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Cathepsin D and plasminogen activator inhibitor type 1 in normal, benign and malignant ovarian tissues: a preliminary report

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Background. *The aim of the present study was to determine the concentration of cathepsin D (Cath D) and plasminogen activator inhibitor type 1 (PAI-1) in normal ovarian tissues, benign and malignant ovarian tumor tissues, and to asses relationship between Cath D and PAI-1 content, and some clinical and pathohistological parameters.*

Materials and methods. *Cath D contents and PAI-1 concentrations were determined (using immunoradiometric ELSA-Cath D assay and commercial IMUDIND[R] ELISA immunoassay, respectively) in 35 samples: 10 normal ovarii, 10 benign, 10 primary malignant and 5 metastatic ovarian tumors.*

Results. *The concentrations of Cath D were significantly higher in malignant (32.89±14.26 pmol/mg protein) and metastatic (31.42±10.24 pmol/mg protein), than in normal (13.68±4.03 pmol/mg protein) and benign (17.89±13.13 pmol/mg protein) ovarian tissues. There was no statistical differences in the concentrations of PAI-1 between normal, benign, malignant and metastatic tumor specimens. The concentrations of Cath D as well as PAI-1 did not correlate to the age of patients, menopausal status, parity, GOG risk group, clinical stage or pathohistological grading.*

Conclusion. *Concentrations of Cath D (but not PAI-1) were significantly increased in malignant and metastatic ovarian tumor tissues when compared to normal and benign ovarian tumor samples; they were independent from pathohistological and clinical parameters.*

Key words: ovarian neoplasms; cathepsin D, plasminogen activator inhibitor type 1

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Introduction

Among gynaecological malignancies, ovarian cancer has been the major cause of death because they are usually presented in advanced clinical stage, with extensive intraab-

dominal metastasis and the unavoidable development of resistance to chemotherapy.[1,2]

Invasion and metastases are multi-step processes that require a complex cascade of interrelated events including extracellular matrix degradation, migration, proliferation and induction of neovascularisation.[3-5] These processes are complicated and only partially understood. Proteases associated with the invasive capacity of tumor cells are metalloproteinases (collagenases, gelatinases, stromelysin), cysteine proteases (cathepsin B, H, L), aspartyl proteases (cathepsin D) and serine proteases (urokinase type plasminogen activator and its activated product, plasmin).

Cathepsin D (Cath D) is a lysosomal acidic protease. It might be involved in tumor growth, invasion and metastasis by different mechanisms: a) by inactivating a secreted growth inhibitor, b) by releasing growth and angiogenesis factors from the extracellular matrix, c) by providing amino acids following phagocytosis of extracellular matrix, d) by degrading extracellular matrix and basal membranes, and e) by activating latent precursors from other proteinases involved in the invasive step of metastatic process.[5-7] Thus, Cath D may facilitate the spread of neoplastic cells and promote tumor invasiveness and metastatic potential through different mechanisms by acting at different levels of the metastatic process.

Positive correlation between Cath D level and aggressiveness of breast tumor, as well as shorter relapse free interval and overall survival of breast cancer patients [5,8,9], have stimulated the investigation on tumors of other origin. So, elevated levels of Cath D have been found in other tumors like melanoma, head and neck carcinoma, genital carcinoma.[6,10-12]

In the multi-step process of invasion, plasminogen/plasmin system plays an important role. Urokinase plasminogen activator (uPA) is a highly specific serine protease that converts plasminogen in plasmin.[4,13] Plasmin degrades fibronectin, laminin and other non-collagenous proteins of the extracellular matrix, and is able to activate latent collagenases. That leads to further degradation of extracellular matrix and invasion and metastasis. UPA activity is controlled by specific plasminogen activator inhibitors (PAI), which include plasminogen activator inhibitor type 1 (PAI-1), plasminogen activator inhibitor type 2 (PAI-2) and protease nexin.[14,15] PAI-1, a serine protease inhibitor (serpin), is the major inhibitor of PA in the plasma. High levels of PAI-1 in tumor extracts from breast, lung and gastric cancerous tissues appear to be highly significant and independent predictors for shorter overall survival.[14]

The importance of proteases in the regulation of ovarian carcinogenesis, invasion and metastases may further promote its clinical application in the detection of early stage of this disease, prognostic assessment of patients with ovarian cancer as well as in the development of new cancer specific treatment modalities. Limited information is available on the prognostic value of cathepsin D and plasminogen activator inhibitor type 1 regarding gynaecogolical malignancies. The aim of the present study was to determine the concentration of Cath D and PAI-1 in normal, benign and malignant ovarian tissues and to asses the relationship between Cath D and PAI-1 content and some clinical and pathohistological parameters.

Materials and methods

Tissue samples

The tissue samples were obtained from fresh specimens removed during surgical procedure at the Department of Obstetrics and Gynaecology of Medical School, University of Zagreb. The Ethics of Research Committee at the Medical School of the University of Zagreb approved the protocol.

Thirty-five tissue samples were analyzed: 10 normal ovarian tissues, 10 benign ovarian

tumor specimens, 10 primary ovarian carcinoma and 5 metastatic ovarian carcinoma samples.

After surgery, the samples were frozen in liquid nitrogen. For biochemical analysis, samples were they were prepared as previously described.[16] Briefly, each specimen was minced, homogenized in lysis buffer, and centrifuged for 45 min at 15000 g. Supernatant (tumor tissue cytosol) was used for biochemical studies.

Cathepsin D determination

Cath D content was measured in the cytosol of homogenized samples using a commercially available solid-phase two site immunoradiometric assay (ELSA-CATH-D, CIS Bio International, Gif-sur-Ivette, France). It detects precursors and mature form of Cath D. The values were normalized according the total protein assessed by Bradford's method[17], and expressed as pmol/mg protein.

Plasminogen activator inhibitor determination

The concentration of PAI-1 inhibitor was determined by immunoassay using a commercially available ELISA kit (IMUBIND^R, American Diagnostica Inc., Greenwhich, USA). It detects latent and active forms of PAI-1 and complexes. The concentrations of PAI-1 were expressed in ng/mg protein.

Statistics

The statistical significance of difference between the concentrations of cathepsin D and PAI-1 in the particular groups was tested by Kruskal-Wallis one way ANOVA test. Their relationship to pathohistological and clinical factors were calculated by Spearman's rank correlation test. Data are reported as mean ± SD. Differences were considered significant at $p<05$.

Results

The pathohistological characteristics of ovarian tumors that were entered into present study are given in Table 1. Their classification and histopathological grade, as well as clinical staging, was defined according to FIGO. Among the patients with benign ovarian tumors, those with cystadenofibroma serosum prevailed, while among the patients with primary malignant tumors, those with cys-

Table 1. Pathohistological characteristics of ovarian tumor specimens

Benign		Primary malignant		Metastatic carcinoma	
teratoma dermoides		cystadenocarcinoma serosum		breast cancer	2
cysticum	1	ovarii	5	carcinoma ventriculi	
cystadenofibroma serosum		cystadenocarcinoma mucinosum		(Krukenberg)	1
ovarii	8	ovarii	2	colorectal carcinoma	1
cysta serosa	1	cystadenocarcinoma		endometrial carcinoma	1
		anaplasticum ovarii	1		
		adenocarcinoma endometrioides			
		ovarii	1		
		cystadenocarcinoma mixtum			
		ovarii	1		

Table 1. Pathohistological characteristics of ovarian tumor specimens

Total	10	
Clinical stage		
I A	1	
IB	1	
IC	4	
III	2	
IV	2	
Tumor grade		
GI (well differentiated)		4
GII (moderately differentiated)		3
GIII (poorly differentiated)		3
Hystological type		
mucinosus	2	
serosus	5	
endometrioid	1	
mixed	1	
anaplastic	1	
Age (mean, range)		
<50	4	
>50	6	
Parity		
0	3	
≥1	7	
GOG(Gynecology Oncology group)		
low risk (stage IA and B, grade 1 or 2)		2
high risk (stage IC or II or grade 3)		8

tadenocarcinoma serosum were prevalent. (Table 1). Primary ovarian carcinomas were further classified as shown in Table 2. More than half of these malignant tumors belong, according to GOG, to high risk group.

The cytosol supernatant obtained after homogenization of ovarian tissue samples was analysed for the content of cathepsin D and plasminogen activator inhibitor type 1. Figure 1 shows the concentrations of cathepsin D in normal, benign and malignant ovarian specimens. Cath D levels were significantly higher in primary malignant (32.89 ± 14.26 pmol/ mg protein) and metastatic (31.42 ± 10.24 pmol/ mg protein) malignant ovarian carcinomas than in normal (13.68 ± 4.03 pmol/ mg protein) or benign (17.89 ± 13.13 pmol/ mg protein) tissue samples (p=0.003 for normal versus primary malignant tissues, p=0.035 for normal versus metastatic malignant tissues, p=0.028 for benign *versus* primary malignant tissues, p=0.029 for benign *versus* metastatic malignant tissues). There was no difference between the levels of Cath D in primary and metastatic ovarian tumor tissues (p=0.995), or between normal and benign tumor tissues (p=0.838).

Figure 2 presents the PAI-1 concentrations in normal, benign and malignant ovarian specimens. Although the mean concentration of PAI-1 was lower in normal (2.96 ± 1.49 ng/mg protein) than in malignant tumor tissues (8.80 ± 10.56 ng/mg protein), this difference was not statistically significant (p=0.170). Also, there was no statistically significant difference between the levels of PAI-1 in primary and metastatic ovarian carcinomas (p=0.864), or between normal and benign tumor tissues (p=0.948).

The concentration of cathepsin D as well as PAI-1 did not seem to correlate to the age of patients, their menopausal status, parity, or GOG risk group. Neither cathepsin D nor PAI-1 concentrations correlated with clinical staging or pathohistological grading.

Discussion

The major cause of death among gynaecological malignancies is ovarian cancer: over 70% of women are diagnosed with incurable advanced-stage disease, defined by widespread intraperitoneal metastases.[1,2] Tumor invasion is a complex, multistep sequence of events based on a cascade of coordinated cellular processes. The combined action of several proteolytic enzymes is involved in tissue

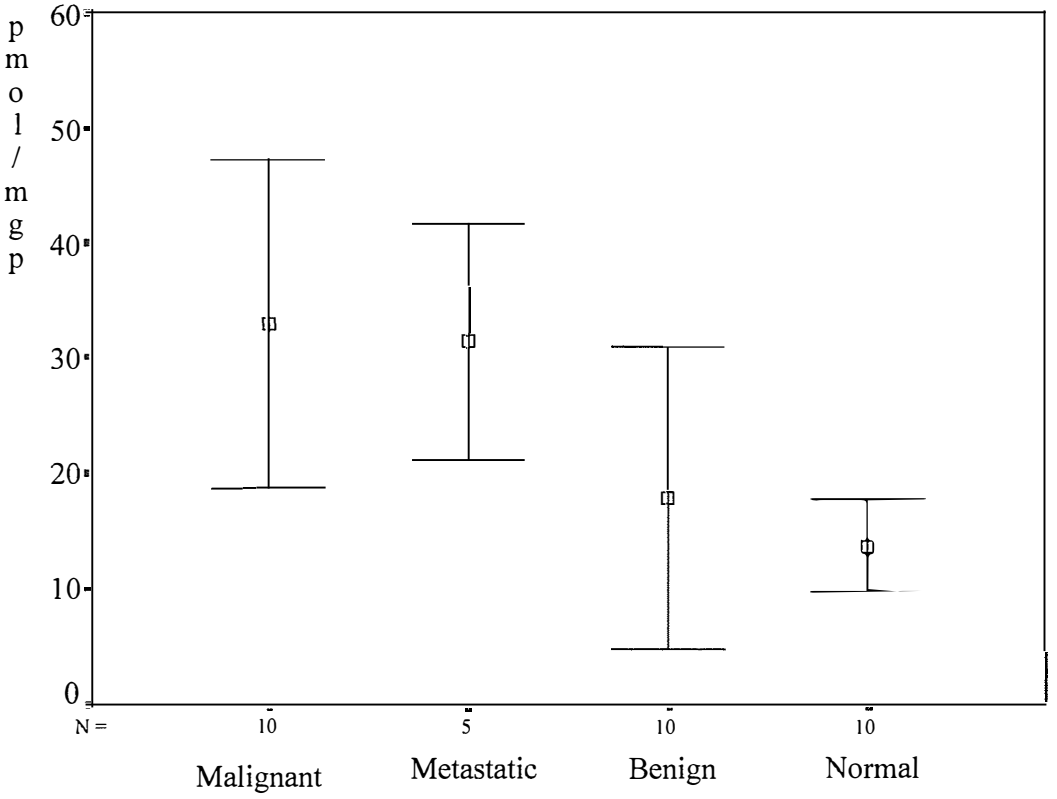

Figure 1. Concentrations of cathepsin D (mean ± SD) in primary and metastatic ovarian carcinomas, benign ovarian tumors and normal ovarian tissues. The number of samples per group are indicated.

degradation and remodeling in both, normal and tumor tissue.[3-5]

The collected evidence have proved that proteases and their inhibitors (including Cath D and PAI-1) may serve as prognostic factors in breast cancer, to predict the outcome of the disease.[5,8,9] Several tumor-associated proteases are important factors also in solid tumors of other origins, such as lung, gastric, colon, genital, head and neck, bladder, and kidney cancers.[4-6,10-12,18,19]

The aim of the present study was to determine and compare the levels of cathepsin D and plasminogen activator inhibitor type 1 in normal, benign, primary malignant and metastatic malignant tumor ovarian tissues. As mentioned in the Introduction, Cath D is a proteolytic enzyme that may be involved in invasion and metastasis through different mechanisms.[5-7]

In the present study, we have determined significantly higher concentrations of Cath D in primary and metastatic ovarian carcinomas than in normal or benign tumor samples (Figure 1), which was is in agreement with literature data.[12,20] It is important to point out that Cath D levels were similar in both, normal and benign tumor tissue samples, suggesting that malignant transformation of ovarian tissue was accompanied with the increased level of this protease. The concentration of Cath D was independent from the pathohistological parameters, such as hystological type or tumor grading, as well as clinical markers, such as clinical stage, age, parity and menopausal status.

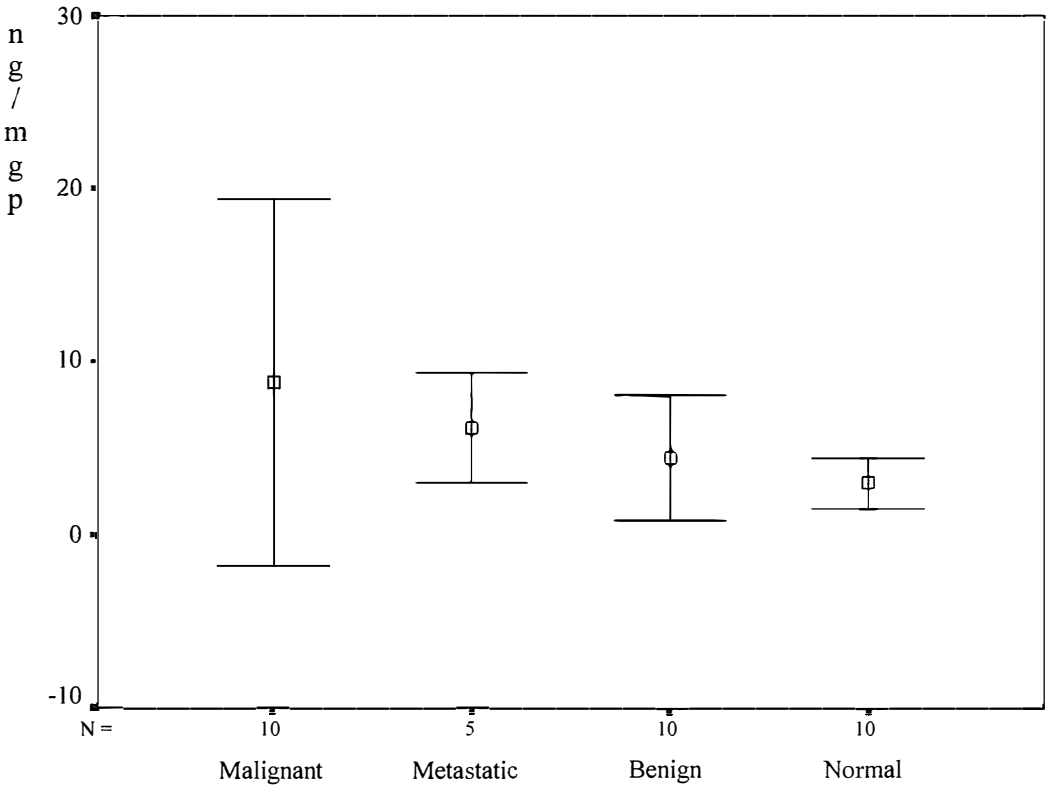

Figure 2. Concentrations of urokinase plasminogen activator inhibitor type 1 (mean ± SD) in primary and metastatic ovarian carcinomas, benign ovarian tumors and normal ovarian tissues. The number of samples per group are indicated.

Urokinase plasminogen activator has important role in the tissue degradation and in the invasiveness of tumor cells.[4,13] In spite of the fact that uPA activity is controlled by a specific inhibitor, such as PAI-1, the increased levels of both, uPA and PAI-1 were found in different tumors: breast cancer[20], gastric cancer[14] and bladder cancer.[21] Furthermore, increased levels of both, uPA and PAI-1 were associated with poor prognosis, and increased relapse rate and shorter survival.

The data in the literature concerning ovarian tumors and PAI-1 levels are contradictory. Some groups have found increased levels of PAI-1 in ovarian cancer[22], and even suggested that uPA and PAI-1 may predict the survival of patients with advanced ovarian cancer.[23] Others did not determine any increased levels of uPA and PAI-1 in ovarian tumors.[20] Our results concerning PAI-1 are closer to the data of Ruppert *et al.*[20] We did not find a significant increase of PAI-1 in malignant ovarian tumor tissues. However, the observed difference in absolute values for mean PAI-1 concentrations between normal and malignant ovarian tissues suggests that in order to draw important conclusions more samples should be examined.

In addition, comparing the levels of Cath D and PAI-1 in normal, benign and malignant tumor tissues, we have not found any correlation between these parameters.

In conclusion, Cath D overexpression in malignant tumor ovarian tissue may be relat-

ed to intraabdominal dissemination of ovarian tumor cells. In order to determine more precisely the diagnostic and prognostic values of Cath D and PAI-1 in ovarian cancer patients, we have started a clinical study with a higher number of patients with ovarian cancer and a longer follow up.

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