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Emerging Roles of Cathepsin E in Host Defense Mechanisms

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Abstract

Cathepsin E is an intracellular aspartic proteinase of the pepsin superfamily, which is predominantly expressed in certain cell types, including the immune system cells and the rapidly regenerating gastric mucosal and epidermal keratinocytes. The intracellular localization of this protein varies with different cell types; i.e., the presence in endosomal organelles, plasma membranes, the endoplasmic reticulum, Golgi complex and the cytosol. The enzyme is also secreted by activated immune system cells and cancer cells. Its strategic expression and localization suggests the association of this enzyme with specific biological functions of the individual cell types. Recent genetic and pharmacological studies have particularly suggested that cathepsin E plays an important role in host defense against cancer cells and invading microorganisms. This review focuses emerging roles of cathepsin E in immune system cells and skin keratinocytes, and in host defense against cancer cells.

Key words: Cathepsin E, Aspartic proteinase, Cancer, Epidermal differentiation, Peptide-mimetic inhibitors and activators

Contents

1. Introduction

Cathepsin E is an intracellular aspartic proteinase of the pepsin superfamily, which is predominantly expressed in certain cell types, including the immune system cells (1-10) and rapidly regenerating gastric mucosal cells (10-14) and epidermal keratinocytes (15-17). Unlike other analogous aspartic proteases, cathepsin E has several notable properties, besides its restricted expression. Differing from the definite localization of other related aspartic proteases, the intracellular localization of cathepsin E varies with different cell types. In antigen presenting cells, such as macrophages, microglia and dendritic cells (2-8), and gastric cells (10-14), the enzyme mainly localized in the endosomal compartments as a mature form. The association of cathepsin E with plasma membrane is observed in erythrocytes (18, 19), intracellular canaliculi of gastric parietal cells (14), renal proximal tubule cells (14), bile canaliculi of hepatic cells (14), intestinal and tracheobranchial epithelial cells (15, 20) and osteoclasts (21). Cathepsin E is also found in the endoplasmic reticulum and Golgi complex (3, 14) and the cytosol (14, 22) of various cell types. Besides its intracellular localization, cathepsin E is also secreted by certain cell types such as activated macrophages (23). Moreover, it has been demonstrated that the expression of cathepsin E in mice varies with different strains (24). Specifically, both protein and message

levels of cathepsin E were profoundly decreased in hemopoietic cells from C57BL/6J mice, compared with 129S2/Sv and Balb/c mice, although the protein levels in gut were similar between C57BL/6J and the other strains. Based on analysis of the promoter region of cathepsin E, the tissue-specific deficiency of cathepsin E in C57BL/6J mice is likely due to the SNP within the PU.1 transcription binding consensus sequence. Taken together, the strategic expression and localization of cathepsin E suggests its important implications for tissue-specific biological functions, including antigen processing (1, 4, 25), neuronal degeneration (26-31) and the generation of various secretory proteins (32-34). The studies from the 1980s have suggested that, overall, cathepsin E contributes to the maintenance of homeostasis by participating in host defense mechanisms. However, because physiologic substrates of cathepsin E have not yet well-defined *in vivo*, precise physiologic functions of this protein remains speculative.

Recent genetic studies using mice lacking or over-expressing cathepsin E have subsequently confirmed and extended these early observations (7, 8, 10, 35-38). As these studies were directed towards identifying the mechanism(s) of the cathepsin E function, the findings obtained might be worthy of remark. In this review, we will focus mainly upon several important points of cathepsin E in considering its physiological functions and possible mechanisms. Besides, compared with cathepsin E, the relevant

analogous cathepsin D studies will also be discussed in terms of biological functions.

2. Cathepsin E in antigen presenting cells

2.1 Roles of cysteine cathepsins and aspartic proteinases in MHC class II-mediated antigen presentation

There is increasing evidence that two classes of intracellular lysosomal proteases, cysteine and aspartic proteases, are involved proteolytic processes required for MHC class II-mediated antigen presentation. Functional studies using specific inhibitors or mice lacking individual cathepsins strongly suggest that the two cysteine cathepsins, cathepsins L and S, are responsible for the terminal degradation of Ii to generate class II-associated Ii peptide (CLIP) during maturation of MHC class II molecules in the thymus and the peripheral lymphoid organs, respectively (39-45). The cysteine protease cathepsin F is also implicated in CLIP generation in peripheral macrophages (46). In addition to the cysteine cathepsins, the two endolysosomal aspartic proteinases, cathepsin D and cathepsin E, were shown to be required for the initial stages of Ii processing, using a series of potent inhibitors (47). Several early studies demonstrated that cathepsin D generated *in vitro* antigenic peptides from ovalbumin (OVA) and hen egg lysozyme that could be presented to T cells (48-50). However, shortly afterward,

studies with splenocytes and macrophages from cathepsin D-deficient (*CatD*$^{-/-}$) mice have revealed that cathepsin D is dispensable for degradation of Ii and processing of a number of exogenous and endogenous antigens (40, 51). By contrast, the experiments using macrophages from cathepsin E-deficient (*CatE*$^{-/-}$) mice have concluded that this proteinase is indispensable for the generation of an antigenic epitope from intact OVA to present to cognate T cells (7, 8). These results are well consistent with those from the previous experiments with microglia from *CatD*$^{-/-}$ mice (4) and with the murine antigen-presenting B cell lymphoma, A20, treated with the cathepsin E-specific *Ascaris* inhibitor (1).

2.2 Cathepsin E in macrophages

Recently, cathepsin E deficiency has been shown to lead to a novel form of lysosome storage disorder in macrophages, manifesting the accumulation of major lysosomal membrane sialoglycoproteins, LAMP-1, LAMP-2 and LIMP-2, and the elevation of lysosomal pH (7). These striking features were also observed with wild-type macrophages by treatment with pepstatin A or cathepsin E-specific *Ascaris* inhibitor. These lysosomal membrane proteins represent more than 50% of the total membrane proteins of endolysosomes (52, 53), and their glycosylation constitutes about 60% (LAMP-1 and LAMP-2) and 20% (LIMP-2) of the total mass of the respective

molecules and the most part is present on the luminal side of endosomes and lysosomes. It is thus believed that these membrane proteins play an important role in the protection of the membrane from degradation by lysosomal hydrolases. Because there was no difference in the vacuolar-type H^+-ATPase activity between wild-type and *CatE*$^{-/-}$ macrophages (7), the elevated lysosomal pH was likely due to the accumulation of these membrane proteins. In this connection it is interesting to note that some types of lysosomal storage disorders including neuronal ceroid lipofuscinosis are associated with the accumulation of lysosomal membrane proteins and/or the elevation of lysosomal pH (54-57). Given that pH is essential for the maintenance of the nature and function of endolysosomal organelles including the normal processing and targeting events of lysosomal proteins, it is most likely that the elevated lysosomal pH interferes with the maturation and fusion events of the organelles involved. Therefore, cathepsin E deficiency appears to result in the impairment of the structural and functional integrity of macrophages. Indeed, cathepsin E deficiency has shown to lead to a significantly increased secretion of soluble lysosomal hydrolases, including cathepsins B, D, S, L, α-mannosidase, β-glucuronidase and β-hexosaminidase (7, 8), and a marked reduction in degradation of phagocytosed OVA (8) and decreased chemotactic responses to MCP-1 and fMLP in macrophages (8, 38). Furthermore, *CatE*$^{-/-}$ macrophages showed a

significant decreased in the cell surface levels of TLR2 and TLR4, which recognize specific components of Gram-positive and –negative bacteria, respectively, despite no significant difference in the total cellular expression levels of these receptors between the wild-type and *CatE*$^{-/-}$ macrophages (36). Additionally, *CatE*$^{-/-}$ macrophages showed a significant decrease in responses to TLR3 ligand (Poly I:C) compared with the wild-type cells (36). Given the preferential localization of TLR3 receptor in endosomal compartments, the decreased responsiveness of *CatE*$^{-/-}$ macrophages to this ligand implies the impairment of early interactions between phagosomes and endosomes. These results thus suggest that cathepsin E deficiency results in the decreased bactericidal activity toward a variety of invading microbial pathogens in macrophages. The cell surface levels of the chemotactic receptors CCR-2 and FPRs, which are receptors for MCP-1 and N-formyl peptides, respectively, and the adhesion receptors CD18 (integrin β2) and CD29 (integrin β1) were also significantly decreased in *CatE*$^{-/-}$ macrophages compared with the wild-type cells (38). Taken together, these findings strongly suggest that cathepsin E deficiency induces profound trafficking defects in cell surface receptors in macrophages, most probably due to the elevation of lysosomal pH. It is thus concluded that cathepsin E in macrophages plays an essential role in immune defense against invading microbial pathogens, chemotaxis, and cell adhesion through

the maintenance of lysosomal pH and normal trafficking and fusion events. A schematic representation of the effects of cathepsin E deficiency on the nature and functions of macrophages is shown in Fig. 1.

2.3 Cathepsin E in dentritic cells

Dendritic cells (DCs) are heterogenous, professional antigen presenting cells that play a central role in the initiation and control of immune responses and probably in the maintenance of tolerance (58, 59). Myeloid precursor cells differentiate into immature DCs, which efficiently engulf a variety of particulate antigens and then transport them into MHC class II endolysosomal compartments for degradation. Subsequently, the immature DCs migrate to the regional lymph nodes where they phenotypically mature and express a number of cell surface molecules for presentation to cognate T cells. It is also known that immature DCs efficiently process and present a variety of particulate antigens, thereby serving as sentinels to immunologic threats. In human myeloid DCs, cathepsin E is found in a perinuclear compartment, which is likely to be associated with the ER, and also a peripheral compartment just beneath the cell membrane (25). Chain and his colleagues have previously shown that cathepsin E has an important role in the MHC class II antigen processing pathway within both human and mouse myeloid DCs using a novel, targeted derivative of the aspartic proteinase inhibitor pepstatin (25).

Unexpectedly, differing from both peritoneal and myeloid macrophages, the nature and functions of both myeloid immature and mature DCs, including intracellular levels of soluble lysosomal hydrolases and lysosomal membrane sialoglycoproteins, lysosomal pH, the cell surface expression of TLRs, chemotactic and adhesion receptors, were not significantly changed by cathepsin E deficiency (8). In addition, there were no significant differences in the destructive potential for phagocytosed OVA between wild-type and *CatE*$^{-/-}$ DCs. Intriguingly, however, there was a marked difference in the capacity for presenting OVA to cognate T cells between wild-type and *CatE*$^{-/-}$ DCs. Whereas cathepsin E deficiency induced a marked decrease in the ability of macrophages to present intact OVA, as well as and its antigenic peptide, to cognate T cells, *CatE*$^{-/-}$ DCs inversely showed a significant increase in the ability of OVA presentation.

Previously, Watts and his colleagues reported that the presentation of two different myoglobin T cell epitopes in DCs was enhanced rather than hindered by the lack of cathepsin D and the residual processing activity in the subcellular fraction of DCs deficient in cathepsin D was completely inhibited by pepstatin, and thereby suggested that aspartic protease(s) besides cathepsin D could be involved in myoglobin antigen presentation in DCs and/or that the reduced activity by cathepsin D deficiency would

produce optimal conditions for its processing and presentation (60). In this regard, it has been reported that the ability of DCs from not only wild-type but also *CatD*$^{-/-}$ mice to present intact OVA, but not an OVA-derived peptide, to cognate T cells is completely blocked by mannosylated BSA-conjugated pepstatin (25). On the basis of these findings, it is concluded that the reduced aspartic protease activity resulting from cathepsin E deficiency in DCs may be compensated by the related aspartic protease(s) or may confer to the optimum conditions for OVA antigen presentation. Given that cathepsin E deficiency did not significantly affect the ability of DCs, like macrophages, to present not only OVA but also its antigenic peptide to cognate T cells, however, it seems likely that cathepsin E is unlikely to be directly involved in antigen processing in these cells and rather plays a crucial role in controlling the endosomal/lysosomal microenvironment and the protein sorting into these compartments. Additional notable features for *CatE*$^{-/-}$ DCs are particularly noteworthy in connection with the enhanced OVA and its antigenic peptide. Namely, the phagocytic activity of both immature and mature DCs, but not myeloid and peritoneal macrophages, toward fluorescent latex particles was significantly increased by cathepsin E deficiency (8). Given that the internalization of particulate antigens by DCs is important for their processing and presentation, the enhanced phagocytic activity of *CatE*$^{-/-}$ DCs is more likely to

contribute to the stimulation of their OVA peptide presentation. In contrast to either type of macrophages, DCs have also revealed a significant increase in the cell surface expression of the costimulatory molecules CD86, CD80 and CD40, which can amplify the response of T cells, by cathepsin E deficiency (8). It is established that T cells require costimulatory signals for optimal activation (61-63). Given that, of the multiple costimulatory signals, the CD80/CD86-CD28 and CD40-CD154 interactions play a key role in the process of T cell priming by DCs (62), the increased expression of CD80, CD86 and CD40 on *CatE*$^{-/-}$ DCs is thus likely to contribute to the enhanced T cell activation. Although the precise mechanism for the increased phagocytic activity and the expression of costimulatory molecules in DCs by cathepsin E deficiency remains to be answered, these observations have provided new insight into the functional diversity of cathepsin E in immune responses of different types of antigen presenting cells. A schematic representation of the effects of cathepsin E deficiency on the nature and functions of DCs is shown in Fig. 2.

3. Cathepsin E and cancer

Recent evidence has demonstrated that the lysosomal proteolytic system, including autophagy, plays an important role in the control of cell death (64). Under physiological

conditions, autophagy especially is implicated in cell survival through the degradation of intra- and extracellular proteins and the removal of old/damaged cellular organelles within the tight compartment of the autolysosome. Autophagy also allows tumor cells to survive under certain stress conditions, including metabolic stress (65) and ER stress resulting from accumulating misfold proteins (66, 67). By contrast, it has also been demonstrated that autophagy induces cancer cell death, and thus defective autophagy is associated with cancer progression (68). Another notable characteristic of cancer cells is lysosomal alterations in connection with cancer cell death. The integrity of the lysosomal membrane in these cells is disrupted in response to various stresses, thereby resulting in the release of lysosomal hydrolases into the cytosol, where not only apoptosis but also apoptosis-like and necrosis-like cell death is induced. Indeed, recent studies have provided evidence that cathepsins released by increased lysosomal membrane permeabilization participate in the execution of cell death that is induced by classic apoptotic stimuli (64).

A variety of intra- and extracellular proteases, including aspartic proteinases and cysteine cathepsins, are highly up-regulated in certain types of cancers (69-84). In addition, there is a change in the localization of the endolysosomal proteases to extracellular spaces. The substrates and functions of cathepsin E might thus change

along with its localization. The proteolytic activity of extracellular aspartic and cysteine cathepsins has long been associated with many types and stages of cancer (73-77, 81-87). Cathepsin E is also known to be up-regulated and secreted in several forms of cancer and thus suggests their clinical utility as a potential biomarker for these cancers (69, 71-80, 88). However, the clinical significance of the increased cathepsin E expression in carcinogenesis has been controversial. For example, increased expression of cathepsin E in premalignant cervical epithelium (71), lung carcinoma (73), pancreatic ductal adenocarcinoma (69, 88) and colorectal sessile serrated adenomas (80) was found to be a marker for bad prognosis, whereas that in lung carcinoma (73), bladder cancer (74), hepatocellular carcinoma (76) is associated with survival as a marker for good prognosis. Additionally, it has been shown that while cathepsin E is normally expressed and localized in chronic the inflammation and ulcer lesions of human stomach, this protein disappeared in the incomplete type of intestinal metaplasia, dysplasia, and well differentiated adenocarcinoma and poorly differentiated adenocarcinoma in this tissue (89). On the other hand, the up-regulation and increased secretion of cathepsin D was observed with breast cancer and prostate cancer, and is associated with a poor prognosis (82-84). In breast cancer, cathepsin D is likely to act as a mitogen, because its promoter region is preferentially up-regulated by estrogen (90). Intriguingly, however, the

proteolytic activity of cathepsin D was dispensable for the progression of breast cancer, because a mutated form devoid of its proteolytic activity also stimulated cancer growth (91). This is likely due to the mitogenic and proliferative activity of the propeptide of cathpsin D (91, 92). In this connection, M6P/IGF-II receptor and low-density lipoprotein receptor-related protein-1 (LRP1) has been identified as a CD binding receptor (93).

In contrast to cathepsin D and cysteine cathepsins, cathepsin E was recently found to have an antitumorigenic activity through the induction of growth arrest and apoptosis in various human prostate carcinoma cell lines without affecting normal cells (37). To determine how and to what extent cathepsin E exerts its antitumorigenic activity, this protein was manipulated in both *in vitro* and *in vivo* experiments using human cancer cell lines, nude mice bearing human cancer cells, and $CatE^{-/-}$ or cathepsin E-overexpressing transgenic mice ($CatE^{Tg}$) bearing syngeneic mouse melanoma cells. These experiments have provided evidence that cathepsin E is a responsible enzyme for specific cleavage of tumor necrosis factor-related apoptosis ligand (TRAIL) at the surface of cancer cells and the consequent generation of a soluble trimeric form of this protein and thereby induces the growth arrest and apoptosis in cancer cells without harming normal cells. Unlike other TNF family members, TRAIL can induce apoptosis

in most transformed cells and some virally infected cells without affecting normal cells (94, 95). Moreover, administration of soluble recombinant TRAIL into mice bearing human tumor xenografts induces significant tumor regression without systemic toxicity (96, 97). Based on the efficacy and safety studies, potential clinical application of recombinant soluble TRAIL in cancer therapy is being explored. In addition, it has been suggested that the TRAIL may be used as an innate effector molecule involved in the elimination of spontaneously arising tumor cells. Although TRAIL has attracted intense interest in cancer therapy, however, an increasing number of cancer cells still remain resistant to TRAIL-mediated apoptosis. An additional problem with *in vivo* use of TRAIL is that a high concentration of this molecule is required to obtain definite therapeutic efficacy, probably owing to the short half-life of soluble TRAIL in plasma. Therefore, there is a need to search for new regimens to enhance sensitization of cancer cells to TRAIL-induced apoptosis. In this regard, cathepsin E appears to have an advantage over TRAIL in inducing apoptosis in cancer cells because this molecule is directly generated within or in the vicinity of tumors. In addition, given that several tumor effector cells including activated T cells, B cells, natural killer cells, DCs and monocytes are known to produce TRAIL, cathepsin E may generate TRAIL from these immune cells. Moreover, cancer cells are known to be eliminated by tumor-infiltrated

effector cells, particularly activated macrophages, through several kinds of mechanisms, e.g., killing by phagocytosis, antigen processing and presentation to T4 lymphocytes, and enhanced secretion of various cytokines that play a crucial role in non-specific host defense. Given the strong association of cathepsin E with the activation and functions of macrophages (7, 23) and the positive correlation of IFN stimulation with the enhanced expression of TRAIL and increased killing activity against cancer cells (98), this enzyme is more likely to exert antitumorigenic activity via not only TRAIL-dependent apoptosis but also tumor-infiltrated, activated macrophage-mediated cytotoxicity. In the meantime, accumulating evidence also shows that, in contrast to their beneficial antitumorigenic activity, many deleterious functions of tumor-infiltrated macrophages have been recognized, such as enhancement of cancer migration and invasion, facilitation of extracellular matrix breakdown and remodeling, promotion of cancer cell motility, and stimulation of angiogenesis (99, 100). These competing functions seem to arise from the pleiotropic nature of the macrophages that participate in immune responses in a polarized manner: classic M1 macrophages produce interleukin (IL) 12 to promote tumoricidal responses, whereas M2 macrophages produce IL10 to help tumor progression (101). In addition, cathepsin E appears to contribute to the inhibition of tumor growth and metastasis through the inhibition of tumor-induced angiogenesis, in

which the enzyme mediates a specific release of endostatin, a potent endogenous angiogenesis inhibitor, from human collagen XVIII (102). More recently, it has also been demonstrated that cathepsin E has a synergistic cytotoxic activity on cancer cells in combination with the anti-cancer agent, even though either of the agents by itself was unable to efficiently induce cell death in these cells, suggesting its therapeutic potential for clinical use (103). A common hurdle that almost all of the anticancer drugs have had not over is their severe side effects. Therefore, the observations that cathepsin E prevents tumor growth and metastasis *in vivo* through multiple mechanisms, including induction of TRIL-induced apoptosis, angiogenesis inhibition, enhanced immune responses and synergistic effects with anticancer drugs may provide a promising strategy for cathepsin E-based cancer therapy. A schematic representation of the multiple mechanisms for the antitumorigenic activity of cathepsin E is shown in Fig. 3.

4. Cathepsin E in skin

The epidermis is a stratified and keratinized epithelium mainly composed of keratinocytes. Epidermal differentiation results in formation of several distinct cell layers characterized by their ultrastructure, mitotic state and expression of specific epidermal differentiation markers. Recent evidence has shown that dermatological

disorders ranging from minor cosmetic problems to life-threatening conditions are commonly due to abnormal differentiation of keratinocytes. Therefore, elucidation of the intracellular molecules involved in the cellular differentiation processes and the regulation of epidermal homeostasis is of special importance for understanding and therapy of these disorders. In human and rat epidermis, cathepsin E is expressed and localized mainly in keratinocytes and in close vicinity to the inner root sheath of hair follicles (104). While cathepsin D is localized mainly on desmosomes of human stratum corneum, cathepsin E is present within the squames (17), suggesting that these two enzymes have different functions in dermal differentiation processes. Cathepsin E deficiency in mice induced abnormal keratinocyte differentiation in the epidermis and hair follicle characterized by the significant expansion of corium and the reduction of subcutaneous tissue and hair follicles (104). In a model of skin papillomas formed in three different genotypes of syngeneic mice, $CatE^{-/-}$. $CatE^{+/+}$ and $CatE^{Tg}$, cathepsin E deficiency induced the significantly reduced expression and altered localization of the keratinocyte differentiation-induced proteins keratin 1 and loricrin. Using primary cultures of keratinocytes from each genotype of mice, cathepsin E deficiency resulted in the delayed differentiation accompanying the reduced expression or the ectopic localization of these differentiation markers, whereas over-expression of this protein

enhanced the rate of keratinocyte terminal differentiation. These findings suggest that cathepsin E in keratinocytes functionally links to the expression of the epidermal differentiation markers, thereby regulating the formation and homeostasis of the epidermis. On the other hand, cathepsin D-deficient mice (*CatD*$^{-/-}$) showed reduced transglutaminase activity 1 activity and reduced protein levels of the cornified envelop proteins involucrin and loricrin (105). Amount and distribution of cornified envelopproteins involucrin, loricrin, filaggrin, and of the keratins K1 and K5 were significantly altered in *CatD*$^{-/-}$ mice. Therefore, both cathepsin E and cathepsin D appear to regulate differentially and cooperatively the formation and homeostasis of the epidermis.

5. Conclusion remarks

A rapidly growing body of evidence demonstrates that cathepsin E is implicated in a variety of immune responses. A wide range of studies have characterized cathepsin E as an indispensable molecule for exogenous antigen processing and presentation. It is of special importance to note that cathepsin E differentially regulates the nature and functions of macrophages and DCs, especially with regards to the cellular levels of major lysosomal membrane sialoglycoproteins, lysosomal pH, and OVA processing and

presentation. In regard to cancer cells, cathepsin E also has some notable difference from other cathepsins. Unlike cathepsin D and cysteine cathepsins, cathepsin E has a potent antitumorigenic activity, which is displayed by multiple mechanisms, including induction of TRIL-induced apoptosis, angiogenesis inhibition, enhanced immune responses and synergistic effects with anticancer drugs. Most strikingly, cathepsin E has no detectable deleterious effect on normal cells. This selectivity may provide a promising strategy for cathepsin E-based cancer therapy. More recently, it has also been demonstrated that cathepsin E in keratinocytes functionally links to the expression of the epidermal differentiation markers, thereby suggesting that this protein may regulate the formation and homeostasis of the epidermis. The recent development in chemical activation of cathepsin E may prove a new therapeutic paradigm in the treatment of dermatological disorders ranging from minor cosmetic problems to life-threatening conditions. One of the most important questions yet to be answered is: what are the specific substrates of cathepsin E predominantly expressed in certain cell types? Knockout or knockdown and drug screens that conducted to identify molecules that interact with cathepsin E might prove effective in identifying its substrates. For further knowledge on, we would like to suggest referring to other previous reviews detailing basic aspects of cathepsin E, including enzymatic properties, regulation of gene

expression, biosynthesis, processing and intracellular trafficking, and role associated with neuronal degeneration, and the references cited therein (106-110).

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References

1. K. Bennett, T. Levine, J.S. Ellis, R.J. Peanasky, I.M. Samloff, J. Kay, B.M. Chain, Antigen processing for presentation by class II major histocompatibility complex requires cleavage by cathepsin E. Eur. J. Immunol. 22 (1992) 1519-1524.
2. K. Nishishita, H. Sakai, E. sakai, Y. Kato, K. Yamamoto, Age-related and dexamethasone-induced changes in cathepsins E and D in rat thymic and splenic cells. Arch. Biochem. Biophys. 333 (1996) 349-358.
3. D.F. Sastradipura, H. Nakanishi, T. Tsukuba, K. Nishishita, H. Sakai, Y. Kato, T. Gotow, Y. Uchiyama, K. Yamamoto, Identification of cellular compartments involved in processing of cathepsin E in primary cultures of rat microglia. J. Neurochem. 70 (1998) 2045-2056.
4. T. Nishioku, K. Hashimoto, K. Yamashita, S-Y. Liou, Y. Kagamiishi, H. Maegawa, N. Katsube, C. Peters, K. von Figura, P. Saftig, N. Katunuma, K. Yamamoto, H. Nakanishi, Involvement of cathepsin E in exogenous antigen processing in primary cultured murine microglia. J. Biol. Chem. 277 (2002) 4816-4822.
5. C.S.K. Yee, Y. Yao, P. Li, M.J. Klemsz, J.S. Blum, C-H. Chan, Cathepsin E: a novel target for regulation by class II transactivator. J. Immunol. 172 (2004) 5528-5534.
6. B.M.Chain, P. Free, P. Medd, C. Swetman, A.B. Tabor, N. Terrazzini, The

expression and function of cathepsin E in dendritic cell. J. Immunol. 174 (2005) 1791-1800.

7. M. Yanagawa, T. Tsukuba, T. Nishioku, Y. Okamoto, K. Okamoto, R. Takii, Y. Terada, K.I. Nakayama, T. Kadowaki, K. Yamamoto, Cathepsin E deficiency induces a novel form of lysosomal storage disorder showing the accumulation of lysosomal membrane sialoglycoproteins and the elevation of lysosomal pH in macrophages. J. Biol. Chem. 282 (2007) 1851-1862.
8. H. Kakehashi, T. Nishioku, T. Tsukuba, T. Kadowaki, S. Nakamura, K. Yamamoto, Differential regulation of the nature and functions of dendritic cells and macrophages by cathepsin E. J. Immunol. 179 (2007) 5728-5737.
9. N. Zaidi, T. Herrmann, D. Baechle, S. Schleicher, J. Gogel, C. Driessen, W. Voelter, H. Kalbacher, A new approach for distinguishing cathepsin E and D activity in antigen-processing organelles. FEBS J. 274 (2007) 3138-3149.
10. T. Kawakubo, A. Yasukochi, T. Tsukuba, T. Kadowaki, K. Yamamoto, Gene expression profiling of mammary glands of cathepsin E-deficient mice compared with wild-type littermates. Biochimie 90 (2008) 396-404.
11. T. Kageyama, K. Takahashi, A cathepsin D-like acid proteinase from human gastric mucosa: purification and characterization. J. Biochem. 87 (1980) 725-735.

12. N. Muto, K. Murayama-Arai, S. Tani, Purification and properties of a cathepsin D-like acid proteinase from rat gastric mucosa. Biochim. Biophys. Acta 745 (1983) 61-69.

13. I,M. Samloff, R.T. Taggart, T. Shiraishi, T. Branch, W.A. Reid, R. Heath, R.W. Lewis, M.J. Valler, J. Kay. Slow moving proteinase; isolation, characterization, and immunohistochemical localization in gastric mucosa. Gastroenterology. 93 (1987) 77-84.

14. T. Saku, H. Sakai, Y. Shibata, Y. Kato, K. Yamamoto, An immunocytochemical study on distinct intracellular localization of cathepsin E and cathepsin D in human gastric cells and various rat cells. J. Biochem. 110 (1991) 956-964.

15. H. Sakai, T. Saku, Y. Kato, K. Yamamoto, Quantitation and immunohistochemical localization of cathepsins E and D in rat tissues and blood cells. Biochim. Biophys. Acta 991 (1989) 367-375.

16. K. Hara, K. Fukuyama, H. Sakai, K. Yamamoto, W.L. Epstein, Purification and immunohistochemical localization of aspartic proteinases in rat epidermis. J. Invest. Dermatol. 100 (1993) 394-399.

17. S. Igarashi, T. Takizawa, T. Takizawa, Y. Yasuda, H. Uchiwa, S. Hayashi, H. Brysk, J.M. Robinson, K. Yamamoto, M.M. Brysk, T. Horikoshi, Cathepsin D, but not

cathepsin E, degrades desmosomes during epidermal desquamation. Brit. J. Dermatol. 151 (2004) 355-361.

18. K. Yamamoto, V.T. Marchesi, Purification and characterization of acid proteinase from human erythrocyte membranes. Biochim. Biophys. Acta 790 (1984) 208-218.
19. K. Yamamoto, M. Takeda, H. Yamamoto, M. Tatsumi, Y. Kato, Human erythrocyte membrane acid proteinase (EMAP): sidedness and relation to cathepsin D. J. Biochem. 97 (1985) 821-830.
20. R. Fiocca, L. Villani, P. Tenti, M. Cornaggia, G. Finzi, C. Riva, C. Capella, J. Bara, I.M. Samloff, E. Solcia, The foveolar cell component of gastric cancer. Hum. Pathol. 21 (1990) 260-270.
21. Y. Yoshimine, T. Tsukuba, R. Isobe, M. Sumi, A. Akamine, K. Maeda, K. Yamamoto, Specific immunocytochemical localization of cathepsin E at the ruffled border membrane of active osteoclasts. Cell Tissue Res. 281 (1995) 85-91.
22. H. Sakai, Y. Kato, K. Yamamoto, Synthesis and intracellular distribution of cathepsins E and D in differentiating murine Friend erythroleukemia cells. Arch. Biochem. Biophys. 294 (1992) 412-417.
23. M. Yanagawa, T. Tsukuba, K. Okamoto, R. Takii, Y. Terada, T. Kadowaki, K. Yamamoto, Up-regulation, enhanced maturation, and secretion of cathepsin E in

mouse macrophages treated with interferon-γ or lipopolysaccharide. J. Oral Biosci. 48 (2006) 218-225.

24. C. Tulone, J. Tsang, Z. Prokopowicz, N. Grosvenor, B. Chain, Natural cathepsin E deficiency in the immune system of C57BL/6J mice. Immunogenetics 59 (2007) 927-935.

25. B.M. Chain, P. Free, P. Medd, C. Swetman, A.B. Tabor, N. Terrazzini, The expression and function of cathepsin E in dendritic cells. J. Immunol. 174 (2005) 1791-1800.

26. H. Nakanishi, T. Tsukuba, T. Kondou, T. Tanaka, K. Yamamoto, Transient forebrain ischemia induces increased expression and specific localization of cathepsins E and D in rat hippocampus and neostriatum. Exp. Neurol. 121 (1993) 215-223.

27. H. Nakanishi, K. Tominaga, T. Amano, I. Hirotsu, T. Inoue, K. Yamamoto, Age-related changes in activities and localizations of cathepsins D, E, B, and L in the rat brain tissues. Exp. Neurol. 126 (1994) 119-128.

28. T. Amano, H. Nakanishi, M. Oka, K. Yamamoto, Increased expression of cathepsins E and D in reactive microglial cells associated with spongiform degeneration in the brain stem of senescence-accelerated mouse. Exp. Neurol. 136 (1995) 171-182.

29. H. Nakanishi, T. Amano, D.F. Sastradipura, Y. Yoshimine, T. Tsukuba, K. Tanabe, I.

Hirotsu, T. Ohono, K. Yamamoto, Increased expression of cathepsins E and D in neurons of the aged rat brain and their colocalization with lipofuscin and carboxy-terminal fragments of Alzheimer amyloid precursor protein. J. Neurochem. 68 (1997) 739-749.

30. K. Tominaga, H. Nakanishi, Y. Yasuda, K. Yamamoto, Excitotoxin-induced neuronal death is associated with response of a unique intracellular aspartic proteinase, cathepsin E. J. Neurochem. 71 (1998) 2574-2584.
31. N. Shigematsu, T. Fukuda, T. Yamamoto, T. Nishioku, T. Yamaguchi, M. Himeno, K.I. Nakayama, T. Tsukuba, T. Kadowaki, K. Okamoto, S. Higuchi, K. Yamamoto, Association of cathepsin E deficiency with the increased territorial aggressive response of mice. J. Neurochem. 105 (2008) 1394-1404.
32. W.E. Lees, S. Kalinka, J. Meech, S.J. Capper, N.D. Cook, J. Kay, Generation of human endothelin by cathepsin E. FEBS Lett. 273 (1990) 99-102.
33. T. Kageyama, M. Ichinose, S. Yonezawa, Processing of the precursors to neurotensin and other bioactive peptides by cathepsin E, J. Biol. Chem. 270 (1995) 19135-19140.
34. F. Henningsson, K. Yamamoto, P. Saftig, T. Reinheckel, C. Peters, S.D. Knight, G. Pejler, A role for cathepsin E in the processing of mast-cell carboxypeptidase A. J.

Cell Sci.118 (2005) 2035-2042.

35. T. Tsukuba, K. Okamoto, Y. Okamoto, M. Yanagawa, K. Kohmura, Y. Yasuda, H. Uchi, T. Nakahara, M. Furue, K. Nakayama, T. Kadowaki, K. Yamamoto, K.I. Nakayama, Association of cathepsin E deficiency with development of atopic dermatitis. J. Biochem. 134 (2003) 893-902.

36. T. Tsukuba, S. Yamamoto, M. Yanagawa, K. Okamoto, Y. Okamoto, K.I. Nakayama, T. Kadowaki, K. Yamamoto, Cathepsin E-deficient mice show increased susceptibility to bacterial infection associated with the decreased expression of multiple cell surface Toll-like receptors. J. Biochem. 140 (2006) 57-66.

37. T. Kawakubo, K. Okamoto, J. Iwata, M. Shin, Y. Okamoto, A. Yasukochi, K.I. Nakayama, T. Kadowaki, T. Tsukuba, K. Yamamoto, Cathepsin E prevents tumor growth and metastasis by catalyzing the proteolytic release of soluble TRAIL from tumor cell surface. Cancer Res. 67 (2007) 10869-10878.

38. T. Tsukuba, M. Yanagawa, K. Okamoto, Y. Okamoto, Y. Yasuda, K.I. Nakayama, T. Kadowaki, K. Yamamoto, Impaired chemotaxis and cell adhesion due to decrease in several cell-surface receptors in cathepsin E-deficient macrophages. J. Biochem. 145 (2009) 565-573.

39. R.J. Riese, P.R. Wolf, D. Bromme, L.R. Natkin, J.A. Villadangos, H.L. Ploegh, H.A.

Chapman, Essential role for cathepsin S in MHC class II-associated invariant chain processing and peptide loading. Immunity 4 (1996) 357-366.

40. J.A. Villadangos, R.J. Riese, C. Peters, H.A. Chapman, H.L. Ploegh, Degradation of mouse invariant chain: roles of cathepsins S and D and the influence of major histocompatibility complex polymorphism. J. Exp. Med. 186 (1997) 549-560.
41. T. Nakagawa, W. Roth, P. Wong, A. Nelson, A. Farr, J. Deussing, J.A. Villadangos, H. Ploegh, C. Peters, A.Y. Rudensky, Cathepsin L: critical role in Ii degradation and CD4 T cell selection in the thymus. Science 280 (1998) 450-453.
42. P. Pierre, I. Mellman, Developmental regulation of invariant chain proteolysis controls MHC class II trafficking in mouse dendritic cells. 93 (1998) 1135-1145.
43. C. Driessen, R.A.R. Bryant, A-M. Lennon-Dumenil, J.A. Villadangos, P.W. Bryant, G-P. Shi, H.A. Chapman, H.L. Ploegh, Cathepsin S controls the trafficking and maturation of MHC class II molecules in dendritic cells. J Cell Biol. 147 (1999) 775-790.
44. G-P. Shi, J.A. Villadangos, G. Dranoff, C. Small, L. Gu, K.J. Haley, R. Riese, H.L. Ploegh, H.A. Chapman, Cathepsin S required for normal MHC class II peptide loading and germinal center development. 10 (1999) 197-206.
45. T.Y. Nakagawa, W.H. Brissette, P.D. Lira, R.J. Griffith, N. Petrushova, J. Stck, J.D.

McNeish, S.E. Eastman, E.D. Howard, S.R.M. Clarke, E.F. Rosloniec, E.A. Elliott, A.Y. Rudensky, Impaired invariant chain degradation and antigen presentation and diminished collagen-induced arthritis in cathepsin S null mice. Immunity 10 (1999) 207-217.

46. G-P. Shi, R.A.R. Bryant, R. Riese, S. Verhelst, C. Driessen, Z. Li, D. Bromme, H.L. Ploegh, H.A. Chapman, Role for cathepsin F in invariant chain processing and major histocompatibility complex class II peptide loading by macrophages. J. Exp. Med. 191 (2000) 1177-1186.

47. M.A. Marric, M.D. Taylor, J.S. Blum, Endosomal aspartic proteinases are required for invariant-chain processing. Proc. Natl. Acad. Sci. USA 91 (1994) 2171-2175.

48. S. Diment, Different roles for thiol and aspartyl proteases in antigen presentation of ovalbumin. J. Immunol. 145 (1990) 417-422.

49. G.M. Rodriguez, S. Diment, Role of cathepsin D in antigen presentation of ovalbumin. J. Immunol. 149 (1992) 2894-2898.

50. J.M. van Noort, M.J.M. Jacobs, Cathepsin D, but not cathepsin B, releases T cell stimulatory fragments from lysozyme that are functional in the context of multiple murine class II MHC molecules. Eur. J. Immunol. 24 (1994) 2175-2180.

51. J. Deussing, W. Roth, P. Saftig, C. Peters, H.L. Ploegh, J.A. Villadangos, Cathepsins

B and D are dispensable for major histocompatibility complex class II-mediated antigen presentation. Proc. Natl. Acad. Sci. USA 95 (1998) 4516-4521.

52. M. Fukuda, Lysosomal membrane glycoproteins. Structure, biosynthesis, and intracellular trafficking. J. Biol. Chem. 266 (1991) 21327-21330.

53. E-L. Eskelinen, Y. Tanaka, P. Saftig, At the acidic edge: emerging functions for lysosomal membrane proteins. Trends Cell Biol. 13 (2003) 137-145.

54. J.M. Holopainen, J. Saarikoski, P.K.J. Kinnunen, I. Järvelä, Elevated lysosomal pH in neuronal ceroid lipofuscinoses (NCLs). Eur. J. Biochem. 268 (2001) 5851-5856.

55. J.A. Schmid, L. Mach, E. Paschke, J. Glössl, Accumulation of sialic acid in endocytic compartments interferes with the formation of mature lysosomes. Impaired proteolytic processing of cathepsin B in fibroblasts of patients with lysosomal sialic acid storage disease. J. Biol. Chem. 274 (1999) 19063-19071.

56. P.J. Meikle, D.A. Brooks, E.M. Ravenscroft, M. Yan, R.E. Williams, A.E. Jaunzems, T.K. Chataway, L.E. Karageorgos, R.C. Davey, C.D. Boulter, S.R. Carlsson, J.J. Hopwood, Diagnosis of lysosomal storage disorders: evaluation of lysosome-associated membrane protein LAMP-1 as a diagnostic marker. Clin. Chem. 43 (1997) 1325-1335.

57. C.T. Hua, J.J. Hopwood, S.R. Carlsson, R.J. Harris, P.J. Meikle, Evaluation of the

lysosome-associated membrane protein LAMP-2 as a marker for lysosomal storage disorders. Clin. Chem. 44 (1998) 2094-2102.

58. J. Banchereau, F. Briere, C. Caux, J. Davoust, S. Lebecque, Y.J. Liu, B. Pulendran, K. Palucka, Immunobiology of dendritic cells. Annu. Rev. Immunol. 18 (2000) 767-811.

59. R.M. Steinman, M.C. Nussenzweig, Avoiding horror autotoxicus: the importance of dendritic cells in peripheral T cell tolerance. Proc. Natl. Acad. Sci. USA 99 (2002) 351-358.

60. C.X. Moss, J.A. Villadangos, C. Watts, Destructive potential of the aspartyl protease cathepsin D in MHC class II-restricted antigen processing. Eur. J. Immunol. 35 (2005) 3442-3451.

61. D.J. Lenschow, T.L. Walunas, J.A. Bluestone, CD28/B7 system of T cell costimulation. Annu. Rev. Immunol. 14 (1996) 233-258.

62. I.S. Grewal, R.A. Flavell, CD40 and CD154 in cell-mediated immunity. Annu. Rev. Immunol. 16 (1998) 111-135.

63. A.J. Coyle, J-C. Gutierrez-Ramos, The expanding B7 superfamily: increasing complexity in costimulatory signals regulating T cell function. Nat. Immunol. 2 (2001) 203-209.

64. G. Kroemer, M. Jäättelä, Lysosomes and autophagy in cell death control. Nat. Rev. Cancer 5 (2005) 886-897.

65. V. Karantza-Wadsworth, S. Patel, O. Kravchuk, G. Chen, R. Mathew, S. Jin, E. White, Autophagy mitigates metabolic stress and genome damage in mammary tumorigenesis. Genes Dev. 21 (2007) 1621-1635.

66. M. Ogata, S. Hino, A. Saito, K. Morikawa, S. Kondo, S. Kanemoto, T. Murakami, M. Taniguchi, I. Tanii, K. Yoshinaga, S. Shiosaka, J.A. Hammarback, F. Urano, K. Imaizumi, Autophagy is activated for cell survival after endoplasmic reticulum stress. Mol. Cell. Biol. 26 (2006) 9220-9231.

67. W.X. Ding, H.M. Ni, W. gao, Y.F. Hou, M.A. Melan, X. Chen, D.B. Stolz, Z.M. Shao, X.M. Yin, Differential effects of endoplasmic reticulum stress-induced autophagy on cell survival. J. Biol. Chem. 282 (2007) 4702-4710.

68. R. Mathew, V. Karantza-Wadsworth, E. White, Role of autophagy in cancer. Nat. Rev. Cancer 7 (2007) 961-967.

69. P. Tenti, S. Romagnoli, E. Silini, R. Zappatore, P. Giunta, G. Stella, L. Carnevali, Cervical adenocarcinomas express markers common to gastric, intestinal, and pancreatobiliary epithelial cells. Pathol Res. Pract. 190 (1994) 342-349.

70. K. Matsuo, I. Kobayashi, T. Tsukuba, T. Kiyoshima, Y. Ishibashi, A. Miyoshi, K.

Yamamoto, H. Sakai, Immunohistochemical localization of cathepsins D and E in human gastric cancer: a possible correlation with local invasive and metastatic activities of carcinoma cells. Hum. Pathol. 27 (1996) 184-190.

71. F. Mota, J.H.C. Kanan, N. Rayment, T. Mould, A. Singer, B.M. Chain, Cathepsin E expression by normal and premalignant cervical epithelium. Am. J. Pathol. 150 (1997) 1223-1229.

72. B. Terris, E. Blaveri, T.T. Crnogorac-Jurcevic, M. Jones, E. Missiaglia, P. Ruszniewski, A. Sauvanet, N.R. Lemoine, Characterization of gene expression profiles in intraductal papillary-mucinous tumors of the pancreas. Am. J. Pathol. 160 (2002) 1745-1754.

73. R. Ullmann, P. Morbini, I. Halbwedl, M. Bongiovanni, M. Gogg-Kammerer, M. Papotti, S. Gabor, H. Renner, H.H. Ropper, Protein expression profiles in adenocarcinomas and squamous cell carcinomas of the lung generated using tissue microarrays. J. Pathol. 203 (2004) 798-807.

74. E. Blaveri, J.P. Simko, J.E. Korkola, J.L. Brewer, F. Baehner, K. Mehta, S. DeVries, T. Koppie, S. Pejavar, P. Carroll, F.M. Waldman, Bladder cancer outcome and subtype classification by gene expression. Clin. Cancer Res. 11 (2005) 4044-4055.

75. P.J. Wild, A. Herr, C. Wissmann, R. Stoehr, A. Rosenthal, D. Zaak, R. Simon, R.

Knuechel, C. Pilarsky, A. Hartmann, Gene expression profiling of progressive papillary noninvasive carcinomas of the urinary bladder. Clin. Cancer Res. 11 (2005) 4415-4429.

76. B.C. Lewis, D.S. Klimstra, N.D. Socci, S. Xu, J.A. Koutcher, H.E. Varmus, The absence of *p53* promotes metastasis in a novel somatic mouse model for hepatocellular carcinoma. Mol. Cell. Biol. 25 (2005) 1228-1237.

77. L. Busquets, H. Guillen, M.E. DeFord, M.A. Suckow, R.M. Navari, F.J. Castellino, M. Prorok, Cathepsin E is a specific marker of dysplasia in APC mouse intestine. Tumor Biol. 27 (2006) 36-42.

78. H.E. Marin, M.A. Peraza, A.N. Billin, T.M. Willson, J.M. Ward, M.J. Kennett, F.J. Gonzalez, J.M. Peters, Ligand activation of peroxisome proliferator-activated receptor β inhibits colon carcinogenesis. Cancer Res. 66 (2006) 4394-4401.

79. J.L. Page, S.C. Strom, C.J. Omiecinski, Regulation of the human cathepsin E gene by the constitutive andorostane receptor. Arch. Biochem. Biophys. 467 (2007) 132-138.

80. M. Caruso, J. Moore, G.J. Goodall, M. Thomas, S. Phillis, A. Tyskin, G. Cheetham, N. Lerda, H. Takahashi, A. Ruszkiewicz, Over-expression of cathepsin E and trefoil factor 1 in sessile serrated adenomas of the colorectum identified by gene expression

analysis. Virchows Arch. 454 (2009) 291-302.

81. H. Rochefort, Cathepsin D in breast cancer: a tissue marker associated with metastasis. Eur. J. Cancer 28A (1992) 1780-1783.

82. G. Ferrandina, G. Scambia, F. Bardelli, P. Benedetti, P. Panici, S. Mancuso, A. Messori, Relationship between cathepsin D content and disease-free survival in node-negative breast cancer patients: a meta-analysis. Br. J. Cancer 76 (1997) 661-666.

83. J.P. Cherry, J.A. Mordente, J.R. Chapman, M.S. Choudhury, H. Tazaki, C. Mallouh, S. Konno, Analysis of cathepsin D forms and their clinical implications in human prostate cancer. J. Urol. 160 (1998) 2223-2228.

84. J.A. Foekens, M.P. Look, B.J. Vries, M.E. Meijer-van Gelder, W.L. von Putten, J.G. Klijin, Cathepsin D in primary breast cancer: prognostic evaluation involving 2810 patients. Br. J. cancer 79 (1999) 300-307.

85. T. Nomura, N. Katunuma, Invovement of cathepsins in the invasion, metastasis and proliferation of cancer. J. Med. Invest. 52 (2005) 1-9.

86. M.M. Mohamed, B.F. Sloane, Cysteine cathepsins: multifunctional enzymes in cancer. Nat. Rev. Cancer, 6 (2006) 764-775.

87. C.M. Overall, O. Kleifeld, Tumor microenvironment-opinion: validating matrix

metalloproteinases as drug targets and anti-targets for cancer therapy. Nat. Rev. Cancer, 6 (2006) 227-239.

88. K. Uno, T. Azuma, M. Nakajima, K. Yasuda, T. Hayakumo, H. Mukai, T. Sakai, K. Kawai, Clinical significance of cathepsin E in pancreatic juice in the diagnosis of pancreatic ductal adenocarcinoma. J. Gastroenterol. Hepatol. 15 (2000) 1333-1338.
89. T. Saku, H. Sakai, N. Tsuda, H. Okabe, Y. Kato, K. Yamamoto, Cathepsins D and E in normal, metaplastic, dysplastic, and carcinomatous gastric tissue: an immunohistochemical study. Gut 31 (1990) 1250-1255.
90. V. Cavailles, P. Augereau and H. Rochefort, Cathepsin D gene is controlled by a mixed promoter, and estrogens stimulate only TATA-dependent transcription in breast cancer cells, Proc. Natl. Acad. Sci. USA 90 (1993) 203–207.
91. M. Glondu, P. Coopman, V. Laurent-Matha, M. Garcia, H. Rochefort, E. Liaudet-Coopman, A mutated cathepsin-D devoid of its catalytic activity stimulates the growth of cancer cells. Oncogene 20 (2001) 6920-6929.
92. V. Vetvicka, J. Vetvickova and M. Fusek, Role of procathepsin D activation peptide in prostate cancer growth. Prostate 44 (2000) 1–7.
93. M. Beaujouin, C. Prébois, D. Derocq, V. Laurent-Matha, O. Masson, S. Pattingre, P. Coopman, N. Bettache, J. Grossfield, R.E. Hollingsworth, H. Zhang, Z. Yao, B.T.

Hyman, P. van der Geer, G.K. Smith, E. and Liaudet-Coopman, Pro-cathepsin D interacts with the extracellular domain of the beta chain of LRP1 and promotes LRP1-dependent fibroblast outgrowth. J Cell Sci. 123 (2010) 3336-3346.

94. S.R. Wiley, K. Schooley, P.J. Smolak, W.S. Din, C-P. Huang, J.K. Nicholl, G.R. Sutherland, T.D. Smith, C. Rauch, C.A. Smith, R.G. Goodwin, Identification and characterization of a new member of the TNF family that induces apoptosis. Immunity 3 (1995) 673-682.

95. R.M. Pitti, S.A. Marsters, S. Ruppert, C.J. Donahue, A. Moore, A. Ashkenazi, Induction of apoptosis by apo-2 ligand, a new member of the tumor necrosis factor cytokine family. J. Biol. Chem. 271 (1996) 12687-12690.

96. H. Walczak, R.E. Miller, K. Ariail, B. Gliniak, T.S. Griffith, M. Kubin, W. Chin, J. Jones, A. Woodward, T. Le, C. Smith, P. Smolak, R.G. Goodwin, C.T. Rauch, J.C.L. Schuh, D.H. Lynch, Tumoricidal activity of tumor necrosis factor-related apoptosis-inducing ligand *in vivo*. Nat. Med. 5 (1999) 157-163.

97. A. Ashkenazi, R.C. Pai, S. Fong, S. Leung, D.A. Lowrence, S.A. Marsters, C. Blackie, L. Chang, A.E. McMurtrey, A. Hebert, L. DeForge, I.L. Koumenis, D. Lewis, L. Harris, J. Bussiere, H. Koeppen, Z. Shahrokh, R.H. Schwall, Safety and antitumor activity of recombinant soluble Apo2 ligand. J. Clin. Invest. 104 (1999)

155-162.

98. T.S. Griffith, S.R. Wiley, M.Z. Kubin, L.M. Sedger, C.R. Maliszewski, N.A. Fanger, Monocyte-mediated tumoricidal activity via the tumor-necrosis factor-related cytokine, TRAIL. J. Exp. Med. 189 (1999) 1343-1354.

99. M.R. Hussein, Tumor-associated macrophages and melanoma tumorigenesis: integrating the complexity. Int. J. Exp. Pathol. 87 (2006) 163-176.

100. J. Condeelis, J.W. Pollard, Macrophages: obligate partners for tumor cell migration, invasion, and metastasis. Cell 124 (2006) 263-266.

101. Y.C. Wang, F. He, F. Feng, X.W. Liu, G.Y. Dong, H.Y. Qin, X.B. Hu, M.H. Zheng, L. Liang, L. Feng, Y.M. Liang, H. Han, Motch signaling determines the M1 versus M2 polarization of macrophages in antitumor immune responses. Cancer res. 70 (1010) 4840-4849.

102. M. Shin, T. Kadowaki, J. Iwata, T. Kawakubo, N. Yamaguchi, R. Takii, T. Tsukuba, K. Yamamoto, Association of cathepsin E with tumor growth arrest through angiogenesis inhibition and enhanced immune responses. Biol. Chem. 388 (2007) 1173-1181.

103. A. Ysukochi, T. Kawakubo, S. Nakamura, K. Yamamoto, Cathepsin E enhances anticancer activity of doxorubicin on human prostate cancer cells showing resistance

to TRAIL-mediated apoptosis. Biol. Chem. 391 (2010) 947-958.

104. T. Kawakubo, A. Yasukochi, K. Okamoto, Y. Okamoto, S. Nakamura, K. Yamamoto. The role of cathepsin E in terminal differentiation of keratinocytes. (2011) in press.

105. F. Egberts, M. Heinrich, J-M. Jensen, S. Winoto-Morbach, S. Pfeiffer, M. Wickel, M. Schunck, J. Steude, P. Saftig, E. Proksch, S. Schütze, Cathepsin D is involved in the regulation of transglutaminase 1 and epidermal differentiation. J. Cell Sci. 117 (2004) 2295-2307.

106. K. Yamamoto, Cathepsin E and cathepsin D: Biosynthesis, processing and subcellular location, in: K. Takahashi (ed.), Aspartic Proteinases: Structure, Function, Biology, and Biomedical Implications, Plenum Press, New York, 1995, pp. 223-229.

107. K. Yamamoto, Cathepsin E and cathepsin D: in V. Turk (Ed.) Proteases: New Perspectives, Birkhäuser Verlag, Basel, 1999, pp. 5971.

108. T. Tsukuba, K. Okamoto, Y. Yasuda, W. Morikawa, H. Nakanishi, K. Yamamoto, New functional aspects of cathepsin D and cathepsin E. Mol. Cells. 10 (2000) 601-611.

109. N. Zaidi, H. Kalbacher, Cathepsin E: A mini review, Biochem. Biophys. Res. Commun. 367 (2008) 517-522.

110. N. Zaidi, C. Hermann, T. Herrmann, H. Kalbacher, Emerging functional roles of cathepsin E. Biochem. Biophys. Res. Commun. 377 (2008) 327-330.

Figure legend

Fig. 1. A schematic representation for the conditions manifested in macrophages by cathepsin E deficiency.

Fig. 2. A schematic representation for the conditions manifested in dendritic cells by cathepsin E deficiency.

Fig. 3. A schematic representation of the multiple mechanisms for the antitumorigenic activity of cathepsin E.

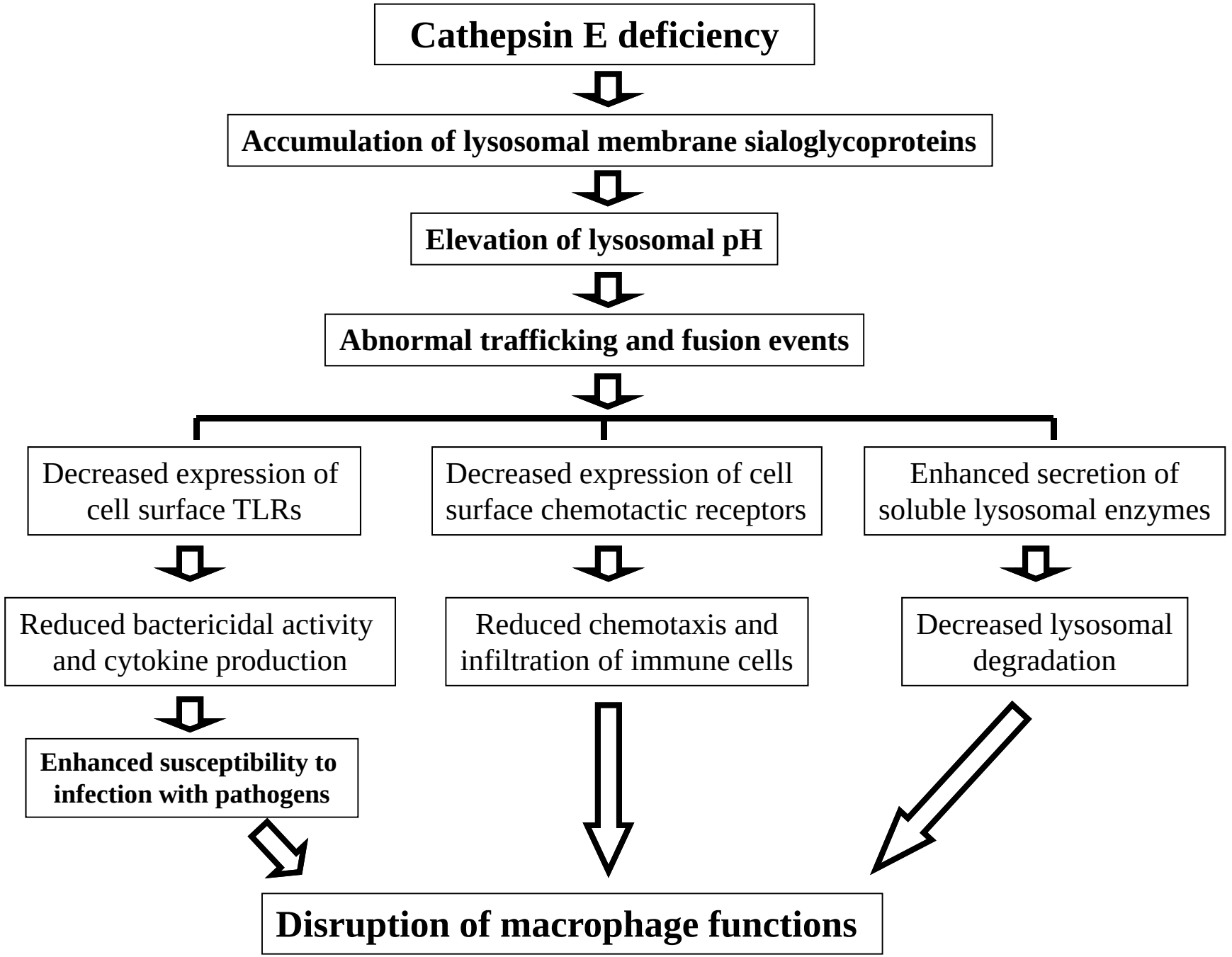

Fig. 1 Yamamoto et al.

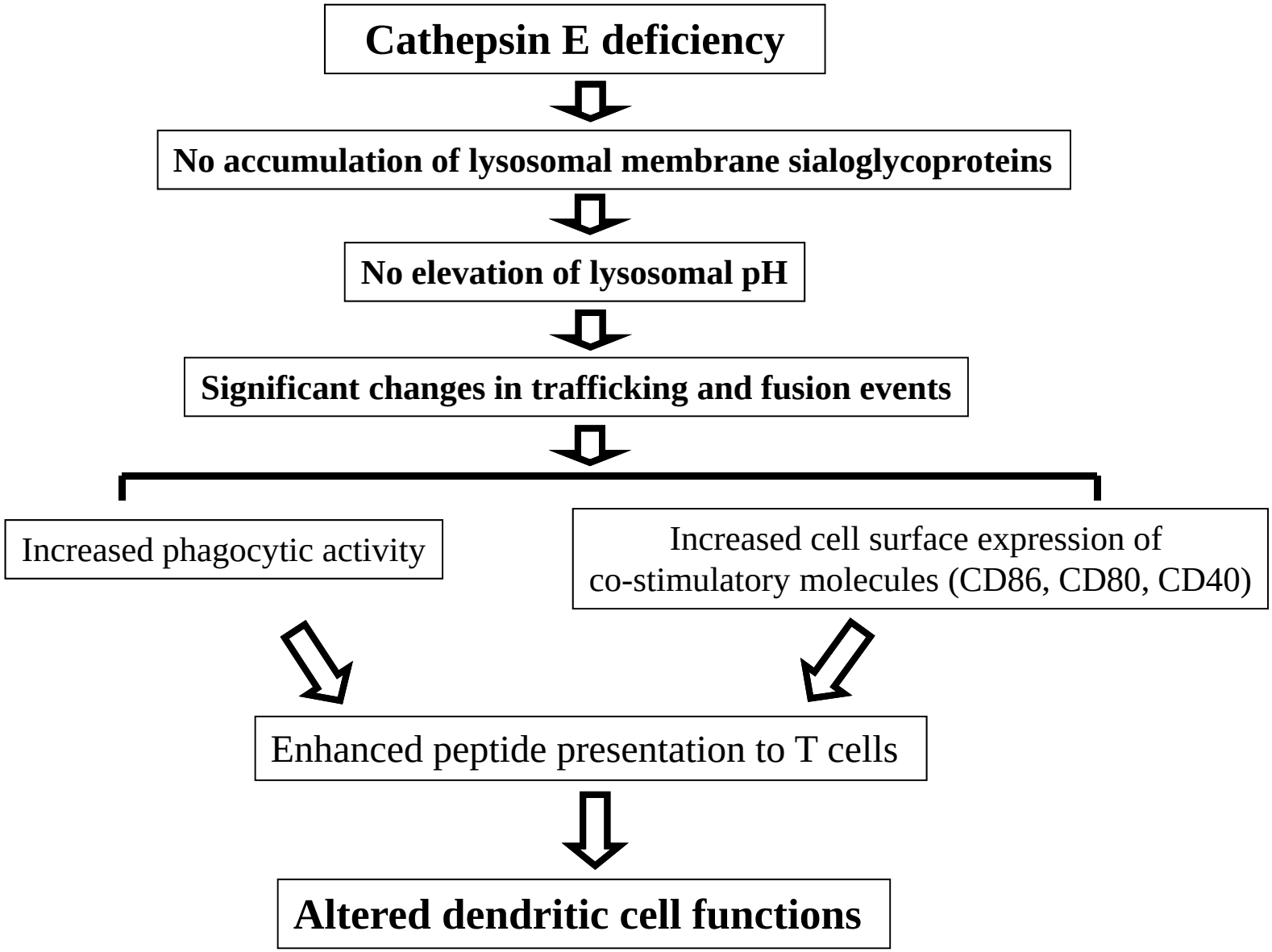

Fig. 2 Yamamoto et al.

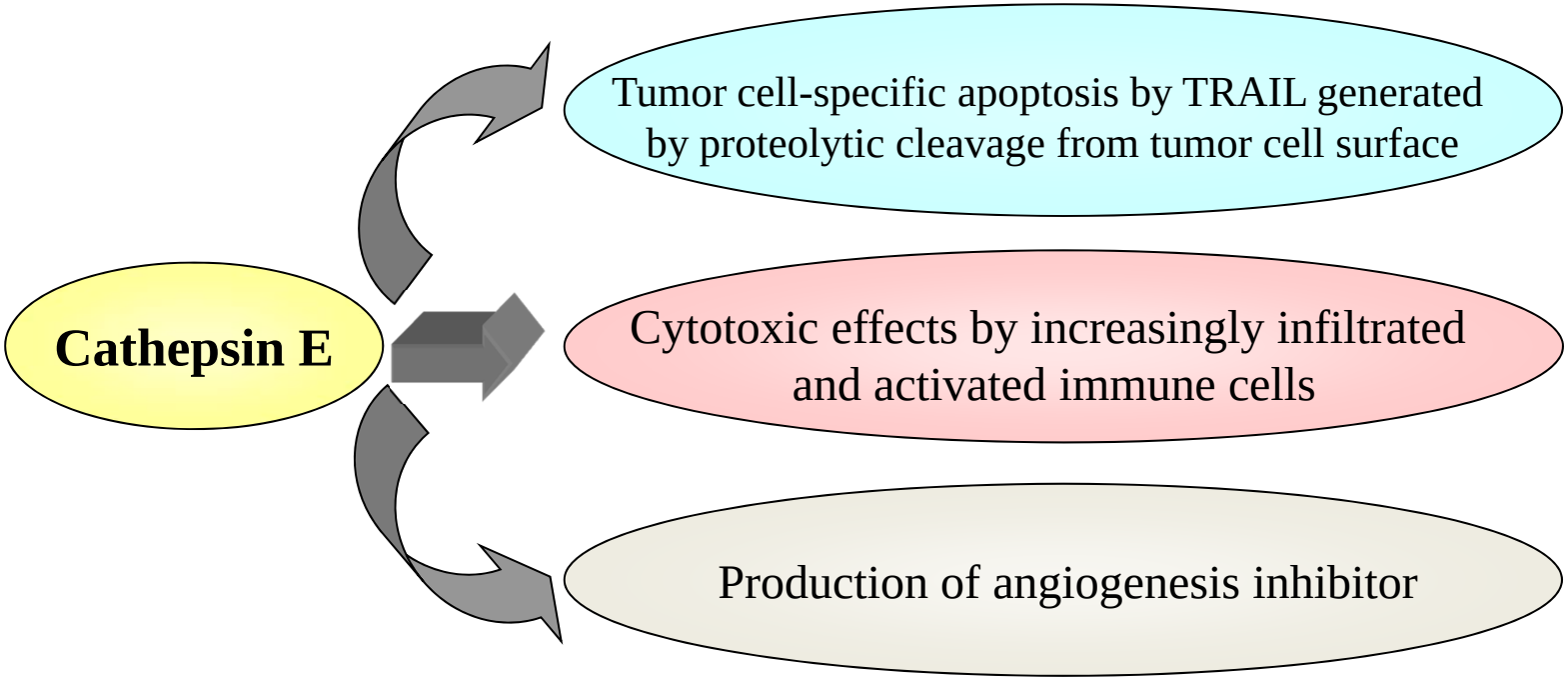

Fig. 3 Yamamoto et al.