Histol Histopathol (2014) 29: 433-438
DOI: 10.14670/HH-29.10.433

http://www.hh.um.es

Histology and Histopathology
Cellular and Molecular Biology

Review

Significance of the tumor protease cathepsin D for the biology of breast cancer

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Summary. Cathepsin D is a protease involved in the metastasis and angiogenesis of mammary carcinomas. This review analyzes the significance of the tumor protease cathepsin D in mammary carcinomas as a tumor marker. We present a systematic overview based on a selective Medline search. Cathepsin D is expressed in mammary carcinomas and exhibits higher expression in invasive ductal carcinomas compared with lobular carcinomas. Nodal positive carcinomas showed reduced cathepsin D expression compared to lymph node metastases, and increased expression has been observed in hormone-receptor negative tumors. Thus, the expression of cathepsin varies between the two histological types. Increased cathepsin D expression in acinar affection has also been described. The lack of an association of cathepsin D with known prognostic factors such as CA15-3, ERalpha and ERbeta does not prevent it from being used as a tumor marker. Cathepsin has already been used along with other genes as a prognostic parameter for carcinoma patients in gene arrays.

Key words: Breast cancer, Cathepsin D, Tumor marker, Prognostic parameter

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Introduction

Max Bergmann was one of the first authors to discuss cathepsins (Greek for 'to digest') in "Science" in 1936. Cathepsins are lysosomal proteases that are activated in an acid-pH environment (pH 4.8; cathepsin D is active below pH 6.2 in vitro) (Capony et al., 1987; Nomura and Katunuma, 2005). Some secreted cathepsins are also active at neutral pH. Lysosomes are digestive cell organelles produced in the Golgi apparatus. Lysosomes contain proteases, lipases, nucleases and carbohydrases. The enzymes are produced in the cytosol and in the endoplasmic reticulum before they accumulate in the lysosomes. Lysosomes digest phagocytized macromolecules and damaged cell structures such as receptors. Lysosomes also repair cell membranes and are involved in apoptosis. Dysfunctions of digestive enzymes can disrupt cellular metabolism and cause various storage diseases. Twelve forms of cathepsin from animal and human cells have been identified.

Cathepsins B, C, H, F, K, L, O, S, V and W are cysteine proteinases. Cathepsins A and G are serine proteinases. Cathepsins D and E (similar to pepsin, renin and HIV-1 proteinases) are referred to as asparte proteinases because they contain aspartic acid in the active center (de Duve, 1983). Cathepsin D is synthesized as a proenzyme in the endoplasmic reticulum and is genetically related to pepsinogen. Cathepsin D is absorbed via mannose 6-phosphate receptors and other unknown receptors in the lysosomes and endocytotically in cancer cells and fibroblasts. The remaining protein blocking the substrate binding site

separates from the binding site in acid environments. Cathepsins B and L undergo similar acidic activation (Wille et al., 2004). Glycosylation follows acidic activation of the enzymes (Liaudet-Coopman et al., 2006; Loeffler et al., 2006). The gene for cathepsin D is located at the far end of chromosome 11p (Faust et al., 1985). Cathepsins B and D appear to exhibit protective roles in Alzheimer's disease in the scission of the amyloid plaques Aß (Hook et al., 2005; Mueller-Steiner et al., 2006; Riemenschneider et al., 2006).

Cathepsin L fulfills a specific function in antigen presentation in thymocytes (Nakagawa et al., 1998). Cathepsin D has recently been detected in human sudor where it appears to modulate the immune defense of the skin (Baechle et al., 2006). Mice that do not form cathepsin D due to gene knockout die within 3 to 4 weeks as a result of malnutrition and akinetic atrophy of the ileum mucosa, intestinal necrosis and apoptotic tissue destruction of the thymus and the spleen and a deficiency of T and B cells. The functioning of the lysosomes was not impaired, and the retina became atrophic, which was interpreted as apoptosis induced by a cathepsin D deficiency. Cathepsin B or L knockout did not cause the same result (Saftig et al., 1995; Koike et al., 2003). Cathepsin is involved in preventing apoptosis under physiological circumstances and inducing apoptosis as a result of oxidative stress or cytostatic agents. During apoptosis, cathepsin is released into the cytosol with its transcription factor p53 (Kagedal et al., 2001; Roberg et al., 2002; Johansson et al., 2003; Liaudet-Coopman et al., 2006).

A reaction inhibitor for cathepsin D in mammals has not been identified. Cathepsins D, B and L are associated with the progression and metastasis of carcinoma (Koblinski et al., 2000; Joyce et al., 2004; Liaudet-Coopman et al., 2006). In malignantly transformed cells, active forms of the intracellular proteinases can also be located on the membrane (Loeffler et al., 2006). The pH near malignant tumors is typically more acidic than physiological pH, but other factors also contribute to the extracellular effectiveness of cathepsin in malignant tumors (Griffiths, 1991). Cathepsins exhibit increased expression in different forms of cancer, including stomach, prostate and breast cancers, which typically involve aggressive tumors (Nomura and Katunuma, 2005). Cysteine proteases are also highly regulated in HPV-16-induced cervical carcinomas (Joyce et al., 2004). Pro-cathepsin D was initially described with regard to breast cancer as a 52 kDA glycoprotein that can be stimulated by estrogens and is secreted by MCF-7 cells. Intermediate (Mr 48 kDa) and mature forms (Mr 34 and 14 kDa) of pro-cathepsin D have also been observed. Increased concentrations of pro-cathepsin D were observed in proliferative and cystic fibrocystic breast diseases (Capony et al., 1987; Rochefort et al., 1987). The following publications from one group also indicate that cathepsin D expression is regulated by estrogens and growth factors in breast-cancer cell lines (Capony et al., 1989a,b; Cavailles et al., 1989; Rochefort et al., 1989).

Exogenous administration of pro-cathepsin D into a breast-cancer cell line causes mitogenic activity, which can be constrained by specific antibodies against the propeptide without reducing the proteolytic activity. The expression of the propetide is stimulated in some breast-cancer cell lines through estrogens (Fusek and Vetvicka, 1994). Transfection of cathepsin D cDNA increases the metastatic potential of tumor cells in nude mice (Garcia et al., 1996). Additional studies report that proteolytic activity is not essential for the mitogenic effect, which appears to be an extracellular, potentially receptor-mediated mechanism. The receptor has not been identified (Glondu et al., 2001; Beaujouin et al., 2006; Liaudet-Coopman et al., 2006). Cathepsin D may constrain the intracellular secretion of antiproliferative proteins by preventing the density-dependent inhibition of cell growth during cell culture (Liaudet et al., 1995). Cathepsin D may also facilitate the intake of extracellular growth factors (Briozzo et al., 1991).

In breast-cancer cell lines, cathepsin D prevents apoptosis. However, under cytotoxic influence, cathepsin D appears to stimulate apoptosis irrespective of its catalytic activity. Therefore, several different mechanisms may be involved (Berchem et al., 2002; Liaudet-Coopman et al., 2006). Cathepsin D also appears to act as a paracrine factor for endothelial and fibroblastic cells. The immediate stromal environment actively influences tumorigenesis by exchanging cytokines, growth factors and proteases such as uPA or matrix metalloproteinases (Liaudet-Coopman et al., 2006). In vitro, cathepsin D also stimulates angiogenesis and constrains apoptosis in tumor cells, which was confirmed immunohistochemically in 102 mammary carcinoma preparations (Gonzalez-Vela et al., 1999; Berchem et al., 2002). Stimulation of angiostatin in prostate carcinomas (much less intense than mammary carcinomas) and specific effects involving prolactin have been reported to inhibit angiogenesis (Morikawa et al., 2000; Tsukuba et al., 2000; Piwnica et al., 2004). Cathepsin D and pro-cathepsin D were detected in the serum of 18 out of 30 female patients with metastasized breast cancer but in none of the carcinoma-free patients (Brouillet et al., 1997).

The following summaries are examples of studies that have shown decreased survival rates corresponding with high cathepsin D levels since the 1990s.

1. High cathepsin D levels in the tumor cytosol corresponded with a significantly shorter period of disease-free and general survival among 331 female breast-cancer patients. In the estrogen receptor-negative group, the correlation was not significant (Duffy et al., 1992).

2. Pujol, Maudelonde et al produced a prospectively designed study in which 123 female patients with primary mammary carcinoma were followed over a

period of 5 years. The cathepsin D concentrations detected immuno-enzymatically in the tumor cytosol only correlated with the lymph node status. High cathepsin D levels, negative progesterone receptor status and lymph node involvement were the most important significant prognostic factors (Pujol et al., 1993).

3. Over a median period of 4 years, a study of 2,810 female breast-cancer patients showed that the prognosis was worse for the patients with higher cathepsin D levels in the tumor cytosol (Foekens et al., 1999).

4. High cathepsin D concentrations in the tumor cytosol (greater than 60 pmol/mg protein) in 138 women over an observation period of 5 years corresponded with a significantly higher risk of early relapses or metastasis (in less than 3 years). For node-negative patients, a significant relationship was also observed for the general and disease-free 5-year survival rates (Scorilas et al., 1999).

5. In a multivariate prospectively designed analysis, Harbeck et al examined 276 female patients with breast cancer for a median follow-up period of 109 months. Harbeck et al analyzed the prognostic and predictive values of uPA, PAI-1 and cathepsins B, D and L and determined the concentrations immunohisto-chemically in the primary tumor tissue. Cathepsin L was the only significant factor for disease-free and general survival in node-positive patients. In node-negative female patients, the most important prognostic and predictive factors were PAI-1 and the grading. For the entire sample, lymph node status, grading, PAI-1 and cathepsin-L were the only statistically significant factors (Harbeck, 2001).

6. In another publication, the same authors refer to an analysis of cathepsins in the tumor cytosol and uPA and PAI-1 in extracts produced by leaching the tumor preparations. This study refers to the same number of patients and follow-up period as the study mentioned previously and found similar results. However, node-negative patients with lower PAI-1 and lower cathepsin D concentrations exhibited a 3.2 % rate of relapse or metastasis compared with 39 % at the higher levels (Harbeck et al., 2000).

7. In 1,033 female patients with primary mammary carcinomas that were followed-up over a median period of 52 months, high cathepsin D levels in the tumor cytosol (>59 pmol/mg protein) were associated with a shorter general survival period in the entire group and with involvement of the lymph nodes (Rodriguez et al., 2005).

Most of the studies focused on the detection of cathepsin D in the cytosol of tumors, which involves an elaborate analytical method that is not always applicable for small tumors in clinical practice. In the NSABP B20 trial, receptor-positive, node-negative female mammary carcinoma patients were randomized for tamoxifen therapy and for chemotherapy. A "recurrence score" (RS) of 21 genes was defined including subgroup invasion CTSL2, which is the coding gene for cathepsin L2, and MMP11, which is the coding gene for stomolysin. The score predicted that the probability of metastatic spread and the benefit of chemotherapy were statistically significant (Paik et al., 2004, 2006). Several authors did not find any correlations between the cathepsin D concentration and known prognosis factors other than the estrogen receptor status (Maudelonde et al., 1988; Spyratos et al., 1989). In estrogen receptor-positive breast-cancer cell lines, stimulation of cathepsin D transcription and expression due to estrogens was demonstrated (Westley and May, 1987; Cavailles et al., 1993). In another study, the cathepsin D level in the tumor cytosol only correlated with the lymph node status, which is consistent with the results published by our group (Pujol et al., 1993). Furthermore, a preceding immunohistochemical analysis showed that cathepsin D expression in the tumor tissue correlated with the lymph node status (Gohring et al., 1996). A more extensive study of 1,003 female patients reported a correlation with the known prognosis factors. The cathepsin D levels were (similar to the previous study) higher in ductal-histological types compared with other types. Prognostic stratification appeared to be possible only for node-positive women. Immunoradiometric measurements in the tumor cytosol were also conducted (Rodriguez et al., 2005).

In a retrospective immunohistochemical study, Joensuu et al. found cathepsin D expression in 80 % of ductal mammary carcinomas and only 54 % of lobular carcinomas among 213 preparations. They found no association with the examined known prognosis factors, but they did observe a high cell proliferation rate (Joensuu et al., 1995). An older study immunohisto-chemically detected cathepsin D in 86 % (48/56) of lobular carcinomas and only 63 % of ductal carcinomas (Domagala et al., 1993). Several studies directly compared the immunohistochemical measurements of cathepsin D with the more elaborate identification in tumor cytosol in freshly frozen tissue (which is only possible with larger tumors). Gohring et al. retrospectively evaluated 270 preparations of primary mammary carcinomas and compared the immunohisto-chemistry with an immunoradiometric assay (IMRA) in the cytosol. The median follow-up period was 68 months. Both methods showed a concordance of 70%. A predictive value referring to the survival was only possible for the immunohistochemistry results (Gohring et al., 1996). Maudelonde published a comparison of 34 breast cancer preparations and found a good correlation (r=0.80) with cathepsin D expression (Maudelonde et al., 1992). In a prospective study, Roger et al. also compared immunohistochemical cathepsin D identification using the same antibody (monoclonal M1G8 antibody) with measurement performed in the tumor cytosol. Cathepsin D expression was increased compared with the

peritumorous tissue in 41 breast-cancer preparations. The correlation was based on the existence of lysosomes and phagosomes in the malignant cells. Immunohistochemical identification correlated significantly with the measurement performed in the cytosol (r=0.76). Ductal tumors also changed color more intensely than lobular tumors (n=35 versus 6). In 40% of the tumors, LAVs (large acidic vesicles, i.e., phagosomes) were detected, and portions of the tumors changed color to positive. In this study, macrophages were additionally detected using antiCD68-antibodies, which exhibited distinct color changes to indicate the presence of cathepsin D. There was no correlation between the quantitative immunohistochemical score and the number of macrophages (Roger et al., 1994). Capony et al demonstrated immunohistochemically 20 to 50 times higher expression for cathepsin D in the mammary carcinomas compared with benign glandular breast tissue or in other cells such as fibroblasts. Macrophages from the tumor stroma also reacted positively (Capony et al., 1989; Roger et al., 1994). Our own studies showed that the expression of cathepsin D significantly higher in the ductal mammary carcinomas compared with lobular carcinomas (Barthell et al., 2007). We also showed that cathepsin D exhibited lower expression in metastasized lymph nodes compared with the corresponding primary tumor (Jeschke et al., 2005). Mammary carcinomas with detected relapse or rather distant metastasis exhibited significantly increased cathepsin D expression compared with mammary carcinomas without metastases (Dian et al., 2012). However, there were no differences between the primary tumors or the distant tumors. More recent recommendations for the treatment of patients with breast cancer at an early stage concluded that the combined immunohistochemical identification of E-cadherin and cathepsin D is sufficient for the recommendation of a treatment (Jacobson-Raber et al., 2011). A number of experimental studies on cell cultures and proteome analyses appear to confirm the increasing significance of this protease (Knopfova et al., 2012; Laurent-Matha et al., 2012; Morimoto-Kamata et al., 2012; Rafn et al., 2012).

Summary and Outlook

This overview shows the fundamental suitability of cathepsin D as a prognosis factor for mammary carcinoma. However, differences in cathepsin D expression among various histological types should be considered. Furthermore, we showed that cathepsin D expression in tumor cells increases along with the metastasis of the lymph nodes. This indicates that cathepsin D is involved in the collapse of the extracellular matrix. Other studies proposed an interaction between cathepsin D expression, proliferation and the hormone-receptor status. According to this study, the cathepsin D concentration of the tumor preparations is independent from the traditional prognosis factors except for the involvement of lymph nodes. In specialist literature, many studies have shown poor prognosis corresponding with high cathepsin D levels, particularly for node-negative female patients (Harbeck et al., 2000). The correlation with poor prognosis may be useful for patients with intermediary risk, in which it is difficult to decide whether to perform chemotherapy. Cathepsin D detection could be considered a diagnostic additional benefit. The advantage of immunohistochemistry is its relative simplicity and belated execution. Further prospectively designed studies should be performed to compare cathepsin D analysis with disease-free and general survival as endpoints.

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Accepted November 20, 2013