SHORT COMMUNICATION

Cathepsin D assay in ovarian cancer: correlation with pathological features and receptors for oestrogen, progesterone and epidermal growth factor

G. Scambia, P. Benedetti, G. Ferrandina, F. Battaglia, G. Baiocchi & S. Mancuso

Department of Gynecology, Catholic University, Rome, Italy.

Summary Using an immunoradiometric assay, Cathepsin-D (Cath-D) concentrations were measured in the cytosol of 68 normal and neoplastic human ovarian tissues. Cath-D levels were higher in malignant tumours than in normal tissue samples (P < 0.01) and benign tumours (P < 0.01). In six out of seven cases, metastatic deposits showed Cath-D concentrations higher than the respective primary tumours. Using of 17 pmol mg⁻¹ protein as cut-off levels, the Cath-D status (high or low) was not related to any pathological parameter. Moreover, no correlation was found between Cath-D levels and receptors for oestrogen, progesterone and epidermal growth factor.

Our results indicate that ovarian tumours produce Cath-D. Further studies are needed to evaluate whether this protein could represent a prognostic factor for this neoplasia.

Research on proteolytic enzymes has recently provoked considerable interest because they may be involved in the process of tumour invasion and metastatization (Goldfarb, 1986). Among these enzymes much attention has been focused on Cathepsin D (Cath-D), a lysosomal aspartyl endopeptidase (Barrett, 1977), which has been found to be identical to the oestrogen induced 52% glycoprotein first described by Westley and Rochefort in MCF-7 cells (Westley & Rochefort, 1979; Morisset et al., 1986). This Cath-D-52 K protein, secreted by human breast cancer cells, displays a mitogenic effect in vitro (Vignon et al., 1986) and a proteolytic effect on extracellular matrix after its autoactivation at acidic pH (Briozzo et al., 1988). Accordingly, it could have a role in the control of the growth and spread of human breast cancer. Moreover, in breast cancer a high cytosolic concentration of Cath-D is associated with a shorter relapse-free survival (Thorpe et al., 1989; Spyridatos et al., 1989).

At present only few data are available on the presence of Cath-D in malignancies other than breast cancer (Garcia et al., 1986; Maudelonde et al., 1990).

In the present study, the concentrations of Cath-D were assayed by an immunoradiometric method in human ovarian cancer cytosols and correlated with other pathological and biological parameters.

Materials and methods

Fifty-three primary ovarian tumours, seven benign tumours and eight normal ovaries were studied. Patient age ranged from 29 to 71 years old (median 53 years). All tissue specimens were frozen on dry ice shortly after surgical removal and stored at −80°C until processed. Tumours were staged according to FIGO criteria and histologically graded from G1 to G3.

Tissue samples were homogenised in ice-cold buffer consisting of 25 mM Tris, 1.5 mM EDTA, 5 mM Na3N, 10 mM monothioglycerol and 20% glycerol. Cytosol and membrane fractions were prepared as previously described (Battaglia et al., 1988, 1989). Oestrogen (ER) and progesterone receptors (PR) were measured by a dextran coated charcoal assay according to EORTC protocol (1980), using 17-B-oestradiol (81 Ci mmol⁻¹) and 3H-ORG-2058 (57 Ci mmol⁻¹) (both from Amersham International plc) as radiolabelled ligands. EGF receptors (EGFR) were assayed on the membrane fraction as previously described (Battaglia et al., 1988) using 1251-EGF (Amersham International plc) as radiolabelled ligand. Cath-D concentration was assayed using a solid phase two site immunoradiometric assay (CIS Bioindustries, Gif-sur-Yvette, France) in which the first monoclonal antibody (D7E3) is coated on the ELSA solid phase and the second one, M1G8, radiolabelled with 125I is used as a tracer (Brouillet et al., 1990). For the Cath-D assay, cytosol protein concentration, measured by the Bradford method (1976) using bovine serum albumin as the standard, was reset to about 1 mg ml⁻¹ before the assay. Cytosols were then diluted 1/40 and 1/80 with the diluent contained in the kit. Radioactivity was measured in a γ counter for 1 min. Intra- and inter-assay variations were 6.4% and 8.5% respectively.

Statistical analysis was performed by Student’s t-test. Chi-square test and Fisher’s exact test were used to evaluate the distribution of Cath-D values according to different variables.

Results

Figure 1 shows the distribution of Cath-D values in normal and neoplastic ovarian specimens. Overall, Cath-D concentration was significantly higher in malignant tumours (mean ± s.e.m., 15.82 ± 1.06 pmol mg⁻¹ protein) than in normal tissue samples (7.30 ± 1.42 pmol mg⁻¹ protein) (P < 0.01) and benign tumours (7.10 ± 1.75 pmol mg⁻¹ protein) (P < 0.01). In seven patients, Cath-D was evaluated in primary tumour and in simultaneous omental metastases. In all cases but one, higher Cath-D concentrations were found in metastatic deposits than in primary tumours.

Table 1 shows the correlation between Cath-D status and different variables in ovarian cancer. To define Cath-D status two arbitrary cut-off values of 12 pmol mg⁻¹ protein and 17 pmol mg⁻¹ protein (corresponding approximately to the mean ± 1 s.d. and the mean ± 2 s.d. of normal samples, respectively) were adopted. Overall, Cath-D levels were found to be above 12 or 17 pmol mg⁻¹ protein in 66% and 36% of the cases, respectively. At both cut-off Cath-D status was not related to any pathological parameter. Moreover, no
correlation was found between Cath-D levels and ER, PR and EGFR content.

Discussion

To our knowledge, this is the first report on the assay of Cath-D in the cytosol of normal and neoplastic ovarian tissue. Interestingly, Cath-D levels were significantly higher in ovarian cancer than in normal ovary. Similar findings have previously been obtained when breast (Garcia et al., 1987) and endometrial cancer (Maudelonde et al., 1990) specimens were compared to their normal counterparts. Moreover, the secretion of Cath-D and other cathepsins is 10-fold greater in breast cancer cells than in normal mammary cells in culture (Rochefort et al., 1987).

It is worth noting that scattered Cath-D levels were found in our ovarian cancer series. Since Cath-D is a proteolytic enzyme which may be secreted by cancer cells to facilitate tumour invasion (Briozo et al., 1988) and may also be an autocrine mitogen (Vignon et al., 1986), the difference in Cath-D content might represent a biochemical characteristic reflecting a different biological aggressiveness. Our finding of high Cath-D levels in metastatic ovarian tumours support the hypothesis that Cath-D production may in some way be linked to tumour progression and invasiveness. In fact, in human breast cancer Cath-D is a powerful independent prognostic factor in predicting relapse-free survival (Thorpe et al., 1989; Spyrratos et al., 1989). The lack of correlation between Cath-D levels and other prognostic parameters such as a stage, histotype, grading and EGFR-R (Bauknecht et al., 1988; Battaglia et al., 1989) could indicate that, like in human breast cancer, the prognostic value of Cath-D could be additive (Maudelonde et al., 1988; Brouillet et al., 1990). It has also to be taken into account, however, that the tumour concentrations of Cath-D may be influenced by the proportion of stromal and inflammatory components (Imort et al., 1983). Further immunohistochemical studies should be addressed to verify this point.

The mechanism of the mitogenic action of Cath-D is unknown. As for other proteases, Cath-D may act indirectly by releasing growth factors, such as TGF alpha, from precursors or from extracellular matrix and/or by activating growth factor receptors (Derynck et al., 1984; Lawrence et al., 1985). However, the lack of correlation between Cath-D and EGFR suggests that the tissue binding capacity for EGFR is not influenced by Cath-D content.

It is well known that Cath-D levels are regulated by oestrogen in human breast cancer cells (Westley & Rochefort, 1979) and by progesterone in rat uterus (Elangoovan, 1980) and human endometrium (Maudelonde et al., 1990). In our series, no correlation was found between Cath-D levels and ER and PR expression. This is consistent with previous

![Figure 1](image.png) Cath-D levels in normal and neoplastic ovarian tissue. Cath-D concentration was measured as detailed in Materials and methods.

| Table I Cath-D status according to different variables in ovarian cancer |
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| **Patients with Cath-D** |
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| n | >12 pmol mg⁻¹ protein | >17 pmol mg⁻¹ protein |
| n (%) | n (%) |
| **Total** | 53 | 35 (66) | 19 (36) |
| **Stage** |  |  |  |
| I | 7 | 6 (86) | 3 (43) |
| II | 3 | 3 (100) | 1 (33) |
| III | 35 | 21 (60) | 12 (34) |
| IV | 8 | 5 (62) | 3 (37) |
| **Histological grading** |  |  |  |
| G1 | 6 | 5 (83) | 1 (17) |
| G2 | 11 | 10 (91) | 4 (36) |
| G3 | 31 | 17 (55) | 14 (45) |
| **Histotype** |  |  |  |
| Serous | 38 | 24 (63) | 12 (31) |
| Mucinous | 5 | 3 (60) | 1 (20) |
| Endometrioid | 6 | 4 (67) | 2 (33) |
| Undifferentiated | 4 | 4 (100) | 4 (100) |
| **Receptors** |  |  |  |
| ER + (>5 fmol mg⁻¹) | 29 | 17 (59) | 10 (34) |
| ER − | 24 | 18 (75) | 9 (37) |
| PR + (>10 fmol mg⁻¹) | 41 | 28 (68) | 16 (39) |
| PR − | 12 | 7 (58) | 3 (25) |
| EGFR − R + (>1.5 fmol mg⁻¹) | 28 | 19 (68) | 11 (39) |
| EGFR − | 25 | 16 (64) | 8 (32) |

*In five cases the histological grading was not available.
findings showing that the concentrations of Cath-D in breast and endometrial cancer are independent from receptor status (Maudelonde et al., 1988, 1990), and that Cath-D is also constitutively produced and secreted in ER-negative breast cancer cells (Garcia et al., 1987). In conclusion our data demonstrate that ovarian tumours contain Cath-D and that this protein could represent a possible prognostic marker in these tumours. This needs to be ascertained by prospective clinical trials which are now under way.

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