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Impact of expression differences of kallikrein-related peptidases and of uPA and PAI-1 between primary tumor and omentum metastasis in advanced ovarian cancer

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Background: Primary tumor levels of serine proteases of the kallikrein-related peptidases (KLK) family as well as urokinase-type plasminogen activator (uPA) and its inhibitor PAI-1 impact disease course in ovarian cancer. The changes in levels of these factors from primary tumor to omentum metastasis ('level differentials') could thus be associated with metastatic processes.

Patients and methods: Protein levels of seven tissue KLK (KLK5–8, 10, 11, 13), uPA, and PAI-1 were determined in extracts of primary tumor tissue and corresponding omentum metastasis of 54 ovarian cancer patients.

Results: Higher level differentials of KLK5–8, 10–11, and uPA were associated with residual tumor >10 mm. Residual tumor and larger level differentials of KLK5–7, 10, and uPA were associated with disease progression in the whole cohort. Remarkably, level differentials of KLK5–8 and 10–11 strongly impacted disease progression even in patients with residual tumor mass ≤10 mm; hence, the observed impact of level differentials in KLK5–7 and 10 on disease progression was not simply attributable to their association with surgical success.

Conclusion: Since they impact both surgical outcome and survival in advanced ovarian cancer, measurement of level differentials could support clinical decisions on surgical and systemic therapy or help in patient selection for novel targeted therapies.

Key words: KLK, metastasis, ovarian cancer, prognosis, uPA/PAI-1

introduction

Every year, almost 22 000 women are newly diagnosed with epithelial ovarian carcinoma (ovarian cancer) in the United States, and >15 000 patients die of this disease. Established clinical prognostic factors in ovarian carcinoma are Fédération Internationale de Gynécologie et d'Obstétrique (FIGO) staging at the time of diagnosis, retroperitoneal nodal status (N), presence of residual tumor mass after primary surgery (R), histomorphological, and nuclear grade of the tumor (G), ascitic fluid volume, and further clinical parameters such as age and performance status. However, clinical prognostic factors currently do not play an important role in selecting chemotherapy regimens. Survival improvements during the last decades are mostly attributable to refined (and more radical) surgical techniques together with combination therapy [1]. Still, more than two-thirds of patients will experience disease recurrence and eventually die of their disease; less than half survive longer than 5 years [2, 3].

There is a substantial body of knowledge of metastatic processes in ovarian cancer at the molecular level, but translation of this knowledge to improved clinical care has lagged. In ovarian cancer patients, the proteolytic factor urokinase-type plasminogen activator (uPA) and its inhibitor PAI-1 have emerged as biomarkers of poor prognosis, indicating an elevated risk of the patient for early disease recurrence and relatively poor survival compared with patients with low levels of these proteolytic factors in their tumor tissue [4]. Furthermore, protein expression of uPA and PAI-1 is significantly elevated in omentum metastases of ovarian cancer FIGO stages III and IV patients compared with primary tumor tissue [5].

Concerning another, related protease family, the kallikrein-related peptidases (KLK), studies using northern blot, RT–PCR,

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and immunoassays have shown that the KLK4–8, 10, 11, and 13–15 are overexpressed in ovarian carcinoma tissues, serum, and/or cell lines.

The single most important known influence of treatment on survival of ovarian cancer patients is surgical success in eliminating residual tumor. A score predicting surgical success could support preoperative risk stratification and identify candidates for alternative therapeutic strategies. Such a score was developed by Dorn et al. [6], combining the clinical factors ascites volume and nuclear grade with primary tumor levels of KLK6 and 13. Considering the common occurrence of loco-regional metastasis in ovarian cancer, the relationship between omentum metastasis and primary tumor levels of biomarkers could supply further clues to the course of these processes.

The biological hypothesis behind our investigation is that tumor cells that are shed and migrate in metastatic processes typical of ovarian cancer might be preferentially associated with different levels of KLK, uPA, or PAI-1 and that individual differences between primary tumor tissues and omentum metastases might be associated with the degree of aggressiveness of these metastastic processes. Important clues in this direction can be seen in the differing levels of proteolytic factors according to disease stage [5]. In the current retrospective study, the aim is to quantify differences in protein levels of the proteolytic factors between primary tumor tissue and omentum metastasis and to explore the association of these differences with progression-free survival (PFS) and overall survival (OS). To this end, it is advantageous to investigate a collective of patients exhibiting a high degree of homogeneity with respect to disease stage and chemotherapy regimens.

patients and methods

patients

Radical tumor debulking surgery was carried out on 54 patients with advanced ovarian carcinoma (FIGO stage III–IV) between 1985 and 1999 at the Department of Obstetrics and Gynecology, Technical University of Munich, as previously described [6]. Following surgery, all patients received adjuvant platinum-containing chemotherapy treatment according to consensus recommendations at that time. None of the patients received neoadjuvant therapy.

tissue extraction and determination of protein content of proteolytic factors by enzymometric analyses (enzyme-linked immunosorbent assay)

Collection and extraction of ovarian cancer tissue specimens were carried out as previously described [7]. Protein levels of seven human KLK were quantified using highly sensitive and specific noncompetitive sandwich-type immunoassays [7]. Protein levels of uPA and PAI-1 were determined using the commercially available ELISA kits Imubind uPA (Product #894) and PAI-1 (Product #821) (American Diagnostica, Stamford, CT), respectively. Total protein content was measured by the BCA Protein Assay reagent kit (Pierce, Rockford, IL). Analyte measurements in extracts of both primary tissues and omentum metastases were expressed in nanogram per milligram protein; analyte levels below sensitivity limits were coded as zero.

statistical methods

Sample distributional characteristics of the proteolytic factors KLK5–8, 10, 11, 13, uPA, and PAI-1 in primary tumor tissue and omentum metastasis were tabulated; the distributions were tested for skewness. For each protease, the Spearman's correlation of protease levels in primary tumor versus omentum metastasis was computed, and for the paired measurements, the (two-sided) Wilcoxon signed-rank statistic was evaluated to test for an overall shift of central tendency.

Level differentials, defined as antigen level in omentum metastasis minus antigen level in primary tumor in units of nanogram per milligram protein, were calculated for each proteolytic factor and for each patient. For subsequent end point analysis, a level differential score (defined as fractional rank between 0 and 1) was also coded for each proteolytic factor.

A binary variable 'residual tumor' was coded as 0 if residual tumor ≤10 mm and as 1 otherwise. An ordinal variable 'ascites' was coded as follows: 2 (volume >500 ml), 1 (volume ≤500 ml), or 0 (no ascites volume data recorded).

End point variables were PFS, OS, presence of residual tumor >10 mm, and chemotherapy response. PFS was defined as time interval in months between surgery and either disease recurrence or distant metastasis; OS was defined as time interval in months between surgery and death.

Univariate Cox proportional hazards regression models for PFS and OS were estimated for all protease level differential scores. Protease level differential scores (fractional ranks) were treated as continuous variables; no cut-offs ('optimal' or otherwise) on level differentials were computed or used. The hazard ratio (HR) associated with each level differential score formally represents the risk of the highest (1.0) versus lowest (0.0) possible rank. The HR between any other pair of percentiles can be reconstructed from the HR (highest/lowest); e.g. the HR of the 75th compared with the 25th percentile is the square root of HR (highest/lowest). Multivariate Cox regression (forward selection) was carried out, including all protease level differential scores as well as the clinical variables 'residual tumor', 'ascites', and 'grade'.

Chemotherapy response was classified as progression, no change, partial remission, or complete remission. Chemotherapy response was considered as a binary variable, because only progression or no change actually occurred in this cohort.

The Mann–Whitney *U* test was applied for associations between continuous variables and binary variables. Logistic regression of the binary variable 'residual tumor' on ranked protease level differentials was also carried out to quantify the effect size as an unadjusted odds ratio in each case.

Analyses were carried out using SPSS (Version 16); *P*-values <0.05 were considered significant.

results

Table 1 summarizes clinical and histomorphological characteristics of this cohort. FIGO stages III and IV were represented, since the cohort involved only patients with omentum metastasis. Following surgery, 31/54 patients had residual tumor <10 mm; of these, 18 had no evidence of residual tumor mass. Median follow-up of patients still alive at time of analysis was 24.5 months.

Table 2 describes the population distribution characteristics of the proteases KLK5–8, 10, 11, 13, uPA, and PAI-1 in both primary tumor and corresponding omentum metastasis. The frequency distributions of the biomarkers both in primary tumor and omentum metastasis were significantly right-skewed (and thus departed strongly from normality), as is also apparent from the percentiles of the distributions. For each antigen, the Spearman's correlation (denoted *r*) of

measurements in primary tumor and omentum metastasis is reported in Table 2 as well. For all the proteases except KLK13, uPA, and PAI-1, a moderately strong, positive correlation between the two measurements was found (ranging from 0.49 to 0.69); the correlation for uPA was significant but weak.

Table 2 also reports the results of the Wilcoxon signed-rank test for the paired measurements of biomarker levels in primary tumor tissues versus omentum metastases, which was employed to test for a significant overall central tendency shift of the frequency distributions. Significant increases from primary

Table 1. Patient characteristics

N				54			
Median age (range)				58.5 (25–85) years			
Median observation time of patients alive (range)				24.5 (1–125) months			
		N	%			*N*	%
FIGO stage				Histological subtype			
	III	39	72.2		Serous	40	74.1
	IV	15	27.8		Mucinous	2	3.7
Nuclear grade					Endometrioid	3	5.6
	G1/G2	16	70.4		Undifferentiated	6	11.1
	G3	38	29.6		Others	3	5.6
Relapsed				Nodal status			
	No	20	37.0		Negative	13	24.1
	Yes	34	63.0		Positive	25	48.1
Deceased					No data	16	27.8
	No	18	33.3	Volume of ascitic fluid	No ascites	9	16.7
	Yes	36	66.7				
Residual tumor mass					≤500 ml	17	31.5
	0 cm	18	33.3		>500 ml	24	44.4
	>0 ≤ 10 mm	13	24.1		No data	4	7.4
	> 10 ≤ 20 mm	11	20.4	Response to chemotherapy			
	> 20 mm	8	14.8		Progression	7	13.0
	No data	4	7.4		No change	24	44.4
					No data	23	42.6

FIGO, Fédération Internationale de Gynécologie et d'Obstétrique.

Table 2. Population distribution characteristics of KLK5, 6, 7, 8, 10, 11, 13, uPA and PAI-1 in both primary tumor and omentum metastasis

An.	*N*	*r*	*P*	Loc.	Mean	SD	Min	25	Med	75	Max	Wilcoxon *P*
KLK5	49	0.69	<0.001	Pr	1.39	2.60	0	0	0.46	1.93	15.32	0.016
				Om	2.56	3.44	0	0	1.14	3.92	12.40	
KLK6	49	0.60	<0.001	Pr	19.7	33.2	0	1.9	9.7	19.5	185.0	0.18
				Om	17.6	20.3	0	1.6	13.1	25.9	98.7	
KLK7	49	0.60	<0.001	Pr	5.23	7.55	0	0.04	1.86	7.00	28.54	0.98
				Om	5.11	10.34	0	0.43	1.46	5.03	60.95	
KLK8	49	0.51	<0.001	Pr	23.4	43.3	0	1.4	8.3	22.2	212.0	0.60
				Om	15.6	24.0	0	0.76	6.0	20.7	117.0	
KLK10	49	0.59	<0.001	Pr	8.87	10.85	0	1.07	3.71	12.00	37.94	0.22
				Om	9.20	11.25	0	1.65	5.82	11.04	51.61	
KLK11	49	0.49	<0.001	Pr	4.48	8.99	0	0.05	1.16	4.61	48.86	0.07
				Om	2.38	4.03	0	0	0.94	2.61	20.48	
KLK13	49	0.26	0.08	Pr	0.30	0.81	0	0	0	0.14	3.81	0.21
				Om	1.33	6.28	0	0	0	0.40	42.57	
uPA	54	0.28	0.038	Pr	1.64	1.93	0.04	0.41	0.91	2.27	10.16	< 0.001
				Om	4.19	4.23	0.37	1.17	2.79	5.42	19.85	
PAI-1	54	0.24	0.08	Pr	24.2	30.2	0.14	6.8	15.7	25.4	163.4	0.001
				Om	49.0	50.8	0.91	15.4	29.2	68.8	262.1	

KLK, kallikrein-related peptidases, uPA, urokinase-type plasminogen activator; An, analyte; measurements in ng/mg protein; *r*, Spearman's correlation; Loc, location; SD, standard deviation; Pr, primary tumor tissue; Om, omentum metastasis; percentiles—min, minimum; 25, 25th percentile; med, median (50th percentile); 75, 75th percentile; max, maximum. *r* and Wilcoxon *P*-value are also shown (see 'statistical methods' section). Shaded *P*-values are significant.

tumor tissue to omentum metastasis were found for KLK5, uPA, and PAI-1. The corresponding increases in the median values were 148%, 207%, and 86%, respectively, and are shown in Figure 1. In the left panel of Table 3 (upper part), univariate impacts of level differentials on PFS are summarized for the cohort as a whole: larger level differentials of KLK5–7, 10, and uPA were significantly and strongly associated with disease progression. The reported HRs refer to the highest (1.0) versus lowest (0.0) fractional rank of each biomarker (see 'statistical methods' section). Level differentials of KLK11, 13, and PAI-1 were not statistically significant for PFS in the cohort as a whole.

Some of the observed univariate impact of level differentials on PFS could have been mediated by their possible association with surgical success. Indeed, the left panel of Table 3 (lower part) quantifies the (expected) significant impact of residual tumor mass on PFS in this cohort. In order to examine this issue more closely and identify the independent significance of level differentials, clinical factors (coding: see 'statistical methods' section) including residual tumor, ascitic fluid volume, and nuclear grade as well as primary antigen levels were entered into a first multivariate Cox block and subsequently level differentials were entered into a second Cox block. The resulting multivariate PFS model contains the following factors: ascites [HR = 2.45, 95% confidence interval (CI) 1.07–5.64], residual tumor (HR = 1.99, 95% CI 0.79–4.97), and fractionally ranked KLK10 level differential (HR = 0.21, 95% CI 0.05–0.94). It appears that in this small subcohort with complete data, a patient with known residual tumor status and ascites would have a more favorable prognosis if KLK10 level differential is higher than the same patient with a lower KLK10 level differential. However, the overall effect of KLK10 level differential is unfavorable (Table 3, left panel) for PFS, presumably due to the increased probability of residual tumor (Table 3, right panel).

In addition, the subcohort of patients with residual tumor ≤10 mm was analyzed separately by Cox regression analysis (right panel of Table 3) for PFS. In this subcohort, level differentials of KLK5–8, 10, and 11 had a significant and very strong impact on PFS, whereas those of KLK13, uPA, and PAI-1 did not. For all KLK with significant impact, larger differentials were associated with disease progression.

In univariate analysis (Table 4), positive level differentials of uPA and of KLK10 were also associated with poorer OS, as was presence of residual tumor. In multivariate analysis for OS, again only presence of residual tumor mass >10 mm remained significant (data not shown). None of the level differentials were significant for OS in the subcohort with residual tumor ≤10 mm (data not shown). The Mann–Whitney *U* test was carried out to test for associations between level differentials and residual tumor. To quantify the effect sizes of these associations in terms of unadjusted odds ratios, logistic regression of the binary variable 'residual tumor' on ranked protease level differentials was also carried out (Table 5): higher level differentials of KLK5–8, 10–11, and uPA were all significantly and strongly associated with residual tumor >10 mm, while level differentials of KLK13 and PAI-1 were not.

No significant association of the biomarker level differentials with response to first-line chemotherapy was seen (Mann–Whitney *U* test).

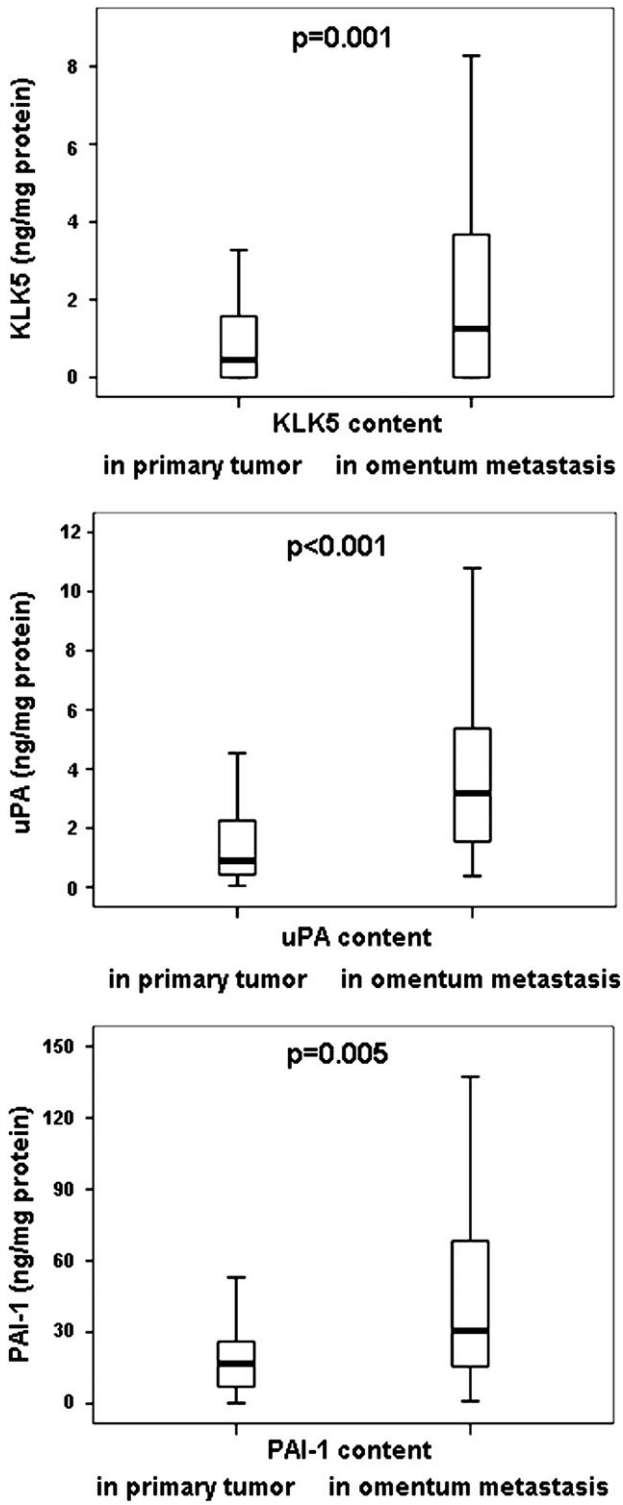

Figure 1. Box plots of antigen concentration in primary tumor tissue extracts and corresponding omentum metastases for kallikrein-related peptidases (KLK5), urokinase-type plasminogen activator (uPA) and PAI-1. The content of all of the analyzed proteolytic factors was higher in omentum metastases than in the primary tumor tissues (bottom boundary: 25th percentile, top boundary: 75th percentile, bars: minimum and maximum values without extremes, horizontal line: median values).

Table 3. Univariate impact of fractionally ranked biomarker level differentials (protein level in omentum minus level in primary tumor) and of residual tumor mass on PFS in the entire cohort and in the subcohort with residual tumor ≤10 mm

PFS	Entire cohort			Residual tumor ≤10 mm		
	P	HR[a]	95% CI	*P*	HR[a]	95% CI
Level differential of serine protease						
KLK5 (fractional rank)	0.045	3.86	1.03–14.4	0.038	26.56	1.21–584.7
KLK6 (fractional rank)	0.006	7.72	1.81–32.9	0.054	18.17	0.96–345.4
KLK7 (fractional rank)	0.033	3.83	1.11–13.1	0.010	98.02	2.97–3230
KLK8 (fractional rank)	n.s.			0.015	59.41	2.22–1592
KLK10 (fractional rank)	0.005	7.31	1.81–29.6	0.048	20.02	1.03–390.8
KLK11 (fractional rank)	n.s.			0.030	20.07	1.34–300.8
uPA (fractional rank)	0.004	4.91	1.67–14.4	n.s.		
Residual tumor						
Presence of residual tumor > 10mm	<0.001	4.64	2.03–10.6	Excluded		

[a]HR represents hazard ratio of highest (1.0) to lowest (0.0) fractional rank of each protease; HR of 75th compared with the 25th percentile is the square root of HR (highest/lowest). Level differentials of PAI-1 and KLK13 were not significant for PFS in the entire cohort or in the subcohort. PFS, progression-free survival; CI, confidence interval; KLK, kallikrein-related peptidases; uPA, urokinase-type plasminogen activator; n.s., not significant.

Table 4. Univariate impact of fractionally ranked level differentials (protein level in omentum minus level in primary tumor) and of residual tumor mass on OS in the entire cohort

OS	Entire cohort		
	P	HR[a]	95% CI
Level differential of serine protease			
uPA (fractional rank)	0.010	4.07	1.39–11.89
KLK10 (fractional rank)	0.047	3.84	1.02–14.49
Residual tumor			
Presence of residual tumor >10 mm	<0.001	7.24	2.72–19.25

[a]HR represents hazard ratio of highest (1.0) to lowest (0.0) fractional rank of each protease; HR of 75th compared with the 25th percentile is the square root of HR (highest/lowest). Level differentials of KLK6, 7, 8, 11, 13, and PAI-1 were not significant for overall survival (OS). None of the level differentials were significant for OS in the subcohort with residual tumor ≤10 mm. CI, confidence interval; KLK, kallikrein-related peptidases.

Table 5. Unadjusted odds ratios of fractionally ranked level differentials (protein level in omentum minus level in primary tumor tissue) on presence of residual tumor >10 mm

Level differential of serine protease	*P*	OR[a]	95% CI
KLK5 (fractional rank)	0.048	10.21	1.02–102.5
KLK6 (fractional rank)	0.020	16.58	1.56–176.3
KLK7 (fractional rank)	0.022	16.17	1.51–173.5
KLK8 (fractional rank)	0.015	20.83	1.80–240.8
KLK10 (fractional rank)	0.013	24.78	1.98–310.7
KLK11 (fractional rank)	0.040	12.13	1.12–130.9
uPA (fractional rank)	0.009	23.54	2.23–248.9

[a]OR represents unadjusted odds ratio of highest (1.0) to lowest (0.0) fractional rank of each protease; the OR of the 75th compared with the 25th percentile is the square root of the listed OR. Level differentials of PAI-1 and KLK13 were not significantly associated with this end point. CI, confidence interval; KLK, kallikrein-related peptidases; uPA, urokinase-type plasminogen activator.

discussion

Taken as a whole, the results of this study seem to support our biological hypothesis that level differentials of KLK5, 6, 7, 8, 10, 11, 13, the serine protease uPA, and its inhibitor PAI-1 between omentum metastasis and primary tumor may be associated with aggressiveness of metastatic processes in ovarian cancer.

The association of higher level differentials with disease aggressiveness manifested itself here in several ways: in poorer surgical success, in PFS, and in OS; patients with higher level differentials of the KLK5–8, 10, 11, and of the serine protease uPA tended to have increased residual tumor mass, which in turn could have mediated disease progression. Interestingly, a very strong impact of several KLK level differentials (KLK5–8, 10, 11) on PFS was also found in the subcohort with residual tumor mass ≤10 mm. Hence, these KLK level differentials characterized the situations in which surgical success did not translate into better PFS. In contrast, uPA level differentials had strong impact on residual tumor and on PFS in the cohort as a whole, but were not significant in the subcohort with residual tumor ≤10 mm. These preliminary results, if confirmed, suggest a clinical relevance in patients with high uPA level differentials but relatively low values of the relevant KLK level differentials: these patients would reap considerable PFS benefit from surgical success in removing as much tumor tissue as possible. Moreover, considering that uPA level differentials were not strongly correlated with those of the kallikreins and that the relevant kallikrein level differentials were well-correlated among themselves, this patient profile could comprise a reasonable proportion of FIGO stage III/IV ovarian cancer patients.

In essence, studying these level differentials may provide a window into the process of disease progression, owing perhaps to the characteristics of cells capable of migration to omentum tissue. In this picture, level differences mark this biological selection process; in fact, the current results indicate that several serine protease level differentials may quantify disease aggressiveness quite effectively.

In recent years, the human tissue KLK family as well as the serine protease uPA and its inhibitor PAI-1 have been identified as playing a key role in tumor invasion and metastasis within a complex proteolytic network. The finding that both uPA and PAI-1 are indicators of poor prognosis in patients experiencing cancer of the ovary or other organs is in contrast to the classical role of PAI-1 as an inhibitor blocking uPA enzymatic action. This surprising feature may be explained by the additional, multifunctional roles of uPA and PAI-1 in cell adherence, cell motility, cell signaling, and cell proliferation. In the case of the kallikreins, recent studies [7, 8] suggest that a complex network of interactions could coordinate activation and regulation of KLK activity. This suggests that it is important to monitor active enzymes as well as potential regulators.

Clinical findings have demonstrated that elevated tumor antigen levels of uPA and/or PAI-1 are conducive to tumor cell spread and metastasis and are associated with poor disease outcome in a variety of solid tumors [9–11]. In breast cancer, uPA and PAI-1 predict response to adjuvant chemotherapy [12], and their measurement in primary tumor tissue supports therapy decision making.

In ovarian cancer, uPA and PAI-1 are involved in tumor progression and have an impact on prognosis. Elevated levels of uPA and/or PAI-1 in plasma, cytosol, and/or ascitic fluid are associated with advanced or more aggressive disease and predict poor patient outcome in ovarian cancer. In a clinical study, uPA correlated with higher FIGO stage and shortened PFS and OS [5]. Several independent research groups have shown that there is a trend toward higher uPA and PAI-1 content from benign tissue to low-malignant-potential tumors and further to invasive ovarian cancer [13–16].

Elevated uPA and PAI-1 levels in omentum metastasis compared with primary tumor tissue have been previously reported [5, 13–15]; in the latter study, Schmalfeldt et al. [5] found in a group of 39 patients with advanced ovarian carcinoma that median uPA concentrations in omentum metastases were about four times higher than in primary tumors, while PAI-1 content increased twofold. However, the current study is the first to quantify the impact of level differentials of these serine proteases on outcome in ovarian cancer.

Several reports have previously indicated an association between deregulated KLK expression and ovarian cancer progression and the potential use of KLK as diagnostic/prognostic biomarkers or therapy targets for this type of cancer. KLK display diverse physiological functions, from the regulation of blood pressure to tissue remodeling, prohormone processing, neural plasticity, and skin desquamation [17]. Recent evidence suggests that KLK may be involved in cascade reactions and that cross-talk may exist with proteases of similar or different catalytic types, such as the plasminogen activation system [18]. KLK with dysregulated expression have been identified as potential markers in a variety of solid tumors. In addition to the established KLK1, 2, and 3, the novel KLK1–15 might also be related to hormonal malignancies such as that of the prostate, testis, breast, and ovary and could serve as new serum and/or tissue biomarkers [19, 20]. The mechanism by which KLK might be involved in the pathogenesis and/or progression of cancer is not yet fully understood, but preliminary reports indicate that several of the KLK participate early and via several cascade-like steps in neoplastic progression. KLK are thought to promote angiogenesis by modulating its activation, facilitating endothelial cell proliferation, migration and capillary-tube formation through direct or indirect extracellular matrix (ECM) degradation. *In vitro* KLK cleave structural components of the subendothelial basement membrane and ECM, and interact with the uPA system and matrix metalloproteinases [18, 21–23]. Angiogenesis may be inhibited by KLK3, 6, and 13 by generating angiostatin-like fragments from plasminogen [24]. Angiostatin is a potent inhibitor of endothelial cell proliferation and angiogenesis *in vivo* [19]. KLK could activate PAR signaling with a consequent stimulatory or inhibitory effect on tumor cell invasion [25].

Cancer metastasis includes cancer cell detachment from their original localization, migration, invasion into surrounding tissue and lymphatic vessels, and evasion to colonize at distant sites of the organism. Involved in this process are proteolytic enzymes like human KLKs, uPA, and PAI-1 [9]. Therefore, studying the levels of these biomarkers in metastatic tissue and in particular their level differentials compared with primary tumor tissue might shed light on their role in metastatic processes. Level differentials are thus not only potential markers of prognosis but also give insight in underlying pathways of metastasis. They have the potential to support clinical therapy decision making by helping to identify those patients who are unlikely to be free of residual tumor after primary radical surgery and thus would be candidates for alternative therapeutic approaches.

In view of the complex interactions of the KLK family and the plaminogen activator system, as well as the current study and previous results such as those of Dorn et al. [7], optimal risk assessment seems to require a multivariate approach taking into account not only individual proteolytic factors but rather a panel of biomarkers including KLK, uPA/PAI-1, clinical and histomorphological parameters, and possibly other tissue and serum cancer biomarkers [26–29].

In conclusion, this study has demonstrated for the first time a significant impact of level differentials (from primary tumor tissue to omentum metastasis) of certain KLK, uPA, and its inhibitor PAI-1 on surgical outcome, disease course, and ultimately survival in ovarian cancer. Further study of these biomarkers could help to improve our understanding of tumor progression and could aid in clinical decision making, including selection of patients for alternative therapeutic approaches such as combining conventional chemotherapy with substances targeting the kallikrein-like peptidase system or the plasminogen activator system.

disclosure

The authors declare no conflict of interest.

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