Procathepsin D: New Target for Treating Cancer

Editorial

Cathepsins are proteases involved in protein degradation in a strong acidic milieu of lysosomes and are subgrouped based on the amino acid present on their active site. Cathepsin D (CD) belongs to a group of aspartic proteases and has been shown to be involved in many processes besides general protein turnover, such as activation and degradation of chemokines, polypeptide hormones, growth factors and their receptors [1-4], antigen processing [5-7], tissue homeostasis [8] and regulation of apoptosis [9,10].

Procathepsin D (pCD), the inactive precursor of lysosomal aspartyl protease cathepsin D, is overexpressed and secreted by several types of tumor and cancerous cell lines. Numerous clinical studies have revealed that the level of pCD/CD represents an independent prognostic factor in a variety of cancers including breast and lung carcinomas [11]. It has been demonstrated that pCD/CD affects multiple stages of tumor progression including proliferation, invasion, metastasis, angiogenesis and apoptosis [12,13]. The mechanism of how pCD/CD affects cancer cells remains unclear. Earlier studies suggested that CD may stimulate cancer growth via its enzymatic activity [14]. Clinical studies conducted in the 1990’s revealed that the CD levels in primary breast cancer cytosols was an independent prognostic parameter that correlates with the incidence of metastasis and short survival [15,16]. Moreover, a meta-analysis of studies on node-negative breast cancer [17] and a Rotterdam study of 2810 patients [18] showed that the higher levels of CD were a marker of aggressiveness. Using the monoclonal antibodies specific for the pro-form, it has been shown that the pCD levels increase in plasma of patients with metastatic breast carcinoma [19]. Various approaches, such as immuno-histochemistry, cytosolic immunoassay, in situ hybridization and Northern and Western blot analyses to detect the CD levels in neoplastic tissues revealed that in a majority of cancers, pCD is overexpressed 2-to-50 fold compared to the control tissue.

However, we and others showed that enzymatically inactive pCD mutants stimulate growth of breast cancer cells both in vitro and in vivo in the same manner as wild-type pCD [20,21]. Moreover, mitogenic effect of pCD on breast cancer cells is blocked by antibodies specific for the propeptide part of pCD [22,23]. Using synthetic peptides corresponding to the activation peptide or mutant pCD with deleted ApPCD, we demonstrated that the ApPCD itself stimulates growth of breast, prostate and lung cancer cells in vitro and in vivo [21,24]. Therefore, a model describing the effects of pCD on cancer cells was proposed [11]. In this model, extracellular pCD binds to cell surface of cancer cells, thus providing a signal that is than transmitted to the cell nucleus to affect gene expression. We identified several differentially expressed genes involved in control of cell cycle progression, cell survival, cell adhesion/angiogenesis, invasion and metastasis in breast cancer cells treated and untreated with ApPCD [25]. Based on results of DNA microarray analysis we hypothesized that one of the main regulators of the pro-mitogenic effect of pCD on cancer cells is NFkB transcription factor.

Members of the NFκB family promote cancer cell growth. NFκB target genes regulating proliferation include cyclin D1, cyclin E and c-Myc [26]. NFκB activation was implicated in epithelial-mesenchymal transition, a process that results in promotion of cancer cell invasion and metastasis. Activation of a so-called mesenchymal program was found to be dependent on NFκB activation in a breast cancer model, and reversal of these processes was triggered by NFκB inhibition [27]. In general, NFκB is believed to represent an important regulator of cancer cell growth, invasion, metastasis and angiogenesis. Similarly, cell growth, invasion, metastasis and angiogenesis are also stimulated by pCD in numerous types of cancers. The fact that pCD and NFκB affects cancer cells in similar ways supports our hypothesis that it is NFκB that mediates the effects of pCD.

Interestingly, both NFκB and pCD/CD possess pro- and anti-apoptotic activities. NFκB is well known for its capacity to protect cells from pro-apoptotic stimuli. NFκB regulates expression or activity of several anti- and pro-apoptotic proteins including members of the Bcl-2 family and protein p53 [28]. However, several pro-apoptotic activities of NF-κB have also been recently described suggesting that the role of NFκB in apoptosis is cell type specific and depends on the type of inducing signal [29]. The pro-apoptotic function of mature CD is namely executed by its protease activity upon its release from lysosomes to cytosol. In contrast, anti-apoptotic activity of extracellular pCD and its propeptide was also reported [30,31]. One can hypothesize that NFκB activation might be involved in anti-apoptotic activity of extracellular pCD. In addition, we observed a significantly increased phosphorylation of IκBα in breast and lung cancer cell lines upon treatment with ApPCD. This suggests that pCD activates NFκB by a signaling pathway that involves IκBs. Upstream kinases and cell surface receptor of this pathway remain to be determined. The relevance of NFκB activity for effects of pCD on breast and lung cancer cells was confirmed by observation that inhibition of NFκB by either
overexpression of dominant negative IκBα or treatment with PDTC reduces the growth-promoting effect of APPCD in both cell types.

Conclusion

Considering the importance of NFκB in carcinogenesis, this suggests that inhibition of NFκB and its signaling pathway offers a potential target of cancer therapy. Our results suggest that inhibitors of NFκB activation may be useful for treatment of pCD-secreting tumors, offering new targets for future treatment.

References

1. Morikawa W, Yamamoto K, Ishikawa S, Takemoto S, Ono M, et al. (2000) Angiostatin generation by cathepsin D secreted by human prostate carcinoma cells. J Biol Chem 275(49): 38912-38920.
2. Woessner JF, Shamberger RJ (1971) Purification and properties of cathepsin D from bovine uterus. J Biol Chem 246(7): 1951-1960.
3. Kudo S, Miyamoto G, Kawano K (1999) Proteases involved in the metabolic degradation of human interleukin-1-beta by rat kidney lysosomes. J Interferon Cytokine Res 19(4): 361-367.
4. Wolf M, Clark-Lewis I, Buri C, Langen H, Liu M, et al. (2003) Cathepsin D specifically cleaves the chemokines macrophage inflammatory protein-1 alpha, macrophage inflammatory protein-1 beta, and SLC that are expressed in human breast cancer. Am J Pathol 162(4): 1183-1190.
5. Kenessey A, Nacharaju P, Ko LW, Yen SH (1997) Degradation of tau by lysosomal enzyme cathepsin D: implication for Alzheimer neurofibrillary degeneration. J Neurochem 69(5): 2026-2038.
6. Benneck M, Marks N, Hashim GA (1975) Metabolic instability of myelin proteins. Breakdown of basic protein induced by brain cathepsin D. Eur J Biochem 52(3): 615-621.
7. Kim YJ, Sapp E, Cuiffo BG, Sobin L, Yoder J, et al. (2006) Lysosomal proteases are involved in generation of N-terminal huntingtin fragments. Neurobiol Dis 22(2): 346-356.
8. Koike M, Shibata M, Ohsawa Y, Nakashishi H, Koga T, et al. (2003) Involvement of two different cell death pathways in retinal atrophy of cathepsin D-deficient mice. Mol Cell Neurosci 22(2): 146-161.
9. Heinrich M, Neumeyer J, Jakob M, Hallas C, Tchikov V, Wintrodt et al. (2004) Cathepsin D links TNF-induced sphingomyelinase to Bid-mediated caspase-9 and -3 activation. Cell Death Differ 11: 550-563.
10. Haendeler J, Popp R, Goy C, Tischler AM, et al. (2005) Cathepsin D and H2O2 stimulate degradation of thioredoxin-1: implication for endothelial cell apoptosis. J Biol Chem 280(52): 42945-42945.
11. Benes P, Vetvicka V, Fusek M (2008) Cathepsin D: Many functions of one aspartic protease. Critical Reviews in Oncology/Hematology 68(1): 12-28.
12. Berchem G, Glondu M, Gleizes M, Brouillet JP, Vignon F, et al. (2002) Cathepsin D affects multiple tumor progression steps in vivo: proliferation, angiogenesis and apoptosis. Oncogene 21(38): 5951-5955.
13. Llauget-Coopman E, Beaujouin M, Deroog D, Garcia M, Glondu-Lassis M, et al. (2006) Cathepsin D: newly discovered functions of a long-standing aspartic protease in cancer and apoptosis. Cancer Lett 237(2): 167-179.
14. Llauget E, Garcia M, Rochefort H (1994) Cathepsin D maturation and its stimulatory effect on metastasis are prevented by addition of KDEL retention signal. Oncogene 9(4): 1145-1154.
15. Thorpe SM, Rochefort H, Garcia M, Freis G, Christensen IJ, et al. (1989) Association between high concentrations of Mw 52,000 cathepsin D and poor prognosis in primary human breast cancer. Cancer Res 49: 6008-6014.
16. Spyratos F, Maudelonde T, Brouillet JP, Brunet M, Defrenne A, et al. (1989) Cathepsin D: an independent prognostic factor for metastasis of breast cancer. Lancet 334(8672): 1115-1118.
17. Ferrandina G, Scambia G, Bardelli F, Benedetti Panici P, Mancuso S, et al. (1997) Relationship between cathepsin-D content and disease-free survival in node-negative breast cancer patients: a meta-analysis. Br J Cancer 76(5): 661-666.
18. Foekens JA, Look MP, Bolt-de Vries J, Meijer-van Gelder ME, van Putten WL, et al. (1999) Cathepsin-D in primary breast cancer: prognostic evaluation involving 2810 patients. Br J Cancer 79(2): 300-307.
19. Brouillet JP, Dufour F, Lemamy G, Garcia M, Slup N, et al. (1997) Increased cathepsin D level in the serum of patients with metastatic breast carcinoma detected with a specific pro-cathepsin D immunoassay. Cancer 79(11): 2132-2136.
20. Goland M, Coopman P, Laurent-Matha V, Garcia M, Rochefort H, et al. (2001) A mutated cathepsin-D allele of its catalytic activity stimulates the growth of cancer cells. Oncogene 20(47): 6920-6929.
21. Ohri SS, Vashishta A, Proctor M, Fusek M, Vetvicka V (2008) The propeptide of cathepsin D increases proliferation, invasion and metastasis of breast cancer cells. Int J Oncol 32(2): 491-498.
22. Fusek M, Vetvicka V (1994) Mitogenic function of human procathepsin D: the role of the propeptide. Biochem J 303: 775-780.
23. Vetvicka V, Vetvickova J, Fusek M (1999) Anti-human procathepsin D activation peptide antibodies inhibit breast cancer development. Breast Cancer Res Treat 57(3): 261-269.
24. Vetvicka V, Vetvickova J, Benes P (2004) Role of enzymatically inactive procathepsin D in lung cancer. Anticancer Res 24(5A): 2739-2743.
25. Benes P, Vashishta A, Saraswat-Ohru S, Fusek M, Pospisilova S, et al. (2006) Effect of procathepsin D activation peptide on gene expression of breast cancer cells. Cancer Lett 239(1): 46-54.
26. Naugler WE, Karin M (2008) NF-kappaB and cancer-identifying targets and mechanisms. Curr Opin Genet Dev 18(1): 19-26.
27. Huber MA, Azoitei N, Baumann B, Grünert S, Sommer A, et al. (2004) NF-kappaB is essential for epithelial-mesenchymal transition and metastasis in a model of breast cancer progression. J Clin Invest 114(4): 569-581.
28. Burstein E, Duckett CS (2003) Dying for NF-kappaB? Control of cell death by transcriptional regulation of the apoptotic machinery. Curr Opin Cell Biol 15(6): 732-737.
29. Fan Y, Dutta J, Gupta N, Fan G, Gélinas C (2008) Regulation of programmed cell death by NF-kappaB and its role in tumorigenesis and therapy. Adv Exp Med Biol 615: 223-250.
30. Brasier AR (2006) The NF-kappaB regulatory network. Cardiovasc Toxicol 6(2): 111-130.
31. Sugalenko V, Muth D, Sugalenko E, Paffhausen T, Schwab M, et al. (2008) Cathepsin D protects human neuroblastoma cells from doxorubicin-induced cell death. Carcinogenesis 29(10): 1869-1877.