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BIOLOGICAL AND CLINICAL SIGNIFICANCE OF CATHEPSIN D IN BREAST CANCER

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Cathepsin D, an aspartyl protease of lysosomes, is overproduced and hypersecreted by breast cancer cells. The prognostic value of its immunoassay in breast cancer cytosol is reviewed from the first retrospective clinical studies available, which show a strong correlation between high concentrations of cathepsin D in the cytosol of primary tumor and further occurrence of metastasis. This new prognostic factor is induced by estrogen in hormone dependent breast cancer but expressed at a high level in hormone independent breast cancer and appears to be independent of other more classical factors. Its value in node negative patients varies according to the studies. In nude mice, transfection of cathepsin D cDNA into tumor cells increases their metastatic potential, suggesting that overexpression of this protease may be one of the factors responsible for metastasis in human breast cancer. The mechanism by which this protease might facilitate metastasis in vivo is still unknown, even though cathepsin D has the potential to initiate a proteolytic cascade, to degrade extracellular matrix and to liberate FGFs like growth factors from the matrix. These studies should stimulate the search for new therapeutical agents in order to inhibit cathepsin D action.

Key words: Breast cancer, proteases, metastasis, lysosomes, estrogens, prognostic marker, mannose-6-phosphate receptor, immunoassay.

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Estrogens play a critical role in the development, growth and dissemination of breast cancer cells (1). At least, part of their action is direct on breast cancer cells via nuclear estrogen receptors. More than 10 years ago, we detected in the conditioned medium secreted by MCF cells and other estrogen regulated breast cancer cells a 52 K protein which was not secreted in the absence of estrogens or after antiestrogen treatment (2). We decided to purify the protein, clone its cDNA and develop monoclonal antibodies.

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The aim and hope were double: First, to characterize an estrogen-induced autocrine growth factor, in fact the purified 52 K protein has some mitogenic activity (3), and secondly, to develop a circulating marker of hormone dependency that could be of use in breast cancer monitoring. In fact, the identification of the 52 K protein proved, 6 years later, to be a good example of serendipity since, rather than a growth factor, we found a lysosomal protease cathepsin D which proved to be a prognostic factor in breast cancer, allowing the prediction of relapse and metastasis rather than hormone dependency (for review, see (4)).

Cathepsin D (E.C. 3.4.23.5) is an aspartyl endoproteinase (5) ubiquitously distributed in all cells at low concentrations. Its normal function is to degrade protein in lysosomes at an acidic pH, and to mature biologically active peptides in endosomes (6). Its optimal pH, although acidic, varies according to the nature of the substrate. Its activity

is specifically inhibited by pepstatin, which binds to and blocks the active site. No natural inhibitor of cathepsin D is known, contrary to other tissular proteinases, and the proenzyme can be auto-actived by partial removal of the pro-fragment when present in an acidic micro-environment (7, 8). The amino acid structure of cathepsin D has been inferred by sequencing its cDNA from human liver and spleen (9), its glycosylation is not precisely known, although it bears 2 N-linked oligosaccharide chains with mannose-6-phosphate signals at their extremities.

What characterizes cathepsin D from breast cancer cells?

The structure appears to be normal in terms of amino acid sequence deduced from sequencing of the corresponding cDNA. In MCF7 cells, there is an amino acid change (Ala → Val) in the pro-peptide (10) but this change was not found in another cell line (11). The glycosylation appears different, with more acidic moieties, in MCF7 cells than in normal mammary cells but this difference may not have functional consequences. The proteolytic activity of cathepsin D from breast cancer cells appears also very similar if not identical to that of cathepsin D from normal cells. The in vitro acidic pH requirement for auto-activation and for activity were also similar. In fact, the major characteristics of breast cancer cathepsin D appears to be quantitative rather than qualitative.

Overexpression of the cathepsin D gene

Overexpression of the cathepsin D gene was found both at the mRNA and protein level and in breast cancer cell lines as well as in breast cancer tissue in patients. Several approaches, such as immunohistochemistry, in situ hybridization, cytosolic immunoassay, northern and western blot analysis, all indicated that in most cases of breast cancer cathepsin D is overexpressed by a factor of 2 up to 50 when compared to its concentration in other cells such as fibroblasts or normal mammary gland cells (12, 13). This overexpression is clearly located in the breast cancer tissue and not in the fibroblasts of the tumor, even though macrophages also produce this enzyme.

Two major questions are raised by this overexpression: mechanism and consequences. Unlike several oncogenes, the mechanism does not seem to involve gene amplification. It is now being studied extensively in our laboratory. In estrogen receptor positive breast cancer, cathepsin D is induced directly by estrogens via ERE(s) located in the 5′ proximal region of the cathepsin D gene (14). This regulation, however, is tissue specific since in the endometrium, progesterone, but not estrogen, induces the protease (15). In vivo, tamoxifen, in the first week of treatment, also induces the enzyme, probably due to its partial agonist activity on this gene (biological flare) (16). Then, the stimulation is no more visible after a longer period of treatment. In estrogen receptor negative breast cancer, the overexpression of cathepsin D gene is not yet explained but could be due to the constitutive production of autocrine or intracrine factors responsible for the induction of the protease. In fact, in MCF7 cells, growth factors acting via the tyrosine kinase pathway are able to induce the cathepsin D mRNA (17).

Increased secretion of pro-cathepsin D

Normally, cathepsin D is routed by lysosomes and maturated in the 34 + 14 kD form (7). In breast cancer cells, up to 50% of pro-cathepsin D can be secreted either spontaneously in ER negative breast cancer cell lines, or following estrogen treatment in ER positive breast cancer cell lines (12). We have studied the mechanism of estradiol-induced increased secretion of pro-cathepsin D in MCF7 cells. We propose that this is at least partly due to the overproduction of pro-cathepsin D which saturates the limited number of mannose-6-phosphate receptor sites (18). In different cell lines, we found an inverse relationship between the amount of mannose-6-phosphate receptors (binding sites and mRNA) and that of cathepsin D secreted by these cell lines. Moreover, estradiol not only induced cathepsin D and IGF-II, another ligand of the 250 kD mannose-6-phosphate receptor, but also down regulated this receptor at the mRNA and protein level. A prediction of this saturating mechanism would be that the other lysosomal pro-enzymes, also routed via the same receptor, would be displaced by cathepsin D and secreted following estrogen treatment. This is what we found when the activities of beta-hexosaminidase and beta-galactosidase were studied. Moreover, estradiol increased cathepsin B secretion without modifying its mRNA level (19).

Other mechanisms for altered targeting, such as a specific decreased affinity of the enzyme for its receptor due to an alteration of the mannose-6-P signal and not to a modification of the receptor have been proposed for other cathepsins (B and L) in other cancers (20). We found no decreased affinity of cathepsin D for the mannose-6-P receptor in the breast cancer cell lines studied (MCF7, ZR75-1, T47D) (21) and pro-cathepsin D bears mannose-6-P signals (8).

Clincial prognostic value of cathepsin D in breast tumors

Total cathepsin D concentration can be assayed in the cytosol of tumor biopsies using a solid-phase double-determinant immunoassay (ELISA or IRMA) with two monoclonal antibodies recognizing two different epitopes of the large chain (34 K) of mature cathepsin D (22). Since these antibodies recognize the same epitopes in the intermediate chain (48 K) and the precursor form (52 K) of the enzyme, total cathepsin D concentration is assayed (23, 24). The assay for total cathepsin D is now commercialized by CIS

Table

First clinical studies on the prognostic value of cytosolic cathepsin D in breast cancer

References	(No.)	Place	Number of patients	Information
Maudelonde et al.	(29)	Montpellier	183	Independent from other prognostic markers
Thorpe et al.	(25)	Copenhagen	400	Shorter relapse-free survival in pre- and postmenopausal patients
Spyratos et al.	(24)	St-Cloud	120	Shorter metastasis-free survival in both node negative and node positive
Tandon et al.	(26)	San Antonio	200	Shorter relapse-free survival and survival in node negative (34K mature form only)
Brouillet et al.	(30)	Montpellier	140	Independent of neu-*erb*-B-2 and *Int*-2, correlated with c-*myc*
Duffy et al. Abst., XIV Int. Congress of Clin. Chem. 1991		Dublin	128	Correlated with urokinase plasminogen activators Shorter overall survival
Romain et al.	(28)	Marseille	85	Shorter survival in nodes positive
Namer et al.	(27)	Nice	237	Shorter overall survival in nodes positive
Henry et al.	(31)	New Castle	94	Good prognostic value Immunohistochemistry
Kute et al. Proc. Amer. Ass. Cancer Cancer Res. 32: 164, 1991		Winston-Salem	177	Shorter relapse free survival in nodes negative
Pujol et al.		Montpellier	123	First prospective studies
Total number of patients with clinical follow-up (excluding 1, 5, 9)			1 470	

Cathepsin D concentrations have been assayed in the cytosol of breast cancer tissue using the two same monoclonal antibodies (D7E3, M1G8) for all studies except study 4 (polyclonal antibodies and assay of the mature 34K form only) and study 9 (polyclonal antibodies to normal cathepsin D by immunohistochemistry in paraffin section).

International (ELSA-cath-D). Approximately 90% of total cathepsin D is extracted by the homogenization procedure used in routine preparations of cytosol (high speed supernatant) for estrogen and progesterone receptor assays (Tris EDTA buffer). This is particularly convenient since the assay may be applied on a small amount (50 μl) of the cytosol routinely prepared for steroid receptor assays. The assay is easy to perform and reliable. The first clinical studies on the prognostic value of cathepsin D concentration in cytosol are summarized in the Table. Two sets of information have been obtained; the most important concerns the value of a high cathepsin D concentration for predicting relapse and metastasis based on retrospective studies analyzed according to Cox's multivariate model. The first study in Copenhagen on patients from the Danish Breast Cancer Cooperative Group (25) and the second study in St-Cloud from the Centre René Huguenin (24), indicated that the predictive value of cathepsin D was useful in both node negative and positive patients. Thereafter the San Antonio group, using polyclonal antibodies to cathepsin D and quantifying the 34 K mature form of cathepsin D by immunoblotting, found predictive values only in node negative patients (26). Subsequently, using the commercially available ELSA-cath-D kit, other groups also found a correlation between total cathepsin D level and overall survival, the significance being in some studies higher for node positive then for node negative patients (27, 28). The reason for these discrepancies might be differences in tissue conservation, selection of patients including the nature of adjuvant therapy and the grading by the pathologist. In all Cox's multivariate studies, cathepsin D ranged within the three first significant prognostic markers. The cut-off level of total cathepsin D concentration, which helped to discriminate between breast cancer with good (low concentration) or poor (high concentration) prognosis, varied depending on the study. The

second set of information shows that cathepsin D concentration and status is generally independent of classical prognostic markers such as node involvement and tumor size, Scarff and Bloom histological grade, age of patients (25–29), as well as more recently used markers such as neu-*erb*-B-2 or *int*-2 oncogene amplification (30). Most of the studies also indicate an absence of correlation of cathepsin D with the estrogen receptor status, contrary to pS2, another estrogen-induced protein, which is consistent with the constitutive high production of cathepsin D in estrogen receptor-negative cell lines. The predictive value of cathepsin D therefore supplements that of other markers and indicates that the overexpression of total cathepsin D is correlated with the frequency of metastasis. To our knowledge, there are no published studies which disagree with the bad prognostic significance of high cathepsin D level in cytosol.

Conflicting results were obtained by immunohistochemistry, where high cathepsin D levels in breast cancer cells indicated a good prognosis (31). However, different antibodies were used and no quantification of staining intensity was attempted. When we quantified, by computer-aided image analysis, the immunostaining obtained with D7E3 monoclonal antibody, we found a good correlation with cytosolic cathepsin D levels (T. Maudelonde et al., submitted for publication). In a recent prospective study on 125 patients of the Cancer Center of Montpellier followed during 5 years, the bad prognostic significance of high cathepsin D level on relapse-free survival was confirmed (P. Pujol et al., submitted for publication).

While these studies have been performed independently and without knowledge of the clinical evolution of patients, other independent studies are required in order to discriminate amongst the increasing number of new prognostic markers, those that are independent of other markers, reliable, most easy to perform, and most useful for selection of patients, for adjuvant therapy. Most of the clinical results currently available, on a total of 1 470 patients followed for at least 5 years (Table), indicate that a high cathepsin D concentration in cytosol of primary breast cancer increases the risk of developing clinical metastasis. Cathepsin D seems to be closely associated with the metastatic ability of the tumor and therefore appears to be promising for determining, in association with other major prognostic parameters, which breast cancer patients will most likely need systemic adjuvant therapy.

A role of cathepsin D in metastasis?

The clinical studies on cathepsin D indicate either that high cathepsin D concentration is an epiphenomenon of the metastatic process or, more excitingly, that it is actually responsible for at least part of this process (4). Direct evidence that overexpression of cathepsin D may promote some step in metastasis has been obtained recently (32). We have transfected a mammalian expression vector of human pro-cathepsin D or the control vector alone into a rat tumorigenic cell line 3YA12, which secretes no cathepsin D activity in vitro. Stable transfectant clones were selected which produced high levels of human cathepsin D and they grew more rapidly in low serum concentrations than the control vector clones. In addition, their metastatic activity in the athymic mouse model was significantly higher than that of control clones. This was the first evidence that increased production of cathepsin D might facilitate metastasis.

Many hypotheses can be raised concerning the mechanism by which cathepsin D may facilitate metastasis. Cathepsin D might act via the mannose-6-phosphate receptor. However, the presence of a coupling mechanism triggered by the activation of this receptor and the nature of the membrane receptor involved in mediating the mitogenic activity of IGF-II and cathepsin D are still debatable (33). The most likely hypothesis is that cathepsin D acts via its proteolytic activity by degrading or activating proteins playing an important role in metastasis. Proteases may facilitate tumor invasion by attacking the basement membrane. In fact, both purified pro-cathepsin D and conditioned media from estrogen-treated MCF7 cells digest extracellular matrix prepared from bovine corneal endothelial cells in vitro. Optimal activity occurs at acidic pH (4 to 5). The degradation of extracellular matrix by secreted proteases present in conditioned media of breast cancer cells is mainly due to cathepsin D, since it is completely inhibited by pepstatin but not by other inhibitors. Several epithelial cancer cell lines have been found to secrete a pepstatin-sensitive protease activity which correlates with cathepsin D antigen concentrations assayed by ELISA (34). It is therefore tempting to propose that high cathepsin D concentrations promote tumor invasion by secretion of cathepsin D out of the cells, thereby facilitating the digestion of the basement membrane. However, this has not yet been demonstrated since autoactivation of secreted pro-cathepsin D in vivo appears to require an acidic micro-environment, which is more frequently encountered within the cells (endosomes, lysosomes) than out of them. Large acidic vesicles containing both mature cathepsin D and endocytosed extracellular matrix have been found at much higher concentrations in breast cancer cells than in normal mammary cells, suggesting that overproduction and derouting of cathepsin D may facilitate digestion of extracellular matrix following its internalization by an endocytotic or phagocytotic process (35).

One consequence of extracellular matrix degradation by cathepsin D would be the liberation of growth factors such as the FGF like growth factors or TGF-beta that are stored in the matrix. We have recently shown that MCF7 cells could liberate ^{125}I bFGF entrapped in extracellular matrix, incorporate it, and respond to its mitogenic activity. Moreover, bFGF uptake was inhibited by pepstatin

suggesting that cathepsin D facilitated the use of bFGF (36). This might play a role in angiogenesis which is an important step of metastasis.

Cathepsin D may also behave as a processing protease able to be autoactivated at high concentrations and low pH and to process and activate other proteases, such as the pro-cathepsin D secreted in various amounts depending on the cancers (37), thus initiating a proteolytic cascade (38) in which plasminogen activators and collagenases would also be activated.

Conclusions

A series of experimental data favor the hypothesis that the overexpression of cathepsin D by breast cancer cells facilitates some steps of invasion and dissemination. High concentrations of cathepsin D in the primary tumor extract are highly correlated with subsequent risk of developing metastasis. This increased accumulation of the protease is secondary to its increased gene expression in both hormone dependent and independent tumors. The consequence of this overexpression is to saturate the mannose-6-P receptor sites and therefore to facilitate secretion of this protease and other lysosomal enzymes. Similar overexpression of cathepsin D can be obtained by transfecting a complete human cDNA sequence under the control of a strong but artificial promoter into an adenovirus transformed rat cell line which is tumorigenic in nude mice, but secretes no cathepsin D. Following transfection with the cathepsin D expression vector, and selection of stable transfectants, the cloned cell lines appear to be more transformed when growing in vitro and more metastatic when injected intravenously into athymic mice. This finding strongly suggests that the overexpression of cathepsin D is actively engaged, more or less directly, in the metastatic process.

In breast cancer patients, the overexpression of cathepsin D gene might be directly due to cathepsin D gene alteration (in the coding sequence or in cis regulatory elements) or might be consecutive to an alteration of trans-acting factor(s) regulating its expression. In the former case, the cathepsin D gene would be considered as a new type of proto-oncogene involved in metastasis. Further studies are in progress to define the molecular alteration responsible for increased cath-D gene expression in breast cancers, as well as the nature of the putative oncogene or suppressor gene cooperating with cath-D to facilitate metastasis.

Other proteases such as plasminogen activator, collagenases, stromelysin, etc., are also thought to play a role in cancer metastasis. However, very few retrospective clinical studies have shown a high correlation between concentrations of these proteases in the primary tumor and the occurrence of metastasis. To our knowledge, in breast cancer only the urokinase-type plasminogen activator appears to be correlated with metastasis (39, 40). In other cancers, other cathepsins (B and L) have been shown to be overexpressed and secreted (37, 41). This would suggest that cancer cells have different strategies, depending on their tissues of origin, for reaching the same proliferation and dissemination goals. The nature of the protease(s) initially involved in triggering invasive steps of carcinogenesis may therefore vary according to the type of cancer, and possibly the species being considered.

The demonstration of a role of cathepsin D in human breast cancer metastasis and the complete understanding of its mechanism might open possibilities for new therapeutical approaches aimed at inhibiting the production and action of cathepsin D at the tumor cell level.

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REFERENCES

1. Beck JS. Oestrogen and the human breast. Edinburgh: The Royal Society of Edinburgh, 1989; 95.
2. Westley B, Rochefort H. A secreted glycoprotein induced by estrogen in human breast cancer cell lines. Cell 1980; 20: 352–62.
3. Vignon F, Capony F, Chambon M, Freiss G, Garcia M, Rochefort H. Autocrine growth stimulation on the MCF7 breast cancer cells by the estrogen-regulated 52K protein. Endocrinology 1986; 118: 1537–45.
4. Rochefort H, Capony F, Garcia M, et al. Estrogen-induced lysosomal proteases secreted by breast cancer cells. A role in carcinogenesis? J Cell Biochem 1987; 35: 17–29.
5. Barrett AJ. Cathepsin D: Purification of isoenzymes from human and chicken liver. Biochem J 1970; 117; 601–7.
6. Diment S, Martin KJ, Stahl PD. Cleavage of parathyroid hormone in macrophage endosomes illustrates a novel pathway for intracellular processing of proteins. J Biol Chem 1989; 264: 13403–6.
7. Erickson AH. Biosynthesis of lysosomal endopeptidases. J Cell Biochem 1989; 40: 31–41.
8. Capony F, Morisset M, Barrett AJ, et al. Phosphorylation, glycosylation and proteolytic activity of the 52K estrogen-induced protein secreted by MCF7 cells. J Cell Biol 1987; 104: 253–62.
9. Faust PL, Kornfeld S, Chirgwin JM. Cloning and sequence analysis of cDNA for human cathepsin D. Proc Natl Acad Sci USA 1985; 82: 4910–4.
10. Augereau P, Garcia M, Mattei MG, et al. Cloning and sequencing of the 52K cathepsin D cDNA of MCF7 breast cancer cells and mapping on chromosome 11. Mol Endocrinol 1988; 2: 186–92.

11. Westley BR, May FEB. Oestrogen regulates cathepsin D mRNA levels in oestrogen responsive human breast cancer cells. Nucleic Acids Res 1987; 15: 3773–86.
12. Capony F, Rougeot C, Montcourrier P, Cavaillès V, Salazar G, Rochefort H. Increased secretion, altered processing, and glycosylation of pro-cathepsin D in human mammary cancer cells. Cancer Res 1989; 49: 3904–9.
13. Rochefort H, Cavaillès V, Augereau P, et al. Overexpression and hormonal regulation of pro-cathepsin D in mammary and endometrial cancer. J Steroid Biochem 1989; 34: 177–82.
14. Cavaillès V, Augereau P, Rochefort H. Cathepsin D gene of human MCF7 cells contains estrogen-responsive sequences in its 5′ proximal flanking region. Biochem Biophys Res Commun 1991; 174: 816–24.
15. Touitou I, Cavaillès V, Garcia M, Defrenne A, Rochefort H. Differential regulation of cathepsin D by sex steroids in mammary cancer and uterine cells. Mol Cell Endocrinol 1989; 66: 231–8.
16. Maudelonde T, Domergue J, Henquel C, et al. Tamoxifen treatment increases the concentration of 52K cathepsin D and its precursor in breast cancer tissue. Cancer 1989; 63: 1265–70.
17. Cavaillès V, Augereau P, Garcia M, Rochefort H. Estrogens and growth factors induce the mRNA of the 52K-pro-cathepsin-D secreted by breast cancer cells. Nucleic Acids Res 1988; 16: 1903–19.
18. Mathieu M, Vignon F, Capony F, Rochefort H. Estradiol down regulates the mannose-6-phosphate/IGF-II receptor gene and induces cathepsin D in breast cancer cells: A receptor saturation mechanism to increase the secretion of lysosomal pro-enzymes. Mol Endocrinol 1991; 5: 815–22.
19. Capony F, Rougeot C, Cavailles V, Rochefort H. Estradiol increases the secretion by MCF7 cells of several lysosomal pro-enzymes. Biochem Biophys Res Commun 1990; 171: 972–8.
20. Dong J, Prence EM, Sahagian GG. Mechanism for selective secretion of a lysosomal protease by transformed mouse fibroblasts. J Biol Chem 1989; 264: 7377–83.
21. Mathieu M, Rochefort H, Barenton B, Prebois C, Vignon F. Interactions of cathepsin D and IGF-II on the IGF-II/M6P receptor in human breast cancer cells and possible consequences on mitogenic activity of IGF-II. Mol Endocrinol 1990; 4: 1327–35.
22. Garcia M, Capony F, Derocq D, Simon D, Pau B, Rochefort H. Monoclonal antibodies to the estrogen-regulated Mr 52 000 glycoprotein: Characterization and immunodetection in MCF7 cells. Cancer Res 1985; 45: 709–16.
23. Rogier H, Freiss G, Besse MG, et al. Two-site immunoenzymometric assay of the 52-kDa-cathepsin D cytosols of breast cancer tissues. Clin Chem 1989; 35: 81–5.
24. Spyratos F, Maudelonde T, Brouillet JP, et al. Cathepsin D: An important marker predicting metastasis in primary breast cancer. Lancet 1989; 2: 1115–8.
25. Thorpe SM, Rochefort H, Garcia M, et al. Association between high concentrations of 52K cathepsin-D and poor prognosis in primary breast cancer. Cancer Res 1989; 49: 6008–14.
26. Tandon A, Clark G, Chirgwin J, McGuire WL. Cathepsin-D and prognosis in breast cancer. N Engl J med 1990; 322: 297–302.
27. Namer M, Ramaiol A, Fontana X, et al. Prognostic value of total cathepsin D in breast tumors. Breast Cancer Res Treat 1991; 19: 85–93.
28. Romain S, Muracciole X, Varette I, Bressac C, Brandone H, Martin PM. La cathepsine D: Un facteur pronostique indépendant dans le cancer du sein. Cancer 1990; 77: 439–47.
29. Maudelonde T, Khalaf S, Garcia M, et al. Immunoenzymatic assay of Mr 52 000 cathepsin D in 182 breast cancer cytosols. Low correlation with other prognostic parameters. Cancer Res 1988; 48: 462–6.
30. Brouillet JP, Theillet C, Maudelonde T, et al. Cathepsin D assay in primary breast cancer and lymph nodes: Relationship with c-*myc*, c-*erb*-B-2 and *int*-2 oncogene amplification and node invasiveness. Eur J Cancer 1990; 26: 437–41.
31. Henry JA, McCarthy AL, Angus B, et al. Prognostic significance of the estrogen-regulated protein, cathepsin D, in breast cancer. Cancer 1990; 65: 265–71.
32. Garcia D, Derocq D, Pujol P, Rochefort H. Overexpression of transfected cathepsin D in transformed cells increases their malignant phenotype and metastatic potency. Oncogene 1990; 5: 1809–14.
33. Griffiths G, Hoflack B, Simons K, Mellman I, Kornfeld S. The mannose-6-phosphate receptor and the biogenesis of lysosomes. Cell 1988; 52: 329–41.
34. Briozzo P, Morisset M, Capony F, Rougeot C, Rocheford H. In vitro degradation of extracellular matrix with Mr 52 000 cathepsin D secreted by breast cancer cells. Cancer Res 1988; 48: 3688–92.
35. Montcourrier P, Mangeat P, Salazar G, Morisset M, Sahuquet A, Rochefort H. Cathepsin D in breast cancer cells can digest extracellular matrix in large acidic vesicles. Cancer Res 1990; 50: 6045–54.
36. Briozzo P, Badet J, Capony F, et al. MCF7 mammary cancer cells respond to bFGF and internalize it following its release from extracellular matrix: A permissive role of cathepsin D. Exp Cell Res 1991; 194: 252–9.
37. Sloane BF, Dunn JR, Honn KV. Lysosomal cathepsin B: Correlation with metastatic potential. Science 1981; 212: 1151–3.
38. Pagano M, Capony F, Rochefort H. La pro-cathepsine D peut activer in vitro la pro-cathepsine D sécrétée par les cancers ovariens. C R Acad Sci Paris 1989; 309: 7–12.
39. Jänicke F, Schmitt M, Hafter R, et al. Urokinase-type plasminogen activator (u-PA) antigen is a predictor of early relapse in breast cancer. Fibrinolysis 1990; 4: 69–78.
40. Duffy MJ, O'Grady P, Devaney D, et al. Urokinase-plasminogen activator, a marker for aggressive breast carcinomas, preliminary report. Cancer 1988; 62: 531–3.
41. Gal S, Gottesman MM. The major excreted protein of transformed fibroblasts is an activable acid-protease. J Biol Chem 1986; 261: 1760–5.