in vivo* studies which show that cathepsin L-deficient mice exhibit distinct defects in MHC class II processing in thymic cortical epithelial cells, impaired myocardial functions, epithelial hyperplasia, hypotrichosis and

progressive hair loss (Reinheckel et al., 2001; Hagemann et al., 2004; Potts et al., 2004; Luft, 2009) and reduced production and secretion of neurotransmitters and hormones (Yasothornsrikul et al., 2003; Funkelstein et al., 2008).

Cathepsin L in bone remodeling

In addition to the biological functions described above, experimental evidence suggests that cathepsin L can also be implicated in the regulation of bone resorption in normal and pathological conditions (Table 1). This hypothesis is supported by many biochemical and immunohistochemical studies which show that this proteinase is present in rodent osteoclasts (Maciewicz et al., 1990; Rifkin et al., 1991; Ohsawa et al., 1993; Tagami et al., 1994; Kakegawa et al., 1995; Furuyama and Fujisawa, 2000a; Goto et al., 2003; Czupalla et al., 2006) and in human osteoclasts (Nakase et al., 2000; Ishibashi et al., 2001; Li et al., 2004; Everts et al., 2006; Perez-Amodio et al., 2006; Charni-Ben Tabassi et al., 2008) where it could contribute to degrade several components of bone extracellular matrix (Maciewicz et al., 1990; Li et al., 2004; Everts et al., 2006; Charni-Ben Tabassi et al., 2008; Lion et al., 2009; Ogawa et al., 2009) (Table 1). Furthermore, immunoelectron microscopy studies reveal that, in rat osteoclasts, cathepsin L is closely associated with the collagen fibrils and bone matrix under the ruffle border of these cells (Goto et al., 2003). Although the expression levels of this proteinase in osteoclasts result in highly variable and are much more lower than that of cathepsin K (Rifkin et al., 1991; Drake et al., 1996; Everts et al., 2006; Perez-Amodio et al., 2006), it has been shown that, *in vitro*, the intracellular activity and secretion of cathepsin L in these cells could be upregulated following the release, in the microenvironment, of cytokines which modulate bone resorption such as parathyroid hormone (PTH) (Tagami et al., 1994; Kakegawa et al., 1995; Furuyama and Fujisawa, 2000b), c-Src tyrosine kinase (c-Src-TK) (Furuyama and Fujisawa, 2000a), interleukin-1α (IL-1α) (Furuyama and Fujisawa, 2000b), interleukin-6 (IL-6) (Damiens et al., 2000; Furuyama and Fujisawa, 2000b), tumor necrosis factor-α (TNF-α) (Kakegawa et al., 1995; Furuyama and Fujisawa, 2000b; Katunuma et al., 2002a; Hashimoto et al., 2006) and interferon-γ (Pang et al., 2005). In particular, some of these studies show that PTH and TNF-α can increase the secretion of a precursor form of cathepsin L into culture medium from a bone cell mixture (Tagami et al., 1994; Kakegawa et al., 1995; Furuyama and Fujisawa, 2000a,b; Katunuma et al., 2002a). The pro-enzyme can then be rapidly converted, under acid conditions, in the mature form and can promote bone pit formation (Kakegawa et al., 1995; Furuyama and Fujisawa, 2000b). Interestingly, these effects are inhibited by E-64, a non-selective cysteine proteinase inhibitor but not by CA-074, a specific inhibitor of cathepsin B (Furuyama and Fujisawa, 2000b; Montaser et al., 2002). By contrast, the possible involvement of cathepsin L in bone resorption is further supported by *in vivo* studies which show that cathepsin L knockout mice (B6;129-Ctsl^{tm1Alpk}) present alterations in bone structure (Potts et al., 2004). However, these alterations are different from the osteopetrotic phenotype type

Table 1 Cathepsin L in bone.

Study	References
Present in rodent osteoclasts	Maciewicz et al., 1990; Rifkin et al., 1991; Ohsawa et al., 1993; Tagami et al., 1994; Kakegawa et al., 1995; Furuyama and Fujisawa, 2000a; Goto et al., 2003; Czupalla et al., 2006
Present in human osteoclasts	Nakase et al., 2000; Ishibashi et al., 2001; Li et al., 2004; Everts et al., 2006; Perez-Amodio, 2006 ((●●**This reference is not listed in ref list pls confirm**●●)); Charni-Ben Tabassi et al., 2008
Colocalization with the collagen fibrils and bone matrix under the ruffle border of rat osteoclasts	Goto et al., 2003
Upregulation of osteoclast cathepsin L activity and secretion by cytokines promoting bone resorption (PTH), c-Src-TK, IL-1α, IL-6, TNF-α	Tagami et al., 1994; Kakegawa et al., 1995; Katunuma et al., 2002a,b●●a or b?●●; Furuyama and Fujisawa, 2000a,b; Pang et al., 2005; Hashimoto et al., 2006
Degradation of bone matrix proteins	Maciewicz et al., 1990; Kakegawa et al., 1995; Everts et al., 2006; Charni-Ben Tabassi et al., 2008; Lion et al., 2009; Ogawa et al., 2009
Altered bone structure in cathepsin L knockout mice	Potts et al., 2004; Kiviranta et al., 2005; Everts et al., 2006
Altered expression levels in non-malignant pathological conditions associated with an enhanced bone turnover	Keyszer et al., 1998; Lang et al., 2004; Potts et al., 2004; Schedel et al., 2004; Kido et al., 2007; Solau-Gervais et al., 2007

observed in cathepsin K-deficient mice (Potts et al., 2004; Kiviranta et al., 2005). In particular, the histomorphometric examination of the bone in the cathepsin L heterozygote and homozygote mice shows a significant reduction of trabecular bone volume, but not that of cortical volume, compared with wild type mice (Potts et al., 2004). This effect was associated with a concomitant reduction in the number of osteoclasts eroding the mineralized cartilagine ●●Should this be cartilage?●● (Potts et al., 2004). These findings could fit well with previous observations from other studies which suggest a specific role for cathepsin L in the process of endochondrial ossification (Nakase et al., 2000; Uusitalo et al., 2000) and further support the hypothesis of a complementary role of this protease in bone resorption (Kakegawa et al., 1995; Furuyama and Fujisawa, 2000a,b; Potts et al., 2004; Kiviranta et al., 2005; Everts et al., 2006; Schurigt et al., 2008; Long and Chagnovich, 2009).

Cathepsin L in metastatic bone disease

Accumulating evidence suggests that cathepsin L, in concert with other proteinases such as cathepsin B, matrix metalloproteinases (MMPs) and, urokinase type plasminogen activator (uPA), could facilitate cancer progression (Chauhan et al., 1991; Wilson and Singh, 2008; Lankelma et al., 2010). This hypothesis is sustained by a consistent body of experimental and clinical observations which show that the intracellular expression levels of cathepsin L are upregulated in many human tumors and that this phenomenon is associated with the onset of more aggressive tumor phenotypes and/or with a poor clinical outcome (Chauhan et al., 1991; Tumminello et al., 1996; Lankelma et al., 2010). It is noteworthy that altered expression levels of cathepsin L have also been observed, in particular, in some primary bone tumors such as osteosarcoma (Park et al., 1996; Haeckel et al., 1999; Damiens et al., 2000; Kirschke et al., 2000; Krueger et al., 2001; Husmann et al., 2008), giant cell tumor of the bone (Park et al., 1996; Lindeman et al., 2004), multiple myeloma (Spens and Haggstrom, 2005), chondrosarcomas (Park et al., 1996; Söderström et al., 2001), in tumors which preferentially metastasize to the bone such as breast cancer (Harbeck et al., 2001; Zajc et al., 2002; Caserman et al., 2006), prostate cancer (Friedrich et al., 1999; Colella et al., 2004; Goo et al., 2009), lung cancer (Kayser et al., 2003), laryngeal cancer (Russo et al., 1995), melanoma (Yang and Cox, 2007), oral cancer (Erdem et al., 2007) and in bone metastases (Park et al., 1996). These findings are intriguingly regarding a possible involvement cathepsin L, also in bone metastasis formation. By contrast, several experimental data support this hypothesis. For instance, Damiens et al. (2000) reported that human osteosarcoma cell lines MG-63 and SaOS2 produced cathepsin L. Interestingly, the intracellular levels of this proteinase were increased by proinflammatory cytokines promoting bone resorption such as IL-1β, IL-6 or TNF-α (Kawamata et al., 1997; Katunuma et al., 2002a; Kwan Tat et al., 2004; Blanchard et al., 2009). More recently, Husmann et al. (2008) showed that, in human osteosarcoma cell lines with different metastatic potential, cathepsin L expression levels were constitutively upregulated in the highly metastatic cell line compared with the low metastatic cell line. This phenomenon was also noted for cathepsins D and K. Conversely, cathepsins B and H expression levels resulted in downregulation, whereas cathepsin V was undetectable either in low or in highly metastatic cell lines (Husmann et al., 2008). These findings fit well with earlier observations of Park et al. (1996) who highlighted that cathepsin L was expressed only in 50% of primary human bone tumors and in 100% of metastatic tumors. Furthermore, clinical investigations carried out in patients with chondrosarcomas demonstrated that the overexpression of cathepsin L in these tumors was significantly associated with their progression (Söderström et al., 2001). Although these data are suggestive of an active involvement of cathepsin L in the pathogenesis of metastatic bone disease, the possible mechanisms by which this proteinase could foster this process remain to be fully unraveled. However, experimental evidence, in this regard, highlights that the enzyme could facilitate the homing and the diffusion of metastatic cells into the bone though multiple mechanisms which are summarized in Table 2. Thus, *in vitro* studies suggest that cathepsin L secreted by malignant cells can directly facilitate the growth and spread of these cells into the bone by degrading bone matrix proteins (Kawamata et al., 1997; Kirschke et al., 2000; Colella et al., 2004; Erdem et al., 2007; Gocheva and Joyce, 2007; Yang and Cox, 2007; Goo et al., 2009; Kielosto et al., 2009; Ogawa et al., 2009). In addition, the upregulation of osteoclast cathepsin L activity induced by proinflammatory cytokines such as PTH and ILs released from tumor cells could further contribute to degrade bone matrix thus allowing the homing of metastatic cells into the bone tissue (Katunuma et al., 2002a; Kwan Tat et al., 2004; Brage et al., 2005; Everts et al., 2006; Blanchard et al., 2009; Lankelma et al., 2010) (Table 1). Furthermore, this proteinase can indirectly promote bone degradation by triggering the activation latent precursor forms of other proteolytic enzymes, such as MMPs and uPA, which are known to be involved in the metastatic cascade and to modulate bone remodeling (Goretzki et al., 1992; Kawamata et al., 1997; Björklund and Koivunen, 2005; Lah et al., 2006; Wilson and Singh, 2008; Lankelma et al., 2010) (Table 1). Interestingly, recent studies suggested that cathepsin L could also facilitate the diffusion of metastatic cells into the bone through specific mechanisms not related with its bone degrading activity. For instance, it has been shown that, similarly as described for cathepsin B and MMPs, cathepsin L can degrade E-cadherin, an adhesion molecule which is downregulated in metastatic tumor cells, thus enhancing their migratory and invasive ability (Table 2) (Gocheva et al., 2006; Lankelma et al., 2010; Martin et al., 2010; Pontes et al., 2010). These findings could fit well with previous *in vitro* observations which showed that the inhibition of cathepsin L mRNA and protein expression by antisense oligonucleotides markedly decreased the motility and invasive ability of MNNG/HOS osteosarcoma cells (Krueger et al., 2001). Yet, cathepsin L could contribute to select more aggressive tumor phenotypes by modulating the intracellular

Table 2 Possible mechanisms by which cathepsin L could facilitate bone metastasis formation.

Mechanism	Effects	References
Bone matrix degradation	Invasion and metastasis	Kawamata et al., 1997; Kirschke et al., 2000; Colella et al., 2004; Erdem et al., 2007; Gocheva and Joyce, 2007; Yang and Cox, 2007; Goo et al., 2009; Kielosto et al., 2009; Ogawa et al., 2009
Upregulation of osteoclast cathepsin L activity induced by cytokines with bone degrading activity released by tumor cells	Bone matrix degradation Invasion and metastasis	Katunuma et al., 2002a; Kwan Tat et al., 2004; Brage et al., 2005; Everts et al., 2006; Lankelma et al., 2010
Activation of latent precursor forms of other proteolytic enzymes involved in the metastatic cascade (MMPs, uPA)	Extracellular matrix degradation Invasion and metastasis	Goretzki et al., 1992; Kawamata et al., 1997; Björklund and Koivunen, 2005; Lah et al., 2006; Lankelma et al., 2010
Degradation of E-cadherin	Reduced adhesion, increased motility and migration of cancer cells	Krueger et al., 2001; Gocheva et al., 2006; Lankelma et al., 2010; Pontes et al., 2010
Modulation of IGF-I	Onset of aggressive tumor phenotype	Nakasaki et al., 2008; Navab et al., 2008; Lankelma et al., 2010
a) Increased expression of cathepsin L in endothelial progenitor cells	Tissue remodeling and tumor neovascularization	Katunuma et al., 2002a,b; Rousselet et al., 2004; Urbich et al., 2005; van Hinsbergh et al., 2006; Keerthivasan et al., 2007;
b) Interactions with VEGF		McMahon and Kwaan, 2008; Chang et al., 2009; Rebbaa et al., 2009;
c) Activation of pro-MMPs and pro-uPA		Deryugina and Quigley, 2010
d) Stimulation of recruitment and activity of blood- or bone marrow-derived cells		

expression levels of growth factors and receptors actively involved in bone remodeling such as insulin growth factor I (IGF-I) (Damiens et al., 2000; Nakasaki et al., 2008; Navab et al., 2008; Lankelma et al., 2010). Intriguingly, recent investigations indicated that cathepsin L can indirectly facilitate bone metastasis formation by fostering tumor angiogenesis (Table 1). In support of this hypothesis, Urbich et al. (2005) reported that bone marrow mononuclear cells or circulating blood-derived progenitor cells required cathepsin L to mediate their invasion activity *in vitro* and neovascularization improvement *in vivo*. Furthermore, cytokines which promote bone pit resorption such as TNF-α have been shown to increase the secretion of pro-cathepsin L in bone marrow cells thus enhancing their invasive activity and facilitating the formation of new blood vessels (Furuyama and Fujisawa, 2000a,b; Katunuma et al., 2002a,b). Moreover, van Hinsbergh et al. (2006) recently demonstrated that cathepsin L, in concert with MMP-9, stimulates the recruitment and action of blood- or bone-marrow derived accessory cells that enhance angiogenesis (Table 1). These data are consistent with those findings which suggested that cathepsin L could foster tumor angiogenesis by activating latent forms of proteolytic enzymes, such as MMPs and uPA, implicated in this process (Goretzki et al., 1992; van Hinsbergh et al., 2006; McMahon and Kwaan, 2008; Wilson and Singh, 2008; Deryugina and Quigley, 2010). Interestingly, Keerthivasan et al. (2007) recently showed that, at least in U87MG glioblastoma cells, vascular endothelial growth factor (VEGF) can upregulate at transcriptional level cathepsin L. Consistent with these observations, Chang et al. (2009) highlighted that in human breast cancer cells, VEGF-A can promote angiogenesis by perturbing the cathepsin-cysteine protease inhibitor balance in venules causing basement membrane degradation. A direct proof of the importance of cathepsin L-VEGF interactions in promoting tumor angiogenesis in different tumors is provided, in part, by some *in vitro* studies which showed that the inhibition of human procathepsin L secretion by anti-cathepsin L monoclonal antibody resulted in an inhibition of tumor-induced angiogenesis in highly metastatic human melanoma cells (Rousselet et al., 2004; Frade et al., 2008). Furthermore, Rebbaa et al. (2009) recently demonstrated that Napsul-IIe-Trp-CHO, a specific inhibitor of cathepsin L, inhibited *in vitro* VEGF secretion by endothelial cell and cathepsin L-mediated degradation of the extracellular matrix. Whereas, *in vivo*, this molecule was able to inhibit tumor growth in nude mice transplanted neuroblastoma.

Cathepsin L inhibitors in the treatment of cancer related bone disease

Experimental findings suggestive of an active role of cathepsin L in the pathogenesis of metastatic bone disease have led, in recent years, to the development of a series of inhib-

itors of this proteinase. These molecules, administered in association with antagonists of other proteinases promoting bone metastasis such as cathepsin K and MMPs (Wilson and Singh, 2008), could contribute to elicit a better therapeutic response in the treatment of this pathological condition. Furthermore, these drug combinations might, eventually, overcome the pitfall of the functional redundancy of cathepsin K and cathepsin L in bone (Nagler and Menard, 2003; Falgueyret et al., 2005; Brix et al., 2008). Another rationale for the development of new specific cathepsin L antagonist relies on the observations that the proteolysis required for tumor invasion occurs mostly extracellularly (Mohamed and Sloane, 2006; Lankelma et al., 2010). This phenomenon could, in part, explain why, during tumor progression, intracellular cathepsin L translocates from lysosomes to the cell membranes and then is actively secreted into the acid extracellular microenvironment of tumors (Chauhan et al., 1998; Katunuma et al., 2002a; Ravanko et al., 2004; Hashimoto et al., 2006; Mohamed and Sloane, 2006; Goo et al., 2009; Ogawa et al., 2009). Therefore, the alternative strategies that can target secreted proteinases such as cathepsin L are actually appealing as the use of molecules which act extracellularly and do not enter cells can overcome potential toxic effects owing to the inhibition of other proteinases present in the lysosomal compartment of normal cells (Hill et al., 1994; Falgueyret et al., 2005; Black and Percival, 2006; Le Gall et al., 2008; Lankelma et al., 2010). This latter phenomenon is, currently, of main concern regarding for instance, the long-term use of some specific inhibitors of cathepsin K in the treatment of bone disease. In fact, these agents, owing to their lysosomotropic nature, by accumulating inside lysosomes, can block the normal activity of other proteinases thus inducing potential adverse effects (Falgueyret et al., 2005; Black and Percival, 2006; Desmarais et al., 2008; Le Gall et al., 2008). In this context, current efforts are specifically directed to design new suitable cathepsin K inhibitors which could be devoid of these potential side effects (Black and Percival, 2006; Desmarais et al., 2008; Le Gall et al., 2008; Brömme and Lecaille, 2009). Most of the cathepsin L targeted agents which have been developed are a series of different classes of antagonists including small molecule inhibitors with a higher selectivity for cathepsin L (Katunuma et al., 2002a,b; Marquis et al., 2005; Sadaghiani et al., 2007; Myers et al., 2008; Palermo and Joyce, 2008; Yadav et al., 2008; Asaad et al., 2009; Bethel et al., 2009; Long and Chagnovich, 2009; Rebbaa et al., 2009; Kishore Kumar et al., 2010), alternative forms of endogenous inhibitors (Brand et al., 2004; Gianotti et al., 2008), antibodies and antisense oligonucleotides (Krueger et al., 2001; Rousselet et al., 2004; Frade et al., 2008; Long and Chagnovich, 2009) or natural products (Lion et al., 2009; Ogawa et al., 2009). Preclinical studies showed that some of these molecules were effective to inhibit, *in vitro*, bone matrix digestion at the level of resorption lacuna and to promote bone mineralization, whereas, *in vivo*, these agents were able to suppress RANKL or M-CSF or TNF-α stimulated bone pit formation and to protect tumor bearing mice from malignant hypercalcemia and to decrease bone metastasis formation (Hill et al., 1994; Katunuma et al., 2002a,b; Brand et al., 2004; Long and Chagnovich, 2009). Interestingly, recent investigations have proposed, as a novel approach, to inhibit proteinase-mediated bone resorption, the use of molecules, such as oligogalacturonic acid (OGA) purified from flax pectin which specifically target the collagenase function of proteases involved in cancer progression, including cathepsin L, without affecting their proteolytic activity (Lion et al., 2009). These authors speculated that, owing to the physiological role of human cathepsins and MMPs in bone collagen degradation (Maciewicz et al., 1990; Li et al., 2004; Everts et al., 2006; Charni-Ben Tabassi et al., 2008), OGA, according to its specific mechanism of action, could be of therapeutic benefit in the treatment of disorders associated with an increased bone resorption such as osteolytic bone metastasis (Lion et al., 2009). In addition, owing to its peculiar mechanism, this compound could have therapeutic advantages over classical active site inhibitors regarding their potential side effects. Although the preclinical studies on the effectiveness of cathepsin L inhibitors in preventing bone metastasis formation are promising, there are some valid concerns regarding the clinical use of these molecules. Some of these concerns rely on recent observations that cathepsin L could also act as pro-apoptotic and tumor suppressive molecules (Lah et al., 2006; Di Piazza et al., 2007; Vasiljeva and Turk, 2008; Hsu et al., 2009; Dennemärker et al., 2010; Lankelma et al., 2010). These findings indicate that, in this case, the use of cathepsin L antagonists could be detrimental. However, experimental evidence highlights that, at least in most human tumors, cathepsin L appears to act mainly as tumor promoting proteinase (Zheng et al., 2004, 2008, 2009; Rebbaa et al., 2009), whereas the tumor suppressor activity of this endopeptidase appeared to be restricted only to some squamous cell carcinomas and gliomas (Lah et al., 2006; Di Piazza et al., 2007; Hsu et al., 2009; Dennemärker et al., 2010; Lankelma et al., 2010). Experimental studies indicate that the pro-apoptotic effects of cathepsin L in these tumors could probably be related to specific functions carried out by this enzyme in the tissue homeostasis of normal counterparts (Lah et al., 2006; Sevenich et al., 2006; Di Piazza et al., 2007; Vasiljeva and Turk, 2008; Hsu et al., 2009; Dennemärker et al., 2010; Lankelma et al., 2010). By contrast, no pro-apoptotic or growth inhibiting activity of cathepsin L on primary bone tumor cells and in bone metastatic cells has been reported so far.

Conclusion and perspectives

Growing evidence highlights that cathepsin L, in concert with other proteinases such as cathepsin K, MMPs and uPA, could contribute to promote bone metastasis formation (Katunuma et al., 2002a,b; Navab et al., 2008; Palermo and Joyce, 2008; Wilson and Singh, 2008; Lankelma et al., 2010). These findings imply that the use of a single molecule directed to inhibit a specific target enzyme might not be effective in preventing and/or eradicating bone metastasis.

Therefore, more effective treatments of this pathological condition should rely on the combination of molecules which can act on different classes of proteolytic enzymes which contribute equally to facilitate the homing and spread of metastatic cells into the bone (Yamamoto et al., 2002; Krol et al., 2003; Palermo and Joyce, 2008; Lion et al., 2009; Long and Chagnovich, 2009; Lankelma et al., 2010). In this scenario, cathepsin L inhibitors could be regarded as additional, potential therapeutic tools in the clinical management of patients with bone metastasis. Furthermore, recent studies which show that cathepsin L appears to act as a pro-survival factor and could contribute to the development of antitumor drug resistance (Zheng et al., 2004; Gocheva et al., 2006; Zajc et al., 2006; Navab et al., 2008; Zheng et al., 2008, 2009; Lankelma et al., 2010) further stress the potential clinical benefit of cathepsin L inhibitors in reverting antitumor drug resistance (Zheng et al., 2004, 2009; Palermo and Joyce, 2008; Rebbaa et al., 2009; Lankelma et al., 2010). Therefore, the combination of cathepsin L antagonists with classical anticancer drugs appears to be a promising therapeutic option also in the treatment of cancer-related bone diseases resistant to conventional antitumor agents (Palermo and Joyce, 2008; Zheng et al., 2008, 2009; Lankelma et al., 2010). Although preclinical studies on the effectiveness of cathepsin L inhibitors in preventing bone metastasis are encouraging, it remains to be established whether these results could be translated to the clinic. Further investigations are needed to better define the pharmacological and toxicological profile of these molecules in humans and their effectiveness in the treatment of metastatic bone disease. These studies could unravel the clinical value of these molecules in the therapeutic management of patients with primary bone cancer and/or skeletal metastasis.

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