Primary Tumor and Metastasis in Ovarian Cancer Differ in Their Content of Urokinase-type Plasminogen Activator, Its Receptor, and Inhibitors Types 1 and 2


Abstract

The relevance of urokinase-type plasminogen activator (uPA) and plasminogen activator inhibitor (PAI) type 1 in predicting the survival probability of patients with advanced ovarian cancer after radical surgery and adjuvant chemotherapy by assessing the patients' primary tumors has recently been shown by us (W. Kuhn et al., Gynecol. Oncol., 55: 401–409, 1994). In the present study, we determined uPA, uPA receptor, PAI-1, and PAI-2 concentrations in primary tumors and tumor-infiltrated omentum and retroperitoneal lymph nodes of ovarian cancer patients. The group consisted of 39 patients with advanced ovarian carcinoma stages Fédération Internationale de Gynécologie et d’Obstétrique (FIGO) III or IV; for comparison 7 patients with early carcinoma stage FIGO I were also included. In metastases of the omentum from ovarian cancer stage FIGO III or IV patients, we noted a 4-fold elevated uPA content, a 2-fold increase in PAI-1, and also a significant increase in uPA receptor and PAI-2 over primary tumors. In metastases of the lymph nodes the levels of the respective antigens were also increased when compared to primary tumors. These data may indicate that elevated levels of components of the fibrinolytic system at sites of metastases may contribute to the aggressive potential of cancer cells by favoring their reimplantation and/or the consolidation of a new tumor stroma.

Introduction

Ovarian carcinoma is one of the most severe gynecological malignancies and because of a long asymptomatic course of the disease displays already advanced stages at diagnosis with extensive cancer spread. This is reflected by the high incidence (70% of the cases) of mortality within 5 years, a fact that stresses the necessity of an early diagnosis for risk selection and the prediction of the course of the disease. Several investigators have demonstrated that the prognosis of patients with advanced ovarian cancer could be improved by radical surgery followed by adjuvant chemotherapy. Although the absence of residual tumor after cytoreductive surgery proved to be a strong prognostic factor for survival, the complete resection of the tumor mass was not sufficient to protect all patients from the recurrence of the disease and subsequent death. Several attempts have been undertaken to improve the prognosis of cancer patients and to establish predictive factors that are related to tumor biology. Numerous factors have been screened, among them the receptors for estrogen, progesterone, and epidermal growth factors, DNA ploidy, S-phase, and oncogenes (1).

In recent years special attention has been paid to tumor cell surface-associated proteolytic enzyme systems regarding their impact on cancer progression and metastasis. The process of tumor invasion and metastasis is controlled by a series of successive events. After local proteolysis of the surrounding extracellular matrix, detachment and spread of tumor cells is facilitated followed by the invasion of the adjacent tissue, crossing of tissue boundaries and the basement membrane. Intravasation and extravasation are followed by reimplantation of tumor cells into the respective target organ, remodeling of a new tumor stroma, and angiogenesis in order to consolidate a secondary tumor at a distant locus. For these events a fine-tuned balance between proteolytic factors, their inhibitors, adhesive proteins, angiogenic factors, oncogenes, and growth factors has to be adjusted (2, 3).

Basic and clinical research in tumor invasion and metastasis have focused on the role of tumor-associated proteases such as metalloproteinases and the serine proteases of the plasmin/plasminogen activator system (2). Metalloproteinases seem to be correlated with tumor cell invasion and metastasis in animal models; however, conclusive data on their clinical significance in humans are still lacking. In contrast, for several different solid cancers a strong prognostic impact of the serine protease uPA and/or its inhibitor PAI-1 as predictors for the course and the outcome of the cancer has been reported. Besides their statistically independent prognostic impact in cancer of the breast (4, 5), lung (6), colon (7), and the gastrointestinal tract (8), significantly elevated levels of both uPA and PAI-1 have been demonstrated in ovarian cancer tissues compared to those in normal ovarian tissue specimens. Patients with advanced ovarian cancer can be subgrouped on the basis of computer-optimized "cutoff" values for uPA and PAI-1: patients with low uPA and PAI-1 (uPA < 0.9 ng/mg protein; PAI-1 < 13.5 ng/mg protein) had a statistically better prognosis than patients with high uPA and PAI-1 antigen levels (9). First indications for a role of the uPA-mediated proteolytic system in tumor invasion and metastasis came in 1976 from Astdedt and Holmberg (10), who demonstrated for the first time high uPA concentrations in cultured ovarian carcinoma tissue. In the following years increased uPA and PAI-1 levels were also found in tumor tissue extracts of patients with ovarian carcinoma (11, 12) and in malignant ascites (13).

uPA is synthesized and secreted by normal and tumor cells. Both the inactive proenzyme form of uPA, pro-uPA, and proteolytically active uPA bind to a specific glycanlipid-anchored receptor (uPA-R, CD 87) on the tumor cell surface. Upon binding of pro-uPA, its activation by other proteases (e.g., plasmin) is facilitated. uPA activates the proenzyme plasminogen into the broad spectrum serine protease plasmin, which may in turn either directly degrade extracellular matrix proteins such as fibronectin, laminin, and proteoglycans or activates certain matrix-degrading enzymes such as procollagenase. The activation of uPA is controlled by its specific inhibitors PAI-1 and PAI-2. Binding of PAI-1 or PAI-2 to uPA-R-bound uPA results in the subsequent internalization of the ternary complex.
complex, thereby regulating cell surface plasmin generation. uPA-R in combination with specific plasmin(ogen) receptors focalizes the plasmin/plasminogen activator system to the tumor cell surface, thus constituting an effective proteolytic enzyme system (2).

Tumor-associated proteolytic enzyme systems are not only important for tumor cell spread from the primary tumor but seem to be crucial parameters for the events at the site of metastases. In this report we investigated the levels of uPA, uPA-R, PAI-1, and PAI-2 in tissue extracts of primary tumors, tumor-infiltrated omentum, and tumor-infiltrated lymph nodes of patients suffering from advanced ovarian carcinoma.

Patients and Methods

Forty-six patients with early (FIGO I) or advanced ovarian cancer stages (FIGO IIc or IV) were enrolled as part of a prospective study on ovarian cancer patients undertaken at the Frauenklinik der Technischen Universität München (Table 1). Patients with stages FIGO II, IIIa, and IIIb were excluded from the present analysis because of a too small number of cases. Patients with advanced ovarian carcinoma received the following radical surgical treatment: longitudinal laparotomy with hysterectomy, bilateral adnexection, appendectomy, infragastric omentectomy, and bilateral pelvic and paraaortic lymphadenectomy. Among the group of patients, seven patients received limited surgical treatment because of their bad health condition. In younger patients (<35 years) with tumor stage FIGO I, less radical surgery was performed in order to preserve the fertility of the patients. Histological examinations of all of the tumor tissue sections were performed at the Institut für Allgemeine Pathologie und Pathologische Anatomie, Technische Universität München (Munich, Germany). Benign ovarian tumors (n = 21), e.g., benign ovarian cysts, serous cystadenoma, or cystadenofibroma served as controls.

Tissue Collection and Extraction. Ovarian cancer tissue specimens from primary tumors, omentum majus, and lymph nodes were collected during surgery, classified by the pathologist, and stored in liquid nitrogen until used. Deep-frozen specimens of 200–500 mg wet weight were pulverized using the Micro-Dismembrator (Braun, Melsungen, Germany) set to maximum power. The resulting powder was immediately suspended in 2 ml TBS (0.02 M Tris-HCl, 0.125 M NaCl, pH 8.5), 1% (v/v) Triton X-100, and the antigen concentrations in primary tumor and omentum metastasis from each patient were evaluated using the Wilcoxon test for paired samples in Fig. 1. In tumor-infiltrated retroperitoneal lymph nodes, an approximately 2-fold increase in the median value of uPA was noted compared to the uPA content in tissue extracts from ovarian cancer patients, e.g., omentum majus and, in addition, in retroperitoneal lymph nodes. The respective tissues were extracted in the presence of the nonionic detergent Triton X-100, and the antigen content of uPA, uPA-R, PAI-1, and PAI-2 was determined using the ELISA. The important new findings in this study can be summarized as follows.

<table>
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<th>Variable</th>
<th>Median age (range)</th>
<th>Stage</th>
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Table 1 Patient data on age, tumor stage, histology, grading, nodal status, and lymphadenectomy.

The following antibodies supplied by American Diagnostica were used at 10 μg/ml: uPA (mAb 3689), uPA-R (mAb 3639), and PAI-1 (mAb 3785). The median concentration of uPA in primary tumor tissue of patients with tumor stage FIGO IIIc or IV was 0.91 ng/mg protein. In extracts of tumor-infiltrated omentum majus, the uPA content was approximately 4-fold higher than in primary tumors exhibiting statistical significance (P = 0.0001). The corresponding values for the uPA content in primary tumor tissue and omentum metastasis of patients with ovarian carcinoma FIGO II or IV are depicted as paired samples in Fig. 1. In tumor-infiltrated retroperitoneal lymph nodes, an approximately 2-fold increase in the median value of uPA was noted compared to the uPA content in tissue extracts from primary tumors; however, statistical significance was lacking (P = 0.68).

The median value for the receptor of uPA, uPA-R, in extracts of primary tumor tissues of the same patients was 2.87 ng/mg protein compared to a median value of 4.36 ng uPA-R/mg protein in tumor-infiltrated omentum majus. This difference was statistically significant (P = 0.05). Values for corresponding uPA-R concentrations in primary tumor and omentum metastasis from each
of the ovarian cancer patients are depicted as paired samples in Fig. 2.
As for uPA, the median uPA-R content in extracts of tumor-infiltrated lymph nodes was increased compared to primary tumors, but lacked statistical significance (P = 0.53).

PAI-1. The median value of PAI-1 in extracts obtained from primary tumor tissues was 16.99 ng/mg protein; in the corresponding sites of metastases (tumor-infiltrated lymph nodes, 35.42 ng/mg protein; metastases of the omentum majus, 34.14 ng/mg protein), an approximately 2-fold increase over extracts of primary tumors was noted. The difference in the PAI-1 concentrations in extracts of primary tumors and omentum majus was statistically significant (P = 0.0009, Table 2), in contrast to only an insignificant increase in extracts of tumor-infiltrated lymph nodes (P = 0.11). The PAI-1 concentration in primary tumor tissue and the corresponding one in omentum metastasis tissue are depicted as paired samples for each of the patients with ovarian cancer FIGO IIIc or IV in Fig. 3.

PAI-2. In extracts obtained from primary tumor tissues of patients with advanced ovarian carcinoma, a low PAI-2 antigen content was determined and reflected by a median value of 0.91 ng/mg protein. In extracts of tumor-infiltrated omentum majus, the median PAI-2 concentration was approximately 7-fold higher than in extracts of primary tumors. This difference was statistically significant (P = 0.004, Table 2). Corresponding values for PAI-2 in primary tumor and omentum metastasis from each of the 33 ovarian cancer patients (FIGO IIIc or IV) are depicted as paired samples in Fig. 4. PAI-2 antigen measurements in extracts of tumor-infiltrated retroperitoneal lymph nodes and primary tumors did not reveal significant differences (P = 0.82). No statistical correlation was noticed among uPA, uPA-R, PAI-1, and PAI-2 content, neither in the primary tumor nor in the metastases.

The concentrations of uPA, uPA-R, and PAI-1 extracts from primary tumor tissue of patients with advanced ovarian cancer FIGO IIIc or IV were significantly higher than in extracts prepared from benign ovarian tumors. PAI-2 levels were almost comparable in extracts of benign and malignant tumors (Table 3). uPA, uPA-R, and PAI-1 levels in extracts of tumor-infiltrated lymph nodes and omentum majus of patients with advanced ovarian carcinoma stages FIGO IIIc or IV were significantly higher than in extracts of tumor-free lymph nodes and omentum majus of patients with early ovarian cancer stage FIGO I. However, PAI-2 levels were not significantly increased in extracts of tumor-infiltrated lymph nodes and omentum majus compared to their tumor-free counterparts of FIGO I patients.

To demonstrate the cellular origin of uPA in advanced ovarian cancer, immunohistochemical stainings were performed in comparison to benign cystadenoma of the ovary (Fig. 5, B and C). All primary tumor specimens (FIGO IIIc/IV) examined in the present study by an ELISA displayed a positive reaction of tumor and stromal cells with mAb 3689 (American Diagnostica) directed to uPA when assessed by immunohistochemistry. A typical example is shown in Fig. 5C. In contrast, although stromal cells of benign ovarian tumors were reactive with the uPA antibody, epithelial cells failed to react (Fig. 5B). To demonstrate the specificity of mAb 3689, uPA-rich kidney tissue specimens were also stained with mAb 3689 (Fig. 5A). As expected, mAb 3689 intensively stained the renal tubule cells, whereas the glomeruli were negative. Tumor cells of primary advanced ovarian carcinomas were also found to be positive for uPA-R (mAb 3639; American Diagnostica) and PAI-1 (mAb 3785; American Diagnostica; data not shown). As for uPA, stromal cells of different origin were found to react with mAbs to uPA-R or PAI-1.

Discussion

Thus far only little information is available concerning differences in the content of uPA, uPA-R, and the inhibitors PAI-1 and PAI-2 in...
A fine-tuned balance among uPA, uPA-R, and PAI-1/2 might be implicated. With respect to PAI-2, a significant increase was observed in cystic fluids of patients with malignant ovarian tumors compared to benign tumors (17). In ascites of patients with ovarian cancer, PAI-2 was found to be an independent factor indicating poor prognosis; however, measurements in tumor tissues were not performed (18). PAI-2 levels are also elevated in tissue extracts from breast carcinomas compared to benign breast tumors, although this increase is of no clinical relevance (19). We observed only low expression of PAI-2 in extracts of primary tumors of ovarian carcinoma and of benign tissue with almost comparable concentrations. These data are in good accordance with data from colon and breast carcinoma (18–20).

![Graph](image1)

Fig. 3. PAI-1 antigen in tissue extracts of primary tumor and omentum metastasis. Paired samples are displayed from each of the 33 patients with ovarian cancer stage FIGO IIIC or IV. Note that there is an increase for the PAI-1 antigen concentration in omentum metastasis over primary tumor in 26 of 33 patients (P = 0.0009).

Fig. 4. PAI-2 antigen in tissue extracts of primary tumors and omentum metastasis. Paired samples are displayed from each of the 33 patients with ovarian cancer stage FIGO IIIC or IV. Note that there is an increase for the PAI-2 antigen concentration in omentum metastasis over primary tumor in 24 of 33 patients (P = 0.004).

![Table](image2)

Table 3 Comparison of uPA, uPA-R, PAI-1, and PAI-2 antigen levels in tissue extracts of benign and malignant ovarian tumors

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Benign ovarian tumors (n = 21)</th>
<th>Ovarian cancer (Primary tumor; n = 39)</th>
<th>Statistical significance (P)</th>
</tr>
</thead>
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<tr>
<td>uPA</td>
<td>(median range) 0.31 (0.01-2.54)</td>
<td>0.91 (0.05-10.16)</td>
<td>0.0001</td>
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<tr>
<td>uPA-R</td>
<td>(median range) 1.63 (0.41-2.83)</td>
<td>2.87 (0.55-8.66)</td>
<td>0.0001</td>
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<tr>
<td>PAI-1</td>
<td>(median range) 4.50 (1.69-15.30)</td>
<td>16.99 (0.14-105.1)</td>
<td>0.0001</td>
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<tr>
<td>PAI-2</td>
<td>(median range) 0.18 (0.01-2.47)</td>
<td>0.25 (0.01-11.34)</td>
<td>n.s.</td>
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</table>

* n, number of patients.

† n = 38.

‡ n.s., not significant.
In the present study an increase in the uPA-R concentration in tumor-infiltrated omentum and lymph nodes of advanced ovarian tumor stages has been observed. In primary colonic carcinomas and their metastases in the liver, an increased presence of uPA-R has been found, too (21). For colon carcinomas it has been demonstrated that a high content of uPA-R in tissue extracts correlated with a poor prognosis for the patients (22). For ovarian carcinomas it remains to be established whether the amount of uPA-R correlates with the progression of tumor growth and malignancy. Interestingly, in ascitic fluid, but also in tumor tissue and blood, a soluble, ligand-free form of uPA-R has been detected in invasive carcinomas. uPA-R may derive from the tumor via a phospholipase and/or proteases-mediated shedding of cell surface-attached uPA-R (23).

High concentrations of uPA, uPA-R, and PAI-1 in malignant ovarian tumor tissue reflect an increased expression of these factors in tumor cells or nonmalignant cells of the tumor stroma. In the present investigation on proteolytic factors in advanced ovarian cancer, by using the same mAbs (3689 and 3639) to uPA and uPA-R, respectively, we could confirm the results by Young et al. (24) that both tumor cells and stromal cells in fact react with the respective antibodies. Moreover, our immunohistochemical results support the assumption that ovarian cancer cells not only contain uPA and uPA-R but also the inhibitor PAI-1.

However, it is still an open question whether the process of metastasis might depend on the selection of tumor cell clones with increased expression of plasminogen activators, or whether the synthesis of uPA and PAI-1 is due to regulatory mechanisms or malignant transformation. In several metastasis models, it has been shown that primary tumors display a heterogeneity with respect to their metastatic potential (25). The occurrence of several types of ovarian carcinomas eliciting different aggressive potential has been reported: in patients with tumor stage FIGO IV accompanied by extensive metastases a smaller intraabdominal tumor mass had frequently been observed compared to patients with tumor stage FIGO III, although they had a comparable retroperitoneal lymph node involvement. These observations might be related to differences in the expression of plasminogen activators and their inhibitors. Thus far only little information is available about the different patterns of protein expression in primary tumors versus metastases during the progression of cancer. In a recent publication on breast cancer, it has been reported that the CD44 variant expression was increased by approximately 15–35% in lymph node metastases compared to primary tumors of breast cancer patients (26). Likewise, elevated levels of uPA, its inhibitors, and/or uPA-R at sites of metastasis may contribute to the spread and invasion of ovarian cancer cells and their capability to reimplant in the peritoneum. The elucidation of the concerted action of the different components of the plasmin/plasminogen activator system and their interdependence involved in tumor metastasis will help to further the understanding of tumor progression and the promotion of malignancy in ovarian carcinoma.

Acknowledgments

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References


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