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Original Paper

Dipeptidyl Peptidase III in Malignant and Non-malignant Gynaecological Tissue

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Exopeptidases, in contrast to endopeptidases (proteinases) have been much less studied in relation to cancer. The aim of this study was to investigate one such enzyme, dipeptidyl peptidase III (DPP III), in gynaecological tissues, by measuring both the enzyme activity and enzyme content. DPP III activity was assessed in normal ($n = 65$), benign ($n = 9$) and malignant ($n = 51$) gynaecological tissues. A statistically significant higher DPP III activity was observed in endometrial ($n = 40$, $P = 4.6 \times 10^{-7}$) and ovarian ($n = 11$, $P = 8.1 \times 10^{-4}$) malignant tumours, whereas no significant difference was detected for leiomyomas ($n = 8$), if compared to the activity in normal tissue. A matched pair analysis of normal and cancerous endometrial tissue confirmed the significance of the DPP III activity increase in the transformed tissue ($n = 7$, $P = 0.022$). Western blot analysis revealed a significantly ($P = 0.014$) increased level of DPP III in endometrial cancer. Further, regression analysis showed a positive correlation between the activity and the content of DPP III in normal tissue ($r = 0.637$, $P = 0.047$) and in endometrial cancer ($r = 0.574$, $P < 0.007$). The increase of the DPP III activity was observed in the endometrial carcinomas of various histological types, grade or the depth of myometrial invasion. The easy-to-perform determination of this exopeptidase activity may serve as a potential indicator of endometrial and ovarian malignancies.

Key words: dipeptidyl peptidase III, human uterus, human ovary, endometrial cancer, ovarian cancer
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INTRODUCTION

PROTEOLYTIC ENZYMES play an important role in various cell functions: mammalian cell growth [1], cell cycle regulation [2], apoptosis [3] and malignant transformation of mammalian cells [4, 5]. Their contribution to physiological tissue remodelling and to some pathological processes, such as periodontal disease, arthritis, cancer invasion and metastasis, as well as cancer cachexia, is well established [6–10]. In the complex process of tumour invasion, the combined action of several different proteolytic enzyme systems including different types of metalloproteinases, serine proteinases (plasmin generated by plasminogen activator), aspartic proteinase cathepsin D, as well as cysteine proteinases cathepsin B and L, and their endogenous inhibitors, has been recognised [9, 11–13]. In contrast to proteinases (endopeptidases), exopeptidases have been much less studied in relation to cell growth and cancer. However, the importance of a cytosolic exopeptidase, a puromycin-sensitive aminopeptidase, in cell cycle regulation has been revealed recently [14]. Also, a role in the invasion of human metastatic tumour cells has been ascribed to the cell-surface aminopeptidase N [15].

Dipeptidyl peptidase III (DPP III) is one of the four well-characterised types of dipeptidyl (amino)peptidases of mammalian cells [16]. It has been extensively purified from human erythrocytes and placenta [17, 18] and partially from bovine pituitary and adrenal glands [19, 20], rat brain [21] and human seminal plasma [22]. The enzyme is predominantly found in cytosol, but for rat brain DPP III, on the basis of activity determination, membrane localisation has also been suggested [21]. Arg-Arg-2-naphthylamide (Arg-Arg-2NA) is a distinctive DPP III synthetic substrate, from

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which this enzyme removes the N-terminal dipeptide. Similarly, starting from the unsubstituted amino-end, DPP III also cleaves some oligopeptides. Enkephalins and angiotensins are among the biologically active peptides which are hydrolysed by purified DPP III [21, 23]. The amino acid sequence, its physiological role and the regulation of this enzyme *in vivo* are not yet known. However, participation in the final steps of intracellular protein catabolism, contribution to degradation of circulating peptides and a regulatory role has been suggested for DPP III [21, 23].

In order to obtain further insight into the function and distribution of human DPP III, we investigated this enzyme in normal and some transformed gynaecological tissues. Here we report for the first time the finding that the level of DPP III activity and its protein content are significantly increased in human gynaecological malignancies.

MATERIALS AND METHODS

Patient samples

The consecutive specimens of gynaecological tissues were collected at surgery or biopsy at the Department of Obstetrics and Gynaecology, School of Medicine, University of Zagreb, Croatia, frozen within 10 min in liquid nitrogen and kept at −196°C until examined. Clinical information was obtained after biochemical analysis of DPP III was completed. Clinical stage, tumour grade and histological type for malignant tumours were determined according to the International Federation of Gynaecologists and Obstetricians (FIGO) staging system (1988). Additional histopathological characteristics included vascular space invasion and determination of the degree of leucocyte infiltration.

Tissue specimens were obtained from 63 patients (mean age 58.3 years, range 16–84 years) undergoing primary surgical treatment. Endometrial cancers included endometrioid (24 tumours); mixed endometrioid and mucinous ($n = 7$); adenosquamous ($n = 3$); serous ($n = 1$); undifferentiated ($n = 1$); mixed endometrioid and serous ($n = 1$) and three were malignant mixed Muellerian tumours. Also 6 serous ovarian cancers and two mucinous, two endometrioid and one dysgerminoma were analysed. Among benign gynaecological tumours analysed, there were eight uterine leiomyomas and one teratoma.

The protocol was approved by the Ethics of the Research Committee at the School of Medicine, University of Zagreb.

Reagents

Arg-Arg-2-naphthylamide·3HCl (Arg-Arg-2NA), Arg-2-naphthylamide·HCl (Arg-2NA) and bestatin were obtained from Sigma Chemical Co. (St Louis, Missouri, U.S.A.). QAE Sephadex A-50 and low-molecular mass electrophoresis calibration kit were obtained from Pharmacia Biotech (Uppsala, Sweden). All other chemicals were purchased from Serva Feinbiochemica GmbH & Co. KG (Heidelberg, Germany), unless stated otherwise.

Tissue processing

Each specimen was minced, then suspended in a 50 mM Tris buffer at pH 7.6 (250 mM sucrose, 134 mM KCl) and homogenised on ice with an Ultra-Turrax T25 homogenizer (Janke & Kunkel, Ika-Labortechnik, Germany) for three 5 s bursts. The homogenate was divided into two equal parts and centrifuged at 4°C for 45 min at 15 000*g*. The supernatant ('tumour cytosol') was used for biochemical studies.

Enzyme and protein assays

The DPP III activity was determined with Arg-Arg-2NA as a substrate as previously described [23], only the reaction was usually performed for 15 min in Tris-HCl buffer with no addition of $CoCl_2$. Hydrolysis of the control substrate Arg-2NA was determined under identical conditions.

One unit of enzyme activity (U) was defined as the amount of enzyme which converts 1 μmol of substrate per minute under the assay conditions. The specific activity of DPP III was expressed in milliunits per mg of the sample protein.

Protein concentration was determined following the Bradfords method [24], using bovine serum albumin as a standard.

DPP III purification

DPP III was purified to homogeneity from human erythrocytes as previously described [23].

Antibody preparation

Rat antiserum to human DPP III was produced by i.p. immunisation of animals with 10–25 μg of the pure human erythrocyte DPP III, followed by a booster dose of 10 μg of the same immunogen on day 28 and bleeding at day 42, as described by Hu and associates [25]. Antibody titres were checked prior to each step of the immunisation protocol, by using dot immunoblotting.

Immunoglobulin G fraction was obtained from rat antisera as a fraction not bound to QAE-Sephadex A-50 column equilibrated in 50 mM ethylene diamine-acetate buffer, pH 7.0, according to Tijssen and Kurstak [26]. It contained 4.1 mg protein/ml and after 1:1000 dilution it was used as a primary antibody for the immunodetection of human DPP III.

Specificity of the prepared immunoglobulin G fraction (primary antibody) from rat antiserum to the purified human DPP III was checked by ELISA and by Western blot analysis. The purified DPP III (4 ng/ml) coated on to the microtitre plate could be clearly detected using this antibody even in the presence of 25 000-fold higher amounts of the human serum proteins. In contrast, even 64-fold higher amounts of DPP III could not be detected with non-specific rat IgG. Similarly, in the Western blot analysis performed with our primary antibody, we did not detect any immunologically reactive protein in normal human serum (when up to 50 μg of protein was applied on the gel), while in parallel even 2 ng of the purified human DPP III could be detected as an immunoreactive band. When non-specific rat IgG (purified from pre-immune rat serum) was used as the primary antibody in the Western blot analysis, no immunoreactivity was observed with 50 ng of the DPP III.

Polyacrylamide gel electrophoresis (PAGE)

PAGE of native proteins was performed by the PhastSystem on a Phast gel gradient 8–25% with native buffer strips at pH 8.5 (all products of Pharmacia, Uppsala, Sweden). 1.5–3μg of protein per lane was applied. After electrophoresis, the lanes on one half of the gel plate were sliced into 2 mm pieces. Each gel slice was incubated at room temperature for 15 h in 200 μl of reaction mixture for detection of DPP III activity. The intensity of the developed colour was estimated visually or on a Multiscan Plus ELISA reader (Labsystem, Helsinki, Finland). The other half of the gel plate was stained with protein dye Coomassie blue R 250 according to the manufacturer's (Pharmacia) instructions.

Slab SDS–PAGE was performed on a Sturdier slab gel electrophoresis unit SE 400 (Hoefer Scientific Instruments, San Francisco, California, U.S.A.) at 15 mA for 4 h at room temperature according to the method of Laemmli [27]. Gels, 0.75 mm thick, composed of a 4% stacking gel and a 7.5% separating gel, were cast. The applied samples (normal or tumour tissue cytosol, purified human DPP III, and low-molecular mass standards), containing 5–10 μg of protein, were visualised for protein bands either by silver staining according to Hempelmann and Kaminsky [28], or by Coomassie blue. When SDS–PAGE was followed by Western blot analysis, 8–66 ng of purified human DPP III mixed with 40 μg of human serum proteins (obtained from the Blood Transfusion Institute, Zagreb, Croatia), 10–15 μg of protein of malignant tumour cytosol or 40–50 μg of normal tissue cytosol proteins were applied on the gel per lane.

Western blot analysis

After SDS–PAGE, one lane with purified DPP III was cut and stained for 10 min with Coomassie blue R 250, and the proteins from other samples were transferred to 0.2 μm pore-sized nitrocellulose membrane (Schleicher and Schuell, Keene, New Hampshire, U.S.A.) for 1 h at 0.8 mA/cm^2 using 2117-250 NovaBlot electrophoretic transfer kit (Pharmacia, Uppsala, Sweden) and continuous buffer system (48 mM Tris, 39 mM glycine and 0.0375% w/v SDS in 20%, v/v methanol, pH approx. 8.7). Alternatively, half of the electrophoresed SDS gel was cut and submitted to protein staining and the other half, containing identical samples, was analysed by Western blotting. Blots were blocked for non-specific binding in 5% w/v bovine serum albumin (Fraction V, Sigma) in washing buffer (140 mM NaCl and 27 mM KCl buffered with 10 mM Na,K-phosphate, pH 7.5, containing 0.1% v/v of Tween 20) for 1 h, rinsed and incubated for 2 h with the primary antibody preparation diluted (1:1000) in the washing buffer containing 1% w/v bovine serum albumin (diluting buffer). After rinsing thoroughly with washing buffer, the blots were incubated for 2 h with horseradish peroxidase-conjugated affinity pure mouse anti-rat immunoglobulins $G_{(H+L)}$, (Jackson Immuno Research, U.S.A.), diluted 1:4000. Finally, rinsed blots were developed in 50 mM Tris-HCl buffer, pH 7.6, containing 0.5 mg 3,3′-diaminobenzidine tetrahydrochloride per ml and 0.0075% (v/v) H_2O_2. Colour development was stopped by immersing the nitrocellulose in water. Colour-developed nitrocellulose blots were optically scanned using the Desk Top Scanner (Pharmacia LKB Image Master DTS) and quantitation of the DPP III immunoreactivity was performed by the use of Image Master Software. Purified DPP III from human erythrocytes was used as an internal standard for all immunoblots.

Statistical analyses

Statistical analyses were performed using the STATGRAPHICS computer program (STSC Inc.). The significance of differences between the mean values was evaluated by Student's *t*-test when the groups consisted of at least 10 data per group. The comparison of median values for groups with number of data < 10 was performed by appropriate non-parametric tests (Wilcoxon's signed rank test for two paired samples, Mann–Whitney U-test for two independent samples). The correlation coefficients were determined by simple linear regression analysis. The probability values < 0.05 were considered to indicate statistical significance. All *P* values were two-tailed.

RESULTS

Identification of DPP III in gynaecological tissue extracts

The rate of hydrolysis of Arg-Arg-2NA by tissue extracts was approximately 2-fold higher at pH 8.6 than at pH 7.0, and it was enhanced by approximately 100% by the addition of 10 μM $CoCl_2$ to the assay mixture. The aminopeptidase inhibitor, bestatin (100 μM), did not influence the reaction rate. These properties, together with a significant fall in the activity observed upon freezing, were consistent with our previously published data on human erythrocyte DPP III [17, 23]. The hydrolysis of control aminopeptidase substrate Arg-2NA was negligible in comparison with the hydrolysis of Arg-Arg-2NA and was strongly inhibited by bestatin. In addition, to confirm the presence of DPP III in the tissue extracts and ensure that, under the assay conditions, the hydrolysis of Arg-Arg-2NA was due solely to the catalytic activity of DPP III, the native- and SDS–PAGE of tissue extracts and DPP III purified from human erythrocytes were performed in parallel. The activity determined after native PAGE of normal and malignant tissue was found in one gel slice only and it coincided with the position of purified enzyme hydrolytic activity and protein. In all the examined tissues, SDS–PAGE and subsequent Western blotting revealed one immunoreactive protein band in the position of $M_r \sim 82\,000$, which was identical to that of purified DPP III. We detected no immunologically reactive DPP III in normal human serum (up to 50 μg) and therefore, we used it in the mixture with the purified DPP III in order to achieve the uniformity of transfer and subsequent blotting conditions. Figure 1(a) shows the results of one Western blot analysis of DPP III in gynaecological tissue cytosols and Figure 1(b) presents the total protein profiles of cytosols from the matched normal and malignant human endometrium obtained by SDS–PAGE. Protein staining showed that the protein ladders obtained from normal and malignant tissue cytosol were very similar, although the specific activity and specific content of DPP III in those tissues differed by at least 3-fold.

DPP III activity in gynaecological tissues

After DPP III was identified as an enzyme responsible for the hydrolysis of Arg-Arg-2NA by tissue cytosols, we determined the specific activity of DPP III using the same substrate in 125 samples originating from: normal tissues (65 samples), benign tumours (9 samples) and malignant tumours (51 samples).

The mean DPP III specific activity in normal tissues, benign tumours (leiomyomas) and malignant tumours was 9.4 mU/mg protein (median 8.0; range 1.1–38.1), 11.2 (median 10.4; range 6.8–17.3) and 39.4 (median 37.0; range 6.9–96.9), respectively, thus revealing a highly significant ($P < 7.3 \times 10^{-15}$) increase in the malignant tumour tissue DPP III activity (Figure 2). The mean DPP III activity of malignant tumour cytosols was four times higher than that of the normal tissue cytosols (ratio $T/N = 4.2$).

No significant difference was observed in the DPP III activity between leiomyomas and normal myometrium or endometrium. One sample of teratoma showed specific activity of DPP III (3.5 mU/mg) in the range of DPP III activity for the normal ovarian tissue.

A comparison of DPP III activity between normal tissues and malignant endometrial or ovarian tissues showed highly significantly greater levels in endometrial tumours ($P = 4.6 \times 10^{-7}$, T/N ratio 2.8) and ovarian tumours ($P = 8.1 \times 10^{-4}$, Mann–Whitney U-test; T/N ratio 5.6) (Figure 2).

Finally, DPP III specific activities were examined in matched pairs of endometrial normal and malignant tissue (Figure 3). Normal tissue counterparts were either adjacent, histologically normal, endometrium (in 'true pairs') or myometrium (in 'apparent pairs'). There was a highly significant difference in the DPP III activity in apparent pairs ($P = 5.4 \times 10^{-12}$; T/N ratio = 4.8). In true pairs, the difference was significant ($P = 0.022$; Wilcoxon's signed rank test), and the average DPP III activity was 3.4 times higher in cancerous than in normal endometrial tissue. The number of ovarian true pairs was insufficient for a statistical analysis.

DPP III protein content in gynaecological tissues

We also investigated whether the (higher) specific activity of DPP III reflects an increased amount of this enzyme in the tissue. Therefore, normal tissues (n = 15) and malignant endometrial tissues (n = 21) were analysed by Western blot analysis using rat anti-DPP III immunoglobulins (see Figure 1a). The determination of DPP III content (expressed as μg DPP III protein/mg of total tissue protein) revealed that the protein content of DPP III was significantly higher in malignant endometrial tissues than in normal tissue (3.6 times as much for the matched paired samples, $n = 15$, $P = 0.014$). A positive correlation was observed ($r = 0.637$, $P = 0.047$) between the specific DPP III activity and DPP III protein content in normal tissue counterpart (myometrium and endometrium) and in the matched endometrial malignant tissues ($r = 0.574$, $P < 0.007$).

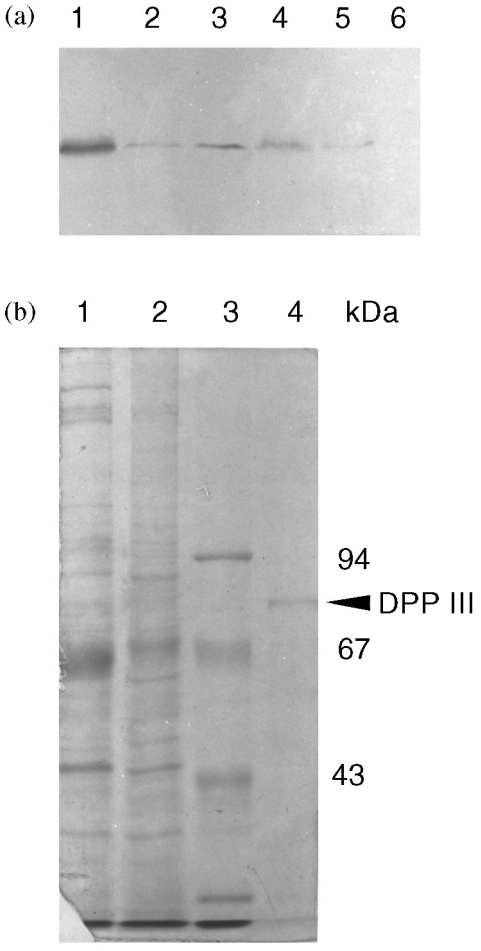

Figure 1. (a) Western blot analysis of DPP III in gynaecological tissue cytosols. Lanes 1 and 4: purified human erythrocyte DPP III (66 and 16.5 ng, respectively); lanes 3 and 5: endometrial cancer tissue (32 and 18.5 μg, respectively); lanes 2 and 6: non-neoplastic endometrial tissue (32 and 18.5 μg, respectively), matched pairs of samples applied in lanes 3 and 5, respectively. (b) Coomassie blue-stained 7.5% polyacrylamide gel after SDS–PAGE. Lane 1: non-neoplastic endometrial tissue, 15 μg (the same sample as in lane 2 in (a)); lane 2: endometrial cancer tissue, matched pair, 15 μg (identical sample as in lane 3 in (a)); lane 3: low-molecular mass markers, 9 μg; lane 4: purified human erythrocyte DPP III, 0.6 μg. kDa, kilodaltons.

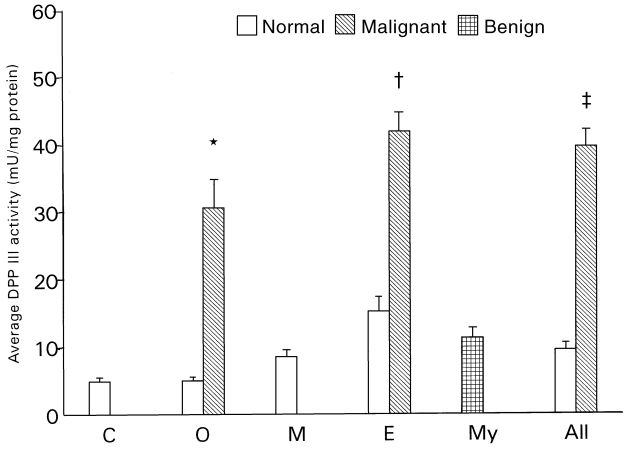

Figure 2. DPP III activities (± SEM) in gynaecological normal and tumour tissues. C, normal cervical tissue (n = 9); O, ovary (normal: n = 7, malignant: n = 11); M, normal myometrium (n = 32); E, endometrium (normal: n = 17, malignant: n = 40); My, uterine leiomyoma (n = 8); All: all normal (n = 65), all malignant (n = 51). *$P = 8.1 \times 10^{-4}$ †$P = 4.6 \times 10^{-7}$ ‡$P < 7.3 \times 10^{-15}$.

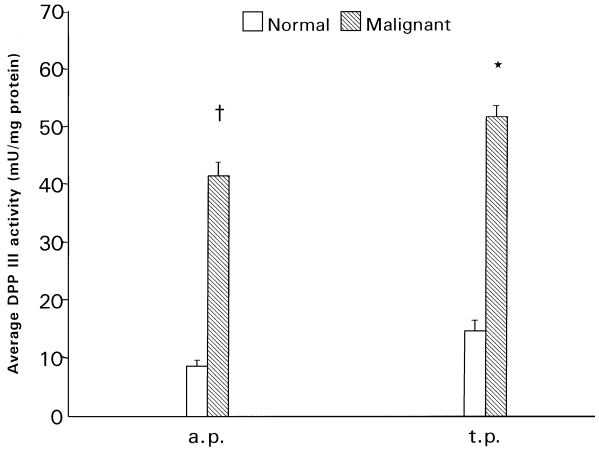

Figure 3. DPP III activities (± SEM) in matched pairs sets of endometrial cancer and normal tissues. a.p., apparent pairs (n = 28): normal tissue is myometrium; t.p., true pairs (n = 7): normal tissue is endometrium. *$P = 0.022$ †$P = 5.4 \times 10^{-12}$.

Table 1. *DPP III activity (± SEM) in matched paired sets of endometrial carcinomas and normal tissues (endometrium or myometrium): distribution according to the biological and histopathological parameters*

Parameter	DPP III activity (mU/mg protein)			
	Tumour	Normal	Ratio T/N	P†
Clinical stage				
I all ($n=28$)	45.5 ± 3.5	10.2 ± 1.1	4.5	2.65×10^{-13}
Ia. ($n=3$)	20.7 ± 1.9	5.1 ± 2.3	4.1	N/A
Ib. ($n=16$)	52.4 ± 4.7	10.6 ± 1.3	4.9	1.14×10^{-9}
Ic. ($n=9$)*	41.5 ± 4.2	11.0 ± 2.6	3.8	1.29×10^{-2}
II ($n=4$)	43.1 ± 4.4	9.3 ± 1.1	4.6	N/A
Tumour grade				
G1 ($n=17$)	42.9 ± 4.7	9.0 ± 1.3	4.8	7.61×10^{-8}
G2 ($n=12$)	50.0 ± 4.7	10.6 ± 2.2	4.7	1.49×10^{-7}
G3 ($n=5$)	36.5 ± 1.1	8.5 ± 0.9	4.3	N/A
Carcinoma type				
Endometrioid ($n=23$)	44.9 ± 3.9	9.2 ± 1.0	4.9	2.69×10^{-11}
Mixed endometrioid and mucinous ($n=6$)*	46.7 ± 5.2	6.7 ± 0.6	7.0	3.60×10^{-2}

*Wilcoxon's signed rank test used. †Normal versus tumour. N/A, numbers too low for statistical analysis.

Relationship between DPP III and biological/clinical parameters in endometrial carcinomas

The DPP III activity was not related to the extent of tumour inflammation, semiquantitatively evaluated as low, medium and high, neither to the lymph-vascular space invasion (data not shown).

When the data on DPP III activity were stratified according to clinical stage (most tumours were of the stage I), histological type and grade, a significant increase in DPP III activity was found in stage Ib and Ic tumours, grade 1 and 2 tumours, and in endometrioid, as well as in mixed endometrioid and mucinous type of endometrial carcinomas, if compared to normal tissue counterpart (Table 1). Although the number of analysed matched endometrial carcinomas of stage Ia and II, and of grade 3, was insufficient for the analysis of significance of differences between matched paired sets, the average values of DPP III activity (± SEM) and T/N ratios (Table 1) seem to indicate the tendency of DPP III specific activity enhancement in the tumour tissue of those groups.

Two stage III endometrial carcinomas (one mixed endometrioid and mucinous type, the other endometrioid and serous type) had a DPP III activity of 37.4 and 7.7 mU/mg, respectively (T/N values were 4.6 and 2.1, respectively), one undifferentiated carcinoma had a DPP III activity of 38.0 mU/mg ($T/N = 4.9$), and three stage I adenosquamous endometrial carcinomas had a mean DPP III activity of 47.6 mU/mg (mean DPP III activity in normal tissue was 11.9 mU/mg).

DISCUSSION

In our study, for the first time, DPP III activities in normal, benign and malignant gynaecological tissues are reported. We have observed no differences in biochemical properties between DPP III purified from human erythrocytes and the enzyme in gynaecological tissues. The correlation between DPP III activity and protein levels suggests that the higher DPP III activity in malignant tissue stems from increased DPP III protein content. Other unpublished data indicated the absence of regulation by an endogenous DPP III activator in tumour tissue or the absence of an inhibitor in normal tissue (M. Abramić, Rudjer Bošković Institute, Croatia). Inflammation within the tumour or blood contamination could not be responsible for the increased DPP III activity, since no positive correlation between these parameters was found (data not shown). There was no linear correlation between patient's age and DPP III activity in normal (endometrium and myometrium) or malignant tissues, if those parameters were compared for all the samples (data not shown).

Final conclusions on differences in the DPP III specific activity regarding the clinical stage, grade, histological type of endometrial carcinoma and patient's age, should certainly wait for larger studies. Our study suggests that the enhancement of DPP III activity occurs in endometrial carcinomas regardless of their histological type, grade or stage.

The role of DPP III in endometrial and ovarian (patho)-physiology is highly speculative, as is that of the two other aminopeptidases detected in these same tissues, i.e. aminopeptidase N and DPP IV [29, 30]. The regulation of local concentrations of biologically active peptides by these membrane-bound ectoenzymes has already been proposed [30]. Yet, it is still not known whether their expression is altered in endometrial or ovarian cancer. However, the accumulated evidence supports the role of aminopeptidase N in tumour cell (renal cell carcinoma, fibrosarcoma and melanoma) invasion [15] and ectopic expression of DPP IV in differentiated thyroid carcinomas has revealed this enzyme as a novel molecular marker [31].

Previously, we have shown *in vitro* that DPP III has a particularly high affinity for the peptide constituents of the renin-angiotensin system (RAS) and that it generates hexapeptide angiotensin IV from octapeptide angiotensin II [23]. Angiotensin IV has been shown to stimulate the expression of plasminogen activator inhibitor type 1 (PAI-1) in endothelial cells [32]. The proteolytic system plasminogen activator (PA), PAI-1, of the human endometrium seems to be involved in degradation of the extracellular matrix and fibrinolysis during implantation and menstruation [33]. As already mentioned in the Introduction, the same enzyme system (PA–PAI-1) contributes to tissue degradation during cancer invasion [11]. The increased level of PAI-1 and/or PA observed in several cancers, including ovarian, appears to be a predictor of short survival [34–36]. Therefore, the relationship of DPP III to malignant growth could originate from its possible *in vivo* regulatory role in RAS, which could cause enhancement of

the tissue concentration of PAI-1. RAS has been recognised as an autocrine/paracrine system in both the ovary and uterus [37, 38].

Matrix metalloproteinases are another group of proteolytic enzymes which are physiologically relevant mediators of endometrial tissue remodelling during the menstrual cycle [39]. Their possible involvement in endometrial malignancy has been indicated [40]. The enhanced level of aspartic proteinase cathepsin D in endometrial cancers has been reported by different authors, including us [41–43], but its prognostic value has yet to be elucidated.

There is a need for more specific tumour markers, as for the utilisation of several markers in parallel [44, 45]. The undoubted difference in enzymatic activities of DPP III between normal and malignant gynaecological tumour tissues suggests that a simple assay of this proteolytic enzyme, if added to the usual pathological examination, might be useful as a biochemical indicator of endometrial and ovarian malignancy.

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