Am J Cancer Res 2018;8(9):1856-1864
www.ajcr.us /ISSN:2156-6976/ajcr0083469

Original Article

Elevated tumor tissue protein expression levels of kallikrein-related peptidases KLK10 and KLK11 are associated with a better prognosis in advanced high-grade serous ovarian cancer patients

Xiaocong Geng[1*], Yueyang Liu[1*], Tobias Dreyer[1], Holger Bronger[1], Enken Drecoll[2], Viktor Magdolen[1], Julia Dorn[1]

[1]Department of Obstetrics and Gynecology, [2]Institute of Pathology, Technische Universität München, Munich, Germany. [*]Equal contributors.

Received August 2, 2018; Accepted August 8, 2018; Epub September 1, 2018; Published September 15, 2018

Abstract: Several members of the KLK family have been proposed to modulate various tumor-relevant processes. Previously, we have shown that in advanced high-grade serous ovarian cancer tissue high KLK11 mRNA levels were significantly associated with prolonged overall and progression-free patients' survival. Furthermore, KLK11 mRNA expression positively correlated with KLK10 mRNA. In the present study, we examined the prognostic value for both KLK10 and KLK11 on the protein expression level by immunohistochemistry (IHC). A cohort encompassing 159 patient tumor samples afflicted with advanced high-grade (FIGO III/IV) serous ovarian cancer, present on tissue microarrays (TMA), was analyzed. For estimation of KLK10 and KLK11 immunoreactivity, an automated digital IHC image analysis algorithm was selected to quantify the antibody staining intensity in the tissues via an immunoreactive score (IRS). In line with the results obtained by mRNA analysis, KLK10 protein expression values were significantly and positively correlated with KLK11 protein expression values. In Kaplan-Meier analyses, both elevated KLK10, KLK11, and the combination of KLK10 and KLK11 protein levels were significantly linked with prolonged overall survival (OS). The addition of KLK10, KLK11 or the KLK10+KLK11 combination IRS to the base model in multivariate Cox analysis demonstrated that high KLK11 and KLK10+KLK11 protein expression levels, apart from clinical parameters, remained favorable independent predictive markers for OS. In conclusion, in the present study, we have validated the coordinate expression of KLK10 and KLK11 in advanced high-grade serous ovarian cancer. Furthermore, both increased KLK10 and KLK11 protein expression is associated with favorable prognosis in this major ovarian cancer subtype. The combined KLK10+KLK11 marker performed even stronger than KLK10 or KLK11 alone.

Keywords: Kallikrein related peptidases, KLK10, KLK11, immunohistochemistry, ovarian cancer

Introduction

Ovarian cancer is the most fatal gynecologic malignant tumor in women of the Western world. Lack of evident early symptoms usually results in late diagnosis, high rate of disease recurrence and inevitably following poor prognosis despite therapeutic efforts with high morbidity and toxicity [1]. Therefore, there is an urgent need to explore effective methods and biomarkers for the evaluation of prognosis and therefore individualization of therapeutic approaches.

The kallikrein-related peptidases (KLK) form a family of secreted serine proteases, encompassing fifteen members clustered on chromosome 19q13.4. Several members of the KLK family have been reported to be involved in ovarian cancer progression and metastasis [2]. Regarding their clinical impact, numerous studies suggested KLKs as prognostic biomarkers in various malignant diseases, including ovarian cancer. However, often contradictory results were reported during the past decade. One reasonable explanation for the discrepancies is that most of these analyses were carried out using heterogeneous patient cohorts, comprising different histological subtypes, which show distinct different molecular behaviors [3]. Therefore, in order to validate the relation between KLKs and tumor patients' survival, we perfor-

Table 1. Association between clinical characteristics of advanced high-grade serous ovarian cancer patients (FIGO III/IV) and tumor biological factors

Clinical parameters	KLK10[a] Low/high	KLK11[b] Low/high	KLK10+KLK11[c] Low/high
Age	P = 0.300	P = 0.328	P = 0.204
≤ 60 years	17/40	12/43	22/33
> 60 years	35/64	26/72	46/49
Residual tumor mass	**P = 0.025**	P = 0.519	**P = 0.037**
0 mm	20/58	19/59	29/47
> 0 mm	31/43	18/53	38/32
Ascitic fluid volume	**P = 0.034**	P = 0.522	P = 0.104
≤ 500 ml	24/64	22/65	36/51
> 500 ml	27/36	16/45	31/27

Chi-square test, significant *p*-values (P < 0.05) are indicated in bold. [a]Dichotomized into low and high levels by the 33th percentile. [b]Dichotomized into low and high levels by the 25th percentile. [c]Dichotomized into low level by KLK10 low and/or KLK11 low, and high level by KLK10 high and KLK11 high. Due to missing values, numbers do not always add up to n = 159.

med the present study cautiously concerning a single histologic subtype, advanced high-grade serous ovarian cancer, which encompasses about 70% of all malignant ovarian cancer cases.

Previously, we assessed the prognostic impact of KLK10 and KLK11 mRNA expression, also restricted to a homogenous patient cohort afflicted with advanced high-grade serous ovarian cancer (n = 139). Our study indicated that higher KLK11 mRNA levels in tumor tissue were significantly associated with prolonged overall and progression-free patients' survival. Furthermore, KLK11 mRNA expression positively correlated with KLK10 mRNA expression [4].

KLK10 is widely present in normal human organs, e.g. breast, prostate, thyroid, and testis and KLK11 is found at the highest level in the prostate, followed by stomach, trachea, and skin. Only weak expression of KLK10 and KLK11 exists in the ovary under physiological conditions compared to the strong expression of KLK10 and KLK11 observed in ovarian cancer [5]. High serum levels of KLK10 were reported to be significantly associated with late-stage high-grade serous ovarian tumors [6] and high serum levels of KLK11 were proposed to distinguish ovarian cancer cases from healthy tissue controls [7].

In view of previous findings, the present study aimed at validating the prognostic value of KLK10 and KLK11 in this major subtype of ovarian cancer on the protein level, and to explore its potential utility in the clinical setting. We assessed KLK10 and KLK11 protein expression levels in tumor tissues of a cohort encompassing 159 patients afflicted with exclusively advanced high-grade serous ovarian cancer by immunohistochemistry (IHC). Expression levels of KLK10 and KLK11 protein on tissue microarrays (TMA) were determined via an automated digital IHC image analysis algorithm allowing analysis of the associations of KLK10 and KLK11 protein expression levels with clinical parameters including overall survival time of patients.

Materials and methods

Patients

159 patients afflicted with advanced high-grade serous ovarian cancer (FIGO stage III/IV), treated between 1991 and 2014 at the Department of Obstetrics and Gynecology, Klinikum rechts der Isar, Technische Universität München, Germany were enrolled in the present retrospective study. The study was approved by the local Ethics Committee. Median patients' age at time of surgery was 65 years (range 33-88 years). All patients initially underwent standard stage-related primary radical debulking surgery. 80 patients (50.3%) were optimally debulked with complete removal of all macroscopically visible tumor manifestations. Following surgery, all of the patients received adjuvant treatment according to consensus recommendations at that time, including platinum-based chemotherapy. None of the patients received neoadjuvant therapy. Median time of follow-up was 29 months for overall survival (OS) (range: 1 to 270 months after primary tumor resection). Clinical factors documented at the time of surgery included histologic subtype, absence or presence of residual tumor mass (0 mm versus > 0 mm, defined as largest abdominal tumor diameter left after surgery) and ascitic fluid volume (≤ 500 ml versus > 500 ml, estimated preoperatively by vaginal ultrasound or intraoperatively) (**Table 1**). During the period of five years follow-up, 71 (44.7%) patients had died.

Immunohistochemistry (IHC)

Formalin-fixed, paraffin-embedded ovarian cancer tissue specimens were retrieved from the archives of the Institute of Pathology of the Technische Universität München, Munich, Germany. Production of the tissue microarrays has been described previously [8, 9]. Dewaxed and rehydrated tissue microarray sections were treated for antigen retrieval via pressure cooking in citrate buffer (pH 6.0, 4 min). After quenching endogenous peroxidase activity with 3% hydrogen peroxide (room temperature, 20 min), the sections were incubated overnight at 4°C with polyclonal rabbit antibodies directed to KLK10 (Sigma HPA017195, 1:200) and KLK11 (Abcam ab131038, 1:500). Interaction of antibodies with the KLK10 and KLK11 target protein was visualized using the polymer one step system based on a horseradish peroxidase-linked reporter assay. Nuclei were counterstained with hematoxylin.

Quantification of immunostaining

For image analysis, the free software ImageJ (Java 1.8.0, 64 bit) downloaded from the NIH website (https://imagej.nih.gov/ij), as well as the IHC Profiler plugin, downloaded from Sourceforge website (https://sourceforge.net/projects/ihcprofiler/) were used. The NDP.View2 scanning system was used to scan the whole stained slides for capturing RGB digital images. Representative images of each tumor tissue core on TMAs were collected and loaded into the ImageJ platform. Using the IHC Profiler plugin for color deconvolution leads to the separation of the antibody (either KLK10 or KLK11) DAB signal from the complimentary image as well as the hematoxylin counterstain. The detailed guidelines and theories pertaining to the use of the plugin have been described by Varghese and co-workers before [10]. The analysis pattern for cytoplasmic proteins with DAB signal was selected, defining the software's threshold and scale settings. Diagnosis of the relevant ovarian tumor cell areas on the TMA cores were identified by consensus of two independent observers. The formula below was used for score assignment of the DAB images.

$$Score = 255 - \sum_{i=1}^{n} InD_i \Big/ \sum_{i=1}^{n} A_i$$

According to the Lambert-Beer law the optical density (OD) is in direct proportion to the staining intensity [11], therefore the optical density of DAB can be used as a surrogate for the antibody signal of tumor cells. InD is defined as the integrated gray density of the specimen transmitting light and A is the computed pixel area.

After calculating the quotient of total gray density (InD) and total pixel area (A), a continuous variable representing the mean staining intensity of the KLKs can be generated ranging from 0 to 255 (0 = darkest color, 255 = brightest color). In order to assign high scores to high staining intensities, the quotient was subtracted from the maximum signal (255).

Statistical analyses

The associations of biological marker expression levels with clinical parameters were evaluated using the Chi-square test. Correlations between continuous variables of tumor biological markers were calculated using the Mann-Whitney U test. For survival analyses, overall survival (OS) of ovarian cancer patients was used as follow-up end points. Associations of tumor biological factors and clinical parameters with patients' survival were analyzed by Cox univariate and multivariate proportional hazards regression models and expressed as hazard ratio (HR) and its 95% confidence interval (95% CI). For the statistical analyses, the observation period was restricted to 60 months. The multivariate Cox regression model was adjusted for established ovarian cancer factors such as age, absence or presence of residual tumor mass, and preoperative ascitic fluid volume. Survival curves were plotted according to Kaplan-Meier, using log-rank tests to test for differences. All statistical analyses were performed with the SPSS statistical analysis software (version 20.0; SPSS Inc., Chicago, IL, USA). *P*-values ≤ 0.05 were considered statistically significant.

Results

KLK10 and KLK11 protein expression patterns in advanced high-grade serous ovarian cancer tumor tissue and their relation to patients' tumor characteristics

We analyzed the clinical impact of KLK10 and KLK11 protein expression in tumor tissue by IHC in 159 patients afflicted with advanced high-grade serous ovarian cancer (FIGO III/IV).

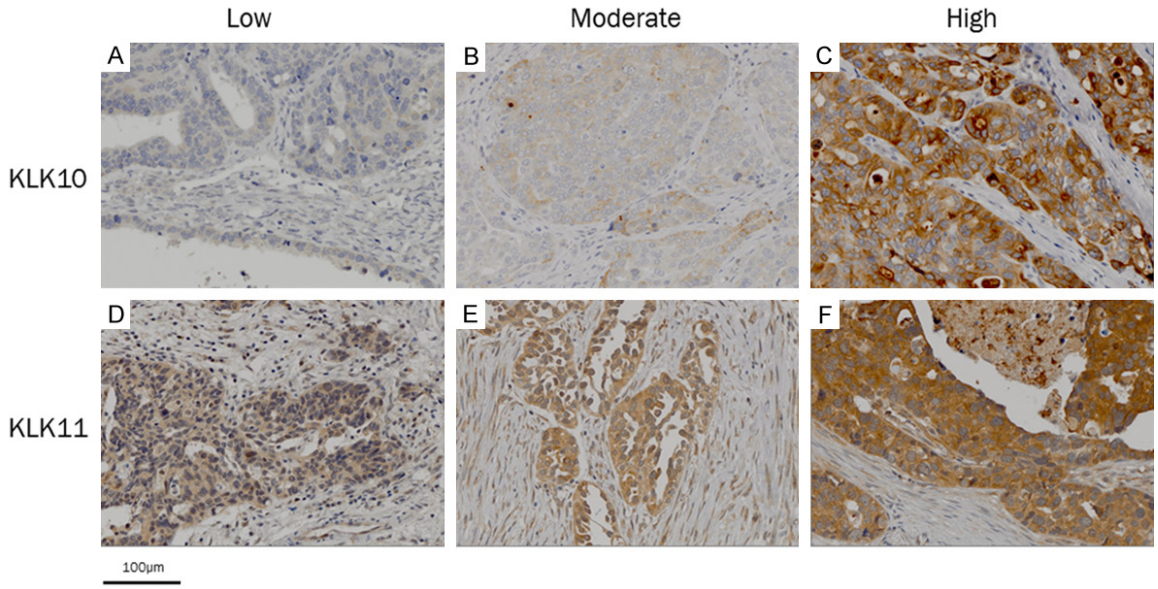

Figure 1. KLK10 and KLK11 immunoexpression in tumor tissue of advanced high-grade serous ovarian cancer patients (FIGO III/IV) specimens. Tissue sections were stained with polyclonal rabbit antibodies directed to KLK10 (Sigma HPA017195) and KLK11 (Abcam ab131038), respectively, applying the polymer one step system based on a horseradish peroxidase-linked reporter assay. Micrographs (A-F) illustrate representative core punches corresponding to low, moderate, and high KLK10 and KLK11 immunoexpression in tumor cells, respectively.

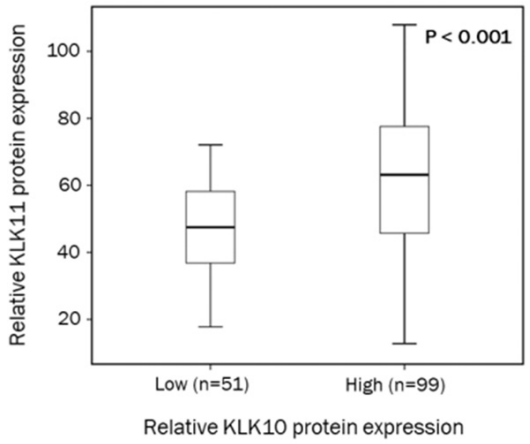

Figure 2. Correlation of KLK10 and KLK11 protein expression in tumor specimens of advanced high-grade serous ovarian cancer (FIGO III/IV) patients. Immunoreactive scores (IRS) were digitally determined applying the assessment tool ImageJ [10]. The scores were dichotomized into low and high protein expression groups by the 33th percentile for KLK10. KLK10 protein expression levels were significantly correlated with KLK11 protein expression levels in tumor tissue (Mann-Whitney test; P < 0.001).

IHC was performed on formalin-fixed, paraffin-embedded tumor tissue microarrays employing KLK10- and KLK11-specific antibodies, following an optimized established staining protocol. We evaluated the expression pattern focusing on tumor cells applying a relative quantitative immunoreactivity score (IRS) based on an automated digital algorithm as described in the Materials & Methods section.

For both KLK10 and KLK11, in the tumor specimens a differential staining pattern of tumor cells with varying intensities from case to case was observed. Tumor cells displayed robust cytoplasmic protein expression, but stromal staining of KLK10 and KLK11 was also present in the extracellular matrix (**Figure 1**). Overall, the IRS values for KLK11 were distinctly higher as those for KLK10. Negative/very low expression (defined by IRS values < 40) was observed in 39.1% of all cases for KLK10 (61 out of 156 cases), whereas all cases (100%) displayed an IRS > 40 for KLK11 (*153* cases). KLK10 IRS values range from 29.64 to 121.51 (median = 42.52), KLK11 IRS values range from 40.96 to 159.92 (median = 96.29), respectively. KLK11, but not KLK10, was also expressed in endothelial and highly inflammatory stroma cells, e.g. fibroblasts and immune cells.

Based on the observed expression pattern of the analyzed KLKs, we categorized the protein expression levels into low versus high groups by the 33th percentile for KLK10, and 25th percentile for KLK11. The relationship between

Table 2. Univariate Cox regression analysis of clinical outcome (overall survival) in advanced high-grade serous ovarian cancer patients (FIGO III/IV) with respect to clinical parameters and the tumor biological factors KLK10, KLK11 and their combination

Clinical parameters	OS		
	No[a]	HR (95% CI)[b]	P
Age			0.337
≤ 60 years	51	1	
> 60 years	93	1.28 (0.78-2.09)	
Residual tumor mass			**< 0.001**
0 mm	72	1	
> 0 mm	68	4.10 (2.38-7.06)	
Ascitic fluid volume			**0.016**
≤ 500 ml	81	1	
> 500 ml	58	1.80 (1.12-2.90)	
KLK10 IRS[c]			**0.035**
Low	48	1	
High	94	0.60 (0.37-0.96)	
KLK11 IRS[d]			**0.024**
Low	36	1	
High	105	0.56 (0.34-0.93)	
KLK10 IRS+KLK11 IRS[e]			**0.001**
Low	64	1	
High	75	0.43 (0.26-0.70)	

Significant *p*-values (*P* < 0.05) are indicated in bold. [a]Number of patients. [b]HR: hazard ratio (CI: confidence interval) of univariate Cox regression analysis. [c]IRS: immunoreactive score, dichotomized into low and high levels by the 33th percentile. [d]Dichotomized into low and high levels by the 25th percentile. [e]Dichotomized into low level by KLK10 low and/or KLK11 low, and high level by KLK10 high and KLK11 high. Due to missing values, numbers do not always add up to n = 144.

low versus high expression levels of these two KLKs is evident in box plot analysis (Mann-Whitney, P < 0.001, **Figure 2**). Based on the observed co-expression pattern of KLK10 and KLK11, we further categorized the protein expression levels into a KLK10 and/or KLK11 low-expressing group (i.e. KLK10 values below the 33th percentile and/or KLK11 values below the 25th percentile) versus a high-expressing group (both, KLK10 values above the 33th percentile and KLK11 values above the 25th percentile) for statistical analyses.

Table 1 depicts the association between the dichotomized biological factors (KLK10, KLK-11, and KLK10+KLK11) and the established clinical parameters in ovarian cancer including age, preoperative ascitic fluid volume, and postoperative residual tumor mass. KLK11 expression values do not differ significantly in relation to these clinical parameters, whereas KLK10 expression values are associated with patients' residual tumor mass (P = 0.025) and ascitic fluid volume (P = 0.034). The combined factor KLK10+KLK11 is significantly related to residual tumor mass (P = 0.037).

Association of clinical parameters and KLKs biological factors with overall survival (OS)

The impact of traditional clinical parameters and KLK protein expression levels on patients' five-years OS was analyzed by univariate Cox regression analysis and is summarized in **Table 2**. Among the clinical factors, as expected, residual tumor mass left after debulking surgery and high preoperative amounts of ascitic fluid indicated significantly shorter OS. Elevated KLK10 and KLK11 protein expression levels were found to be significant predictive factors for longer OS (HR = 0.60, 95% CI = 0.37-0.96, P = 0.035; HR = 0.56, 95% CI = 0.34-0.93, P = 0.024), displaying an about two-fold decreased probability of death in both the high KLK10 and high KLK11 expressing group. Interestingly, high KLK10+KLK11 values performed as an even more pronounced excellent favorable predictive marker for OS (HR = 0.43, 95% CI = 0.26-0.70, P = 0.001) indeed pointing to a co-operative function of KLK10 and KLK11. The findings are visualized by the respective Kaplan-Meier survival curves in **Figure 3**.

The independence of the prognostic value for OS of the KLKs was studied by multivariate Cox hazard regression analysis, including the factors age, ascitic fluid volume, and presence of residual tumor mass (base model) (**Table 3**). In the base model, residual tumor mass was the only clinical parameter representing a predictive marker for OS (HR = 3.58, 95% CI = 1.99-6.43, P < 0.001), while the pre-operative ascitic fluid volume lost its prognostic significance for OS when adjusted to multivariate analysis. Among the tumor biological factors (added separately to the base model), KLK10 lost its prognostic significance, whereas KLK11 values significantly contributed to the base model for OS (HR = 0.53, 95% CI = 0.30-0.92, P = 0.023).

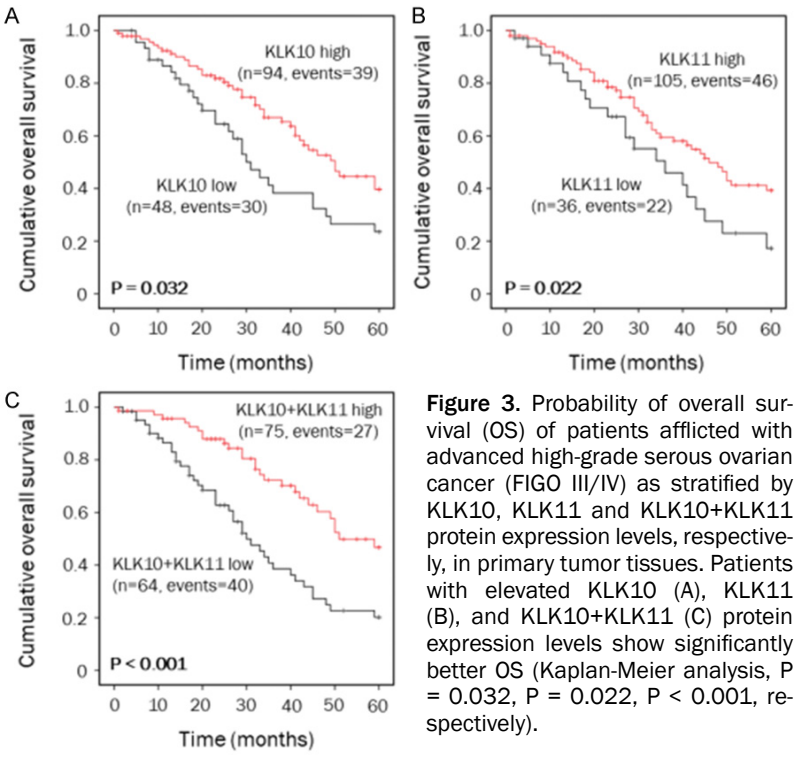

Figure 3. Probability of overall survival (OS) of patients afflicted with advanced high-grade serous ovarian cancer (FIGO III/IV) as stratified by KLK10, KLK11 and KLK10+KLK11 protein expression levels, respectively, in primary tumor tissues. Patients with elevated KLK10 (A), KLK11 (B), and KLK10+KLK11 (C) protein expression levels show significantly better OS (Kaplan-Meier analysis, P = 0.032, P = 0.022, P < 0.001, respectively).

Finally, the KLK10+KLK11 combination remains to be independently significant for OS (HR = 0.44, 95% CI = 0.26-0.75, P = 0.002) as well (**Table 3**).

Discussion

In the present study, we examined a homogeneous cohort of 159 patients afflicted with advanced high-grade serous ovarian cancer (FIGO III/IV). The protein expression levels of KLK10 and KLK11 were investigated by IHC on a collection of tumor tissue microarrays using an automated algorithm. The prognostic values of KLK10, KLK11 protein expression levels and their combination for patient clinical outcome were estimated by univariate and multivariate survival analyses.

Assessment of protein expression by immunohistochemistry allows recognition of the protein localization and furthermore can be easily used in clinical practice. Recently, automated scoring systems have been suggested because of their advantages over the manual ones, including no variability through different observers, efficient manipulation discerning minute differences, which might be overseen by eye, and objectively stable results based on computer calculations. Previous studies supported the digital utilities in IHC staining. As an example, Kolin and co-workers [12] have reported that KLK11 protein expression represents an unfavorable prognostic marker for gastric cancer when determined in TMA tissues applying an optimized algorithm. The digital algorithm used in the present study, which combines the IHC Profiler plugin with the publicly available software ImageJ, was originally described by Varghese et al [10]. Herein, we first implemented this algorithm for the protein analysis in TMA tissues of ovarian cancer patients and established a simple numeric formula to calculate the continuous variable for statistical analyses. Still, the challenge of applying automated algorithms in clinical routine remains an issue due to the lack of universally recognized platforms and principles.

Previously, we reported that KLK11 mRNA expression levels in tumor tissue positively correlated with KLK10 mRNA expression levels (r_s = 0.647, P < 0.001) [4]. Consistently, low (high) KLK10 protein expression levels were significantly associated with low (high) KLK11 protein expression levels (Mann-Whitney, P < 0.001) in the present study. Interestingly, a KLK10 and KLK11 co-expression pattern has been observed also in non-small-cell lung as well as breast cancer [13, 14]. The significant positive correlation indicates that a similar regulatory mechanism might underlie gene expression and secretion of both proteins. In fact, a common steroid hormone (especially estrogen and androgen)-dependent mechanism was proposed for coordinated gene expression of kallikrein-related peptidases [15, 16].

In the present study, we demonstrate that both high KLK10 and KLK11 protein expression levels as well as their combination are significantly associated with an increased five year-OS in univariate survival analysis. Furthermore, in multivariable Cox regression analysis, KLK-11 and KLK10+KLK11 remained independent

Table 3. Multivariate Cox regression analysis of clinical outcome (overall survival) in advanced high-grade serous ovarian cancer patients (FIGO III/IV) with respect to clinical parameters and the tumor biological factors

Clinical parameters	OS		
	No[a]	HR (95% CI)[b]	P
Age			0.964
≤ 60 years	49	1	
> 60 years	82	1.01 (0.60-1.71)	
Residual tumor mass			**< 0.001**
0 mm	68	1	
> 0 mm	63	3.58 (1.99-6.43)	
Ascitic fluid volume			0.749
≤ 500 ml	78	1	
> 500 ml	53	1.09 (0.65-1.84)	
KLK10 IRS[c]			0.088
Low	45	1	
High	86	0.64 (0.38-1.07)	
KLK11 IRS[d]			**0.023**
Low	35	1	
High	96	0.53 (0.30-0.92)	
KLK10 IRS+KLK11 IRS[e]			**0.002**
Low	62	1	
High	69	0.44 (0.26-0.75)	

Biological markers were added separately to the base model of clinical parameters age, residual tumor mass, and ascitic fluid volume. Significant *p*-values ($P < 0.05$) are indicated in bold. [a]Number of patients. [b]HR: hazard ratio (CI: confidence interval) of multivariate Cox regression analysis. [c]Dichotomized into low and high levels by the 33th percentile. [d]Dichotomized into low and high levels by the 25th percentile. [e]Dichotomized into low level by KLK10 low and/or KLK11 low, and high level by KLK10 high and KLK11 high.

favorable markers (P = 0.023; P = 0.002), whereas KLK10 showed a trend towards significance only (P = 0.088). Several other studies explored the prognostic value of KLK10 and KLK11, respectively, in ovarian cancer patients. Similar to what we found, Borgoño et al [17] reported a favorable prognostic impact of elevated KLK11 protein levels in tumor tissues of ovarian cancer patients. It should be noted, however, that there were controversial reports describing both KLK10 [18, 19] and KLK11 [20] as unfavorable prognostic markers in ovarian cancer. As we discussed before [4], these results, at least in part, could be explained by the use of rather inhomogeneous patient cohorts encompassing different histological and molecular ovarian cancer sub-types, whereas in the present study, a homogeneous cohort encompassing only advanced high-grade serous ovarian cancer (FIGO III/IV) patients was analyzed.

Except the survival outcome, adoption of KLK10 and KLK11 as favorable indicators was reported for evaluating other clinical parameters in malignancy diseases. Positive KLK11 protein expression in tumor tissues of gastric cancer patients indicated a higher sensitivity to chemoradiotherapy, leading to significantly better prognosis [21]. In colorectal cancer, researchers incorporated KLK11 into a predictive genetic model for estimating response sensitivity to FOLFOX4 chemotherapy in patients with synchronous liver metastasis [22]. The combination of the urinary levels of KLK10 (uKLK10) with tumor location and tumor size allowed distinguishing operable gastric cancer patients from inoperable ones [23]. An immunopeptidomic landscape analysis of epithelial ovarian cancers (EOCs) unveiled KLK10 as one of the most presented antigens by HLA class I molecule exclusively on ovarian cancer cells. This study demonstrated that KLK10, capable of exposing itself to immune cells as an epitope in EOC, showed possible capacity for further testing as pharmacological target for immunotherapy [24].

Possible explanations of the favorably biological role of KLK10 and KLK11 in malignancies have been explored. Pépin and coworkers [25] found that enhancement of KLK10 expression in the ovarian cancer cell line ES-2 was sufficient to significantly reduce the ability of the tumor cells to form colonies. Moreover, mice injected with ES-2 clones overexpressing KLK10 significantly survived longer as compared to the control group. Elevated KLK10 expression was found to decelerate proliferation of SGC-7901 gastric cancer cells [26]. Furthermore, overexpression of KLK-10 in PC3 prostate cancer cell did not only suppress proliferation, but also glucose metabolism accompanied with an increasing apoptosis rate by regulating expression of Bcl-2 and HK-2 [27]. Increased KLK11 mRNA levels in laryngeal cancer may not only inhibit tumor growth but also angiogenesis [28]. Taken together, it is tempting to speculate that KLK10 and KLK11 might play an inhibitory role in ovarian cancer progression by delaying several neoplastic process. However, the knowledge of precise biochemical mechanisms remains limited.

All KLKs are secreted as inactive zymogens, requiring activation via proteolytic removal of a pro-peptide [29]. In addition to protease cascades formed by the KLKs themselves, they are simultaneously implicated and activated in other proteolytic networks, interacting with crucial proteases like MMPs, meprins and urokinase plasminogen activator (uPA) [2, 30]. Therefore, it could be worth trying to support the impact of KLK10 and KLK11 in advanced serous ovarian cancer by regulating their stimulators, i.e. the pro-KLK10- and pro-KLK11-cleaving proteases. Although it is not yet possible to speculate on the potential of KLK10 and KLK11 as favorable pharmaceutical target in ovarian cancer, it is still reasonable that KLK10 and KLK11 may perform as promising markers for prognosis in advanced ovarian cancer to adjust, individualize, or decrease therapy to spare patients from invasive therapy.

In conclusion, we established a protocol for IHC analysis of KLK10 and KLK11 in the tumor tissue of advanced high-grade serous ovarian cancer (FIGO III/IV) patients applying an automated digital algorithm. We show that KLK10 and KLK11 are coordinately expressed on the protein level, which was previously observed on the mRNA level as well. Both high KLK10 and high KLK11 protein expression levels can be considered as favorable prognostic factors for patients' OS. While KLK11 was found to be independently associated with OS, the use of KLK10 in combination with KLK11 allowed a better identification of patients with favorable prognosis.

Acknowledgements

The present study was supported in part by grants from the German Research Foundation (DFG), DO 1772/1-1, AV 109/4-1, BR4733/3-1 and MA1236/10-1, respectively, and the DFG and the Technical University of Munich (TUM) in the framework of the Open Access Publishing Program.

Disclosure of conflict of interest

None.

Address correspondence to: Viktor Magdolen, Department of Obstetrics and Gynecology, Technische Universität München, Munich, Germany. E-mail: viktor.magdolen@tum.de

References

[1] Jayson GC, Kohn EC, Kitchener HC, Ledermann JA. Ovarian cancer. Lancet 2014; 384: 1376-88.

[2] Dorn J, Beaufort N, Schmitt M, Diamandis EP, Goettig P, Magdolen V. Function and clinical relevance of kallikrein-related peptidases and other serine proteases in gynecological cancers. Crit Rev Clin Lab Sci 2014; 51: 63-84.

[3] Kurman RJ, Shih Ie M. The dualistic model of ovarian carcinogenesis: revisited, revised, and expanded. Am J Pathol 2016; 186: 733-47.

[4] Geng X, Liu Y, Diersch S, Kotzsch M, Grill S, Weichert W, Kiechle M, Magdolen V, Dorn J. Clinical relevance of kallikrein-related peptidase 9, 10, 11, and 15 mRNA expression in advanced high-grade serous ovarian cancer. PLoS One 2017; 12: e0186847.

[5] Uhlén M, Fagerberg L, Hallström BM, Lindskog C, Oksvold P, Mardinoglu A, Sivertsson A, Kampf C, Sjostedt E, Asplund A, Olsson I, Edlund K, Lundberg E, Navani S, Szigyarto CA, Odeberg J, Djureinovic D, Takanen JO, Hober S, Alm T, Edqvist PH, Berling H, Tegel H, Mulder J, Rockberg J, Nilsson P, Schwenk JM, Hamsten M, von Feilitzen K, Forsberg M, Persson L, Johansson F, Zwahlen M, von Heijne G, Nielsen J, Ponten F. Tissue-based map of the human proteome. Science 2015; 347: 1260419.

[6] Luo LY, Yousef G, Diamandis EP. Human tissue kallikreins and testicular cancer. APMIS 2003; 111: 225-32; discussion 32-3.

[7] McIntosh MW, Liu Y, Drescher C, Urban N, Diamandis EP. Validation and characterization of human kallikrein 11 as a serum marker for diagnosis of ovarian carcinoma. Clin Cancer Res 2007; 13: 4422-8.

[8] Seiz L, Dorn J, Kotzsch M, Walch A, Grebenchtchikov NI, Gkazepis A, Schmalfeldt B, Kiechle M, Bayani J, Diamandis EP, Langer R, Sweep FC, Schmitt M, Magdolen V. Stromal cell-associated expression of kallikrein-related peptidase 6 (KLK6) indicates poor prognosis of ovarian cancer patients. Biol Chem 2012; 393: 391-401.

[9] Dorn J, Gkazepis A, Kotzsch M, Kremer M, Propping C, Mayer K, Mengele K, Diamandis EP, Kiechle M, Magdolen V, Schmitt M. Clinical value of protein expression of kallikrein-related peptidase 7 (KLK7) in ovarian cancer. Biol Chem 2014; 395: 95-107.

[10] Varghese F, Bukhari AB, Malhotra R, De A. IHC Profiler: an open source plugin for the quantitative evaluation and automated scoring of immunohistochemistry images of human tissue samples. PLoS One 2014; 9: e96801.

[11] Commoner B, Lipkin D. The application of the beer-lambert law to optically anisotropic systems. Science 1949; 110: 41-3.

[12] Kolin DL, Sy K, Rotondo F, Bassily MN, Kovacs K, Brezden-Masley C, Streutker CJ, Yousef GM. Prognostic significance of human tissue kallikrein-related peptidases 11 and 15 in gastric cancer. Tumour Biol 2016; 37: 437-46.
[13] Planque C, Ainciburu M, Heuze-Vourc'h N, Regina S, de Monte M, Courty Y. Expression of the human kallikrein genes 10 (KLK10) and 11 (KLK11) in cancerous and non-cancerous lung tissues. Biol Chem 2006; 387: 783-8.
[14] Yousef GM, Yacoub GM, Polymeris ME, Popalis C, Soosaipillai A, Diamandis EP. Kallikrein gene downregulation in breast cancer. Br J Cancer 2004; 90: 167-72.
[15] Kroon E, MacDonald RJ, Hammer RE. The transcriptional regulatory strategy of the rat tissue kallikrein gene family. Genes Funct 1997; 1: 309-19.
[16] Lawrence MG, Lai J, Clements JA. Kallikreins on steroids: structure, function, and hormonal regulation of prostate-specific antigen and the extended kallikrein locus. Endocr Rev 2010; 31: 407-46.
[17] Borgoño CA, Fracchioli S, Yousef GM, Rigault de la Longrais IA, Luo LY, Soosaipillai A, Puopolo M, Grass L, Scorilas A, Diamandis EP, Katsaros D. Favorable prognostic value of tissue human kallikrein 11 (hK11) in patients with ovarian carcinoma. Int J Cancer 2003; 106: 605-10.
[18] Luo LY, Katsaros D, Scorilas A, Fracchioli S, Piccinno R, Rigault de la Longrais IA, Howarth DJ, Diamandis EP. Prognostic value of human kallikrein 10 expression in epithelial ovarian carcinoma. Clin Cancer Res 2001; 7: 2372-9.
[19] Luo LY, Katsaros D, Scorilas A, Fracchioli S, Bellino R, van Gramberen M, de Bruijn H, Henrik A, Stenman UH, Massobrio M, van der Zee AG, Vergote I, Diamandis EP. The serum concentration of human kallikrein 10 represents a novel biomarker for ovarian cancer diagnosis and prognosis. Cancer Res 2003; 63: 807-11.
[20] Shigemasa K, Gu L, Tanimoto H, O'Brien TJ, Ohama K. Human kallikrein gene 11 (KLK11) mRNA overexpression is associated with poor prognosis in patients with epithelial ovarian cancer. Clin Cancer Res 2004; 10: 2766-70.
[21] Unal D, Tasdemir A, Oguz A, Eroglu C, Cihan YB, Turak EE, Karaman H, Soyuer S. Is human kallikrein-11 in gastric cancer treated with surgery and adjuvant chemoradiotherapy associated with survival? Pathol Res Pract 2013; 209: 779-83.
[22] Lu X, Pan J, Li S, Shen S, Chi P, Lin H, Huang Y, Xu Z, Huang S. Establishment of a predictive genetic model for estimating chemotherapy sensitivity of colorectal cancer with synchronous liver metastasis. Cancer Biother Radiopharm 2013; 28: 552-8.
[23] Shimura T, Ebi M, Yamada T, Yamada T, Katano T, Nojiri Y, Iwasaki H, Nomura S, Hayashi N, Mori Y, Kataoka H, Moses MA, Joh T. Urinary kallikrein 10 predicts the incurability of gastric cancer. Oncotarget 2017; 8: 29247-57.
[24] Schuster H, Peper JK, Bosmuller HC, Rohle K, Backert L, Bilich T, Ney B, Loffler MW, Kowalewski DJ, Trautwein N, Rabsteyn A, Engler T, Braun S, Haen SP, Walz JS, Schmid-Horch B, Brucker SY, Wallwiener D, Kohlbacher O, Fend F, Rammensee HG, Stevanovic S, Staebler A, Wagner P. The immunopeptidomic landscape of ovarian carcinomas. Proc Natl Acad Sci U S A 2017; 114: E9942-E9951.
[25] Pépin D, Shao ZQ, Huppe G, Wakefield A, Chu CW, Sharif Z, Vanderhyden BC. Kallikreins 5, 6 and 10 differentially alter pathophysiology and overall survival in an ovarian cancer xenograft model. PLoS One 2011; 6: e26075.
[26] Huang W, Tian XL, Wu YL, Zhong J, Yu LF, Hu SP, Li B. Suppression of gastric cancer growth by baculovirus vector-mediated transfer of normal epithelial cell specific-1 gene. World J Gastroenterol 2008; 14: 5810-5.
[27] Hu J, Lei H, Fei X, Liang S, Xu H, Qin D, Wang Y, Wu Y, Li B. NES1/KLK10 gene represses proliferation, enhances apoptosis and down-regulates glucose metabolism of PC3 prostate cancer cells. Sci Rep 2015; 5: 17426.
[28] Patsis C, Yiotakis I, Scorilas A. Diagnostic and prognostic significance of human kallikrein 11 (KLK11) mRNA expression levels in patients with laryngeal cancer. Clin Biochem 2012; 45: 623-30.
[29] Goettig P, Magdolen V, Brandstetter H. Natural and synthetic inhibitors of kallikrein13 related peptidases (KLKs). Biochimie 2010; 92: 1546-67.
[30] Beaufort N, Plaza K, Utzschneider D, Schwarz A, Burkhart JM, Creutzburg S, Debela M, Schmitt M, Ries C, Magdolen V. Interdependence of kallikrein-related peptidases in proteolytic networks. Biol Chem 2010; 391: 581-7.