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New insights into procathepsin D in pathological and physiological conditions

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Abstract

Procathepsin D is a major glycoprotein that is secreted from numerous types of cancer cells including breast, lung and prostrate carcinomas. It affects multiple stages of tumorigenesis that include proliferation, invasion, metastasis and apoptosis. Previous studies showed that the mitogenic effect of procathepsin D on cancer cells was mediated through its propeptide or activation peptide. Recent studies have also implicated the possible use of procathepsin D/activation peptide as a marker of cancer progression. Considering the broad range of functions of procathepsin D, the present review summarizes the three major potentials of procathepsin D-cancer progression, tumor marker and wound healing.

Keywords: Procathepsin D, cancer, wound healing, aspartic proteinase.

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Procathepsin D in Cancer Progression

Despite decades of intensive research, cancer remains one of the most dangerous diseases in the developed world. In the United States alone, thousands of people die each year from a variety of cancers. The incidence of various tumors and cancers is increasing at an alarming rate despite great achievements and decades of intensive, labor-consuming and expensive research. According to the National Cancer Institute estimates, slightly less than one-in-two men and a little more than one-in-three women in the U.S. are likely to contract cancer in their lifetime. Although some success in surgical removal of primary tumors has been achieved, it is difficult to terminate the spread of metastatic cancer or to predict which early stage cancers are potentially metastatic. Therefore, there has been an intense interest in methods for identifying patients with higher recurrence risk. Among the many, procathepsin D (pCD) is one of the independent prognostic factors that has gained considerable attention in recent years [1-8].

Independent studies established an essential role of pCD in cancer development [9, 10]. The first study showing increased levels of cathepsin D (CD; processed form of pCD) in several human neoplastic tissues was reported in the mid-eighties [11]. Subsequent clinical studies demonstrated a correlation between pCD/CD levels and tumor size, tumor grade, tumor aggressiveness, incidence of metastasis, prognosis and a degree of chemoresistance in a variety of solid tumors [12-14]. Various studies employing several different approaches such as immunohistochemistry, *in situ* hybridization, cytosolic assay, Northern and Western blot analyses indicated that in most breast cancer tumors, pCD was overexpressed at least 2-to-50 fold. Importantly, the overexpression was demonstrated both at the mRNA and protein levels [1].

The mitogenic effect of secreted pCD on breast cancer cells was first demonstrated by Vignon et al. [11]. Subsequent additional studies showed that secreted pCD functioned as an autocrine growth factor in breast [15, 16], prostrate [17, 18], ovarian [19] and lung cancer cells [20, 21]. In contrast, decreased expression of pCD by antisense gene transfer [22], RNA interference [23] or ribozymes [24] resulted in decreased growth of breast cancer cells *in*

vitro and *in vivo*. Consistent with this, tumor growth was also inhibited by utilization of anti-pCD antibodies *in vivo* and *in vitro* [15, 18, 25, 26].

In addition to this autocrine mitogenic effect, pCD is also implicated in paracrine communication with surrounding cells. Berchem's group demonstrated that pCD not only stimulated parent cancer cell proliferation but also tumor angiogenesis by a paracrine mechanism [27]. Consistent with this study, Liaudet-Coopman et al. showed that pCD secreted from cancer cells possibly stimulated proliferation, survival, motility and invasive potential of surrounding fibroblasts by activation of the ras-MAPK pathway indicating pCD's role in paracrine communication [28]. Supporting these data, we demonstrated substantial secretion of cytokines especially IL-4, IL-8, IL-10, IL-13 and MIP-1β from both cancer cell lines and fibroblasts after addition of pCD which promoted the growth of both cell types [29].

Direct association of secreted pCD with cancer invasion and metastasis has also been demonstrated by independent studies. The first direct role of pCD in cancer metastasis was demonstrated in rat tumor cells where over-expressed pCD increased the metastatic potential of these cells [30]. Alternatively, downregulation of pCD expression by antisense gene inhibited lung metastasis of breast cancer cells but had no effect on invasion *in vitro* [22]. However, Tedone et al. showed that downregulation of pCD expression resulted in decreased invasion of MCF-7 cells *in vitro* [31]. In addition, Sivaparvathi et al. observed that anti-CD antibodies inhibited glioblastoma cell invasion through Matrigel [32]. Consistent with this data, our studies with decreased pCD secretion from breast and lung cancer cells directly influenced their ability to cross the Matrigel membrane *in vitro* and *in vivo* [21, 23, 24, 33].

Berchem et al demonstrated that secreted pCD stimulated tumor angiogenesis in tumor xenograft in athymice nude mice [27]. Supporting this, we recently observed several regulators of angiogenesis being differentially expressed in breast cancer cells treated with the activation peptide of pCD [34]. These studies strongly suggest that pCD may also be involved in angiogenesis during cancer progression. Since the control of angiogenesis is a balance between positive and negative angiogenic factors, additional experiments are required to define the exact role of pCD in angiogenesis.

Supporting the diverse functions of pCD in cancer progression, microarray analysis of activation peptide (AP) treated breast cancer cells showed differential expression of genes involved in cell cycle progression, cell survival, cell adhesion, angiogenesis, invasion and metastasis [34]. In contrast, decreased expression of these genes in response to ablated pCD using RNA interference further confirmed the role of pCD in cancer progression [23].

Despite extensive documentation regarding pCD's role in cancer progression, the underlying mechanism remains largely unknown. Independent studies have shown that the secreted pCD binds to the surface of the breast cancer cells through a receptor with possible downstream signalling [25, 35]. Based on these observations, we proposed a model for the mechanism of pCD action in which overexpressed pCD escapes its normal targeting pathway and is secreted from the cancer cells. The secreted pCD interacts via its activation peptide to an unidentified cell surface receptor present on its surrounding cells. This interaction releases a signal resulting in differential expression of cancer promoting genes that includes various cytokines that stimulate tumor growth.

Collectively, these studies not only define pCD as a tumor marker of cancer progression but also underlie pCD as a potential target for cancer therapy if selective inhibition of pCD interaction with a cellular receptor could be achieved.

Procathepsin D as a Tumor Marker

Current diagnostic assays for cancers are antigen-based and rely on the detection of circulating proteins that are associated within a particular cancer. These assays are based on the expression, synthesis and release of specific proteins by tumor cells either by active secretion or shedding or as a consequence of cell death (either by necrosis, apoptosis, or autophagy). Regardless, these antigenic proteins must "escape" the primary site of disease, saturate the antigen-processing capacity of the individual's immune components, gain access to the circulation, and reach a sufficient steady-state concentration to be detected by enzyme- or radiolabel-based immunoassays. These events usually occur well after the initial establishment of disease. Thus, despite the fact that certain specific antigenic epitopes exhibit common recognition sites among patients with the same tumor types, the use of these antigen-based cancer assays has not been widely accepted into clinical practice and many individual countries differ in the use of these potential diagnostic factors.

Research performed in both our laboratory and others has demonstrated the presence of anti-pCD autoantibodies [36]. As these antibodies are specific to pCD only and do not recognize mature CD [37, 38], they represent an ideal target for comparison of the pCD presence and cancer progression.

As pCD/CD is supposed to be contained inside the cells, it is possible that the body will react to their presence by formation of specific autoantibodies. Subsequently, their level might correlate with the stage of breast, lung and prostate cancer, corresponding with the increased number of pCD-releasing cancer cells and thus offer a cost-effective, non-invasive screening test. As the release of pCD was observed in numerous cancer types [15-21], one can assume that the specific autoantibodies will also be formed in additional types of cancer. In our preliminary experiments, we found the elevated levels of

anti-pCD autoantibodies in lung, prostate and stomach cancer. We assume that the amount of the APpCD/pCD in the patient's serum will change with the progress of the cancer disease, in correlation with the increased number of pCD-releasing cancer cells. Since the levels of autoantibodies corresponded with stages of breast cancer, it is clear that there is a high clinical potential in evaluation of specific anti-pCD autoantibodies as biological marker.

Procathepsin D in Wound Healing

In past decades, more and more interest was focused on the possible additional roles of pCD's. Independent studies showed the potential role of pCD in wound healing, tissue remodeling [39] and programmed cell death - apoptosis [40, 41]. Epidermis is the barrier between the body and external environment which is constantly exposed to various forms of environmental and physical stresses. Keratinocytes are the basic elemental cells that form the epidermis and play a critical role in normal regeneration and healing processes. Skin healing is dependent upon several processes that involve inflammation, protein synthesis, matrix deposition, migration and subsequent proliferation of keratinocytes [42, 43]. Numerous proteins that include proteolytic enzymes such as matrix metalloproteinases [44], interstitial collagenase [45] and cathepsin B [46] are secreted from keratinocytes. During the wound healing process, these proteolytic enzymes may play a role in motility of keratinocytes by remodeling of extracellular matrix for migration of keratinocytes to peripheral layers of epidermis. Indeed, the study conducted by Katz and Taichmann [47], focusing on the proteins secreted by cultured human epidermal keratinocytes, showed CD being one of the secreted proteins.

In skin, increased levels of the mature form of CD was shown in basal keratinocytes during hyperproliferative skin disorders such as psoriasis [48]. In addition, the involvement of different isoforms of CD in the epidermal cell differentiation was suggested. The presence of pCD was shown in the spinous layer and the active forms were present in stratum corneum, where they played a role in epidermal desquamation [49, 50]. Although the role of CD in epidermal differentiation has been defined, the presence of pCD at different stages of differentiation is still unclear. Moreover, most of these studies were performed using cell lysates where all the isoforms are present, thus making clear distinction of the roles played by individual isoforms virtually impossible.

To better define the role of pCD in differentiation, our recent study demonstrated active secretion of pCD by human keratinocytes cell line HaCaT. Subsequent experiments showed that exogenous addition of purified pCD enhanced the proliferation of HaCaT cells. This proliferative effect of pCD was inhibited by monoclonal antibody against the activation peptide of pCD. Supporting this, treatment of HaCaT cells with pCD or its activation peptide resulted in the secretion of a set of cytokines that promoted the growth of cells in a paracrine manner. The role of secreted pCD and its mechanism of action were further studied in a scratch wound model. While the presence of pCD and its activation peptide enhanced the regeneration of monolayer, this effect was reversed by the addition of anti-AP antibody. Finally, expression and secretion of pCD was upregulated in HaCaT cells exposed to various stress conditions. Taken together, our results strongly suggest that the secretion of pCD is not only linked to cancer cells but also plays an essential role in the normal physiological conditions such as wound healing and tissue remodeling [51].

Conclusion

While many functions of pCD in the physiological and pathological processes could be attributed to its enzymatic activity, this review clearly establishes that some of the functions of pCD are independent of its protease activity and rely on the ability of pCD to interact with other important molecules. Therefore, it seems inevitable that searching for pCD-interacting partners should be conducted to explore the mechanism of pCD actions.

References

1. Rochefort H. Biological and clinical significance of cathepsin D in breast cancer. Acta Oncol 1992; 31:125-130.
2. Johnson MD, Torri JA, Lippman ME, et al. The role of cathepsin D in the invasiveness of human breast cancer cells. Cancer Res 1993; 53: 873-877.
3. Pujol P, Maudelonde T, Daures JP, et al. A prospective study of the prognostic value of cathepsin D levels in breast cancer cytosol. Cancer 1993; 71: 2006-2012.
4. Schultz DC, Bazel S, Wright LM, et al. Western blotting and enzymatic activity analysis of cathepsin D in breast tissue and sera of patients with breast cancer and benign breast disease and of normal controls, Cancer Res 1994; 54: 48-54.
5. Garcia M, Platet N, Liaudet E, et al. Biological and clinical significance of cathepsin D in breast cancer metastasis. Stem Cells 1996; 14: 642-50.
6. Billgren AM, Rutqvist LE, Johansson H, et al. The role of cathepsin D and PAI-1 in primary invasive breast cancer as prognosticators and predictors of treatment benefit with adjuvant tamoxifen. Eur J Cancer 2000; 36: 1374-1380.
7. Gaci Z, Bouin-Pineau MH, Gaci M, et al. Prognostic impact of cathepsin D and c-erbB-2 oncoprotein in a subgroup of node-negative breast cancer patients with low histological grade tumors. Int J Oncol 2001; 18: 793-800.
8. Fusek M, Vetvicka V. Aspartic Proteinases. Physiology and Pathology. CRC Press, Boca Raton, 1995.
9. Podhajcer OL, Bover L, Bravo AL, et al. Expression of cathepsin D in primary and metastatic human melanoma and dysplastic nevi. J Invest Dermatol 1995; 104: 340-344.

10. Bazzett LB, Watkins CS, Gercel-Taylor C, et al. Modulation of proliferation and chemosensitivity by pCD and its peptides in ovarian cancer. Gynecol Oncol 1999; 74: 181-187.
11. Vignon F, Capony F, Chambon M, et al. Autocrine growth stimulation of the MCF 7 breast cancer cells by the estrogen-regulated 52 K protein. Endocrinology 1986; 118: 1537-1545.
12. Leto G, tumminello FM, Crescimanno M, Flandina C, Gebbia N. Cathepsin D expression levels in nongynecological solid tumors: clinical and therapeutic implications. Clin Exp Metastasis 2004; 21: 91-106.
13. Ioachin E. Immunohistochemical tumour markers in endometrial carcinoma. Eur J Gynaecol Oncol 2005; 26: 363-371.
14. Cunat S, Hoffmann P, Pujol P. Estrogens and epithelial ovarian cancer. Gynecol Oncol 2004; 94: 25-32.
15. Fusek M, Vetvicka V. Mitogenic function of human procathepsin D: the role of the propeptide. Biochem J 1994; 303: 775-780.
16. Vetvicka V, Vetvickova J, Fusek M. Effect of human procathepsin D on proliferation of human cell lines. Cancer Lett 1994; 79: 131-135.
17. Vetvicka V, Vetvickova J, Fusek M. Effect of procathepsin D and its activation peptide on prostate cancer cells. Cancer Lett 1998; 129: 55-59.
18. Vetvicka V, Vetvickova J, Fusek M. Role of procathepsin D activation peptide in prostate cancer growth. Prostate 2000; 44: 1-7.
19. Bazzett LB, Watkins CS, Gercel-Taylor C, Taylor DD Modulation of proliferation and chemosensitivity by procathepsin D and its peptides in ovarian cancer. Gynecol Oncol 1999; 74: 181-187.
20. Vetvicka V, Vetvickova J, Benes P. Role of enzymatically inactive procathepsin D in lung cancer. Anticancer Res 2004; 24: 2739-2743.
21. Vashishta A, Ohri SS, Proctor M, Fusek M, Vetvicka V. Role of activation peptide of procathepsin D in proliferation and invasion of lung cancer cells. Anticancer Res 2006; 26: 4163-4170.
22. Glondu M, Liaudet-Coopman E, Derocq D, et al. Downregulation of cathepsin D expression by antisense gene transfer inhibits tumor growth and experimental lung metastasis of human breast cancer cells. Oncogene 2002; 21: 5127-5134.
23. Ohri SS, Vashishta A, Proctor M, Fusek M, Vetvicka V. Depletion of procathepsin D gene expression by RNA interference: A potential therapeutic target for breast cancer. Cancer Biol Ther 2007; 6: 1081-1087.
24. Vashishta A, Ohri SS, Proctor M, Fusek M, Vetvicka V. Ribozyme-targeting procathepsin D and its effect on invasion and growth of breast cancer cells: an implication in breast cancer therapy. Int J Oncol 2007; 30: 1223-1230.
25. Vetvicka V, Vetvickova J, Hilgert I, Voburka Z, Fusek M. Analysis of the interaction of procathepsin D activation peptide with breast cancer cells. Int J Cancer 1997; 73: 403-409.
26. Vetvicka V, Vetvickova J, Fusek M. Anti-human procathepsin D activation peptide antibodies inhibit breast cancer development. Breast Cancer Res Treat 1999; 57: 261-269.
27. Berchem G, Glondu M, Gleizes M, et al. Cathepsin D affects multiple tumor progression steps in vivo: proliferation, angiogenesis and apoptosis. Oncogene 2002; 21: 5951-5955.
28. Liaudet-Coopman E, Beaujouin M, Derocq D, et al. Cathepsin D. newly discovered functions of a long-standing aspartic protease in cancer and apoptosis. Cancer Lett 2006; 237: 167-179.
29. Fusek M, Vetvickova J, Vetvicka V. Secretion of cytokines in breast cancer cells: the molecular mechanism of pCD proliferative effects. J Interferon Cytokine Res 2007; 27: 191-199.
30. Garcia M, Derocq D, Pujol P, Rochefort H. Overexpression of transfected cathepsin D in transformed cells increases their malignant phenotype and metastatic potency. Oncogene 1990; 5: 1809-1814.
31. Tedone T, Correale M, Barborossa G, Casavole V, Paradiso A, Reshkin SJ. Release of the aspartyl protease cathepsin D is associated with and facilitates human breast cancer cell invasion. FASEB J 1997; 11: 785-792.
32. Sivaparvathi M, Sawaya R, Chintala SK, Go Y, Gakaslan ZL, Rao JS. Expression of cathepsin D during the progression of human gliomas. Neurosci Lett 1996; 208: 171-174.
33. Vetvicka V, Benes P, Fusek M. Procathepsin D in breast cancer: what do we know? Effects of ribozymes and other inhibitors. Cancer Gene Ther 2002; 9: 854-863.
34. Benes P, Vashishta A, Saraswat-Ohri S, Fusek M, Pospisilova S, Tichy B. Effect of procathepsin D activation peptide on gene expression of breast cancer cells. Cancer Lett 2006; 239: 46-54.
35. Laurent-Matha V, Farnoud MR, Lucas A, Rougeot C, Garcia M, Rochefort H. Endocytosis of pro-cathepsin D into breast cancer cells is mostly independent of mannose-6-phosphate receptors. J Cell Sci 1998; 111: 2439-2549.
36. Chinni SR, Gercel-Taylor C, Connor GE, Daylor DD. Cathepsin D antigenic epitopes identified by the humoral responses of ovarian cancer patients. Canc Immunol Immunotherap 1998; 46: 48-54.
37. Benes P, Vetvicka V, Fusek M. Cathepsin D – Many functions of one aspartic protease. Crit Rev Oncol Hematol 2008; 68: 12-28.
38. Vashishta A, Ohri SS, Vetvicka V. Pleiotropic effects of cathepsin D. Endocrine, Metabolic, Immune Disoders-Drug Targets, 2009; 9: 385-391.
39. Safting P, Hetman M, Schmahl W. Mice deficient for the lysosomal proteinase cathepsin D exhibit progressive atrophy of the intestinal mucosa and profound destruction of lymphoid cells. EMBO J 1995; 14: 3599-3608.
40. Deiss LP, Galinka H, Beriss H, Cohen O, Kimchi A. Cathepsin D protease mediates programmed cell

death induced by interferon-gamma, Fas/APO-1 and TNF-alpha. EMBO J 1996; 15: 3861-3870.
41. Bidere N, Lorenzo HK, Carmona S, et al. Cathepsin D triggers Bax activation, resulting in selective apoptosis-inducing factor (AIF) relocation in T lymphocytes entering the early commitment phase to apoptosis. J Bil Chem 2003; 278: 31401-31411.
42. Kanzler MH, Gorsulowsky DC, Swanson NA. Basic mechanisms in the healing cutaneous wound. J Dermatol Surg Oncol 1986; 12: 1156-1164.
43. Clark RA. Biology of dermal wound repair. Dermatol Clin 1993; 11: 647-666.
44. Stamenkovic I. Extracellular matrix remodeling: the role of matrix metalloproteinases. J Pathol 2003; 200: 448-464.
45. Saarialho-kere UK, Vaalamo M, Airola K, Niemi KM, Oikarinen AI, Parks WC. Interstitial collagenase is expressed by keratinocytes that are actively involved in reepithelialization in blistering skin disease. J Invest Dermatol 1995; 104: 982-988.
46. Buth H, Wolters B, Hartwig B, et al. HaCaT keratinocytes secrete lysosomal cysteine proteinases during migration. Eur J Cell Biol 2004; 83: 781-795.
47. Katz AB, Taichmann LB. A partial catalog of proteins secreted by epidermal keratinocytes in culture. J Invest Dermatol 1999; 112: 818-821.
48. Chen SH, Arany I, Apisarnthanarax N, et al. Response of keratinocytes from normal and psoriatic epidermis to interferon-gamma differs in the expression of zinc-alpha(2)-glycoprotein and cathepsin D. FASEB J 2000; 14: 565-571.
49. Horikoshi T, Arany I, Rajaraman S, et al. Isoforms of cathepsin D and human epidermal differentiation. Biochimie 1998; 80: 605-612.
50. Igarashi S, Takizawa T, Yasuda Y, et al. Cathepsin D, but not cathepsin E, degrades desmosomes during epidermal desquamation. Br J Dermatol 2004; 151: 355-361.
51. Vashishta A, Ohri SS, Vetvickova J, Fusek M, Ulrichova, J, Vetvicka V. Procathepsin D secreted by HaCaT keratinocyte cells-A novel regulator of keratinocyte growth. Eur J Cell Biol 2007; 86: 303-313.