Biol. Chem., Vol. 389, pp. 781–786, June 2008 • Copyright © by Walter de Gruyter • Berlin • New York. DOI 10.1515/BC.2008.089

Short Communication

Quantitative RT-PCR analysis and immunohistochemical localization of the kallikrein-related peptidases 13 and 14 in lung

Chris Planque[1,2,3], Claire Bléchet[1,2], Aida Ayadi-Kaddour[4], Nathalie Heuzé-Vourc'h[1,2], Pascal Dumont[5], Serge Guyétant[1,2], Eleftherios P. Diamandis[3], Faouzi El Mezni[4] and Yves Courty[1,2,*]

[1] INSERM U618 'Protéases et Vectorisation Pulmonaires', Faculté de Médecine, F-37000 Tours, France
[2] IFR 135 'Imagerie Fonctionnelle', Faculté de Médecine, F-37000 Tours, France
[3] Mount Sinai Hospital and Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto M5T 3L9, Ontario, Canada
[4] UR du Poumon et du Médiastin, Hôpital A. Mami de l'Ariana, 2080 Ariana, Tunis, Tunisia
[5] Service de Chirurgie Thoracique, CHRU de Tours, F-37000 Tours, France

* Corresponding author
e-mail: courty@univ-tours.fr

Abstract

Expression of the *KLK13* and *KLK14* genes was examined at the mRNA and protein levels in a cohort of 57 patients with non-small-cell lung cancer (NSCLC). The mRNA levels, assessed by real-time RT-PCR, were significantly different in malignant tissues compared to adjacent non-malignant tissues (*KLK13*, $p=0.006$; *KLK14*, $p=0.022$). *KLK13* and *KLK14* mRNA overexpression in tumors (1/3 of the patients) was associated with a positive nodal status in multivariate analysis ($p=0.018$ and $p=0.069$, respectively). KLK13 and KLK14 were localized in the cytoplasm of epithelial cells of normal bronchus and NSCLC, as determined by immunohistochemistry. Moreover, positive staining was significantly associated with adenocarcinoma histotype (KLK13, $p=0.014$) and tumor size (KLK14, $p=0.048$). Although the results are marginally significant, patients with high KLK13 expression at the mRNA or protein level had lower overall survival.

Keywords: kallikrein-related peptidases; kallikreins; lung cancer; real-time RT-PCR; serine proteases; tissue microarray.

The human tissue kallikrein-related peptidase gene family (*KLK*) is a group of 15 closely related genes, located in tandem on chromosome 19 (q13.4). Kallikrein genes range between 4.4 and 10.5 kb in length and share common features, including exon/intron organization, conserved intronic phases and exon length (Yousef and Diamandis, 2001). Each of these genes codes for a secreted serine protease with either trypsin- or chymotrypsin-like activity. Kallikrein-related peptidases have been implicated in various physiological processes ranging from cellular homeostasis to tissue remodeling. It is well documented that multiple KLKs participate in the skin desquamation and semen liquefaction cascades (Pampalakis and Sotiropoulou, 2007). KLKs have also been implicated in cellular signaling through differential actions on the proteinase-activated receptors (Oikonomopoulou et al., 2006). Although numerous investigations over the past decade have highlighted various aspects of KLK functions, the multiple roles of each KLK in cell biology remain to be elucidated (Emami and Diamandis, 2007).

Deregulation of kallikreins was observed in numerous pathological conditions including cancer and non-cancer disease states such as skin and neurodegenerative disorders (Paliouras et al., 2007). Although studies revealed that several KLKs were expressed in normal lung tissue (Petraki et al., 2006; Shaw and Diamandis, 2007), their expression in lung cancer has not been studied in detail. A microarray study revealed that *KLK11* was overexpressed in lung neuroendocrine tumors with less favorable outcome (Bhattacharjee et al., 2001). In other non-small-cell lung cancer (NSCLC) subtypes, the expression profile of *KLK10* and *KLK11* did not correlate with patient survival (Planque et al., 2006). Our group showed that the *KLK5* gene was expressed at a higher level in squamous cell carcinoma than in adjacent non-malignant tissue, whereas *KLK7* expression was reduced in adenocarcinoma (Planque et al., 2005). Finally, *in vitro* and *in vivo* studies revealed that high expression of *KLK8* confers a favorable clinical outcome in NSCLC, which might be associated with KLK8-mediated suppression of tumor cell invasiveness (Sher et al., 2006). In the present study we determined for the first time KLK13 and KLK14 expression at the mRNA and protein levels in an NSCLC series and correlated these results to pathological parameters.

KLK13 and *KLK14* mRNA expression was analyzed by real-time RT-PCR (Table 1) in a set of matched tissue samples from 57 patients undergoing surgical treatment for primary NSCLC classified as adenocarcinoma or squamous cell carcinoma. Protein expression was assessed by immunohistochemistry (IHC) using a small-scale tissue microarray (TMA) containing specimens from the same set of patients. The mRNA expression of

Table 1 Parameters for real-time PCR reactions.

	KLK13	*KLK14*	*18S*
Forward primer	5′-GTGCCAACATCCAACTTCG-3′	5′-TGGGTCATCACTGCTGCTC-3′	5′-CGCGGTTCTATTTTGTTGGTTTT-3′
Reverse primer	5′-CCCTCACAGGAGTCTTTGC-3′	5′-CTCCTCAGGTTGTGCTTGC-3′	5′-TTCGCTCTGGTCCGTCTTGC-3′
Acquisition temperature (°C)	83	81.5	84
Amplicon size (bp)	115	71	121
Detection limit	<10	$<10^2$	<10
Quantification range	$10–10^8$	$10^2–10^9$	$10–10^9$
Linearity (r)	0.997	0.996	0.998
Intra-assay CV (%)			
Mean	1.14	1.29	0.91
Range	0.37–2.35	0.15–2.96	0.27–2.25
Inter-assay CV (%)			
Mean	2.79	2.83	3.30
Range	1.57–5.47	0.98–5.83	2.67–4.00

The *KLK13* and *KLK14* primers were designed to target two exons. Real-time PCR was carried out in a 25-μl reaction mixture containing SYBR Green I dye (1/50 000; Roche Diagnostics GmbH, Meylan, France), 100 ng of reverse-transcribed total RNA, 0.2 μM (*KLK14*) or 0.4 μM (*KLK13*) of each primer and 1 U of FastStart *Taq* DNA polymerase (Roche Diagnostics GmbH) according to the manufacturer's instructions. The cycling conditions were as follows: 95°C for 5 min, followed by 45 cycles of 95°C for 10 s, 59°C (*KLK14*) or 68°C (*KLK13*) for 15 s, and 72°C for 20 s. The cycling conditions for 18S rRNA were as previously described (Planque et al., 2005). Fluorescent product was measured using the i-Cycler iQ detection system (Bio-Rad, Marnes-la-Coquette, France) in single acquisition mode for 15 s after each cycle. Serial dilutions of plasmid DNA corresponding to the classical *KLK13* and *KLK14* transcripts were used to establish the PCR parameters. The detection limit and quantification ranges are given in molecules per reaction. Intra- and inter-assay variability was assessed by repeating PCR several times and was based on crossing point variation.

KLK13 ($p=0.006$) and *KLK14* ($p=0.022$) was significantly different in malignant lung tissues compared to matched non-malignant controls (Figure 1). *KLK13* overexpression of at least three-fold was observed in 18 out of the 57 tumor samples (31.6%), whereas *KLK14* was increased in 35.1% of the cancerous samples (Table 2). Mean expression of *KLK13* and *KLK14* was 47- and 81-fold higher, respectively, in cancerous lung tissues. Conversely, underexpression was found in 31.6% (*KLK13*) and 17.5% (*KLK14*) of primary lung tumors (Table 2). The relationships between various clinicopathologic variables and the mRNA expression status of *KLK13* and *KLK14* were examined with the χ^2 test. Patients with *KLK13* ($p=0.025$) or *KLK14* ($p=0.090$) overexpression were more likely to have a positive nodal status. Although marginally significant, underexpression of *KLK13* ($p=0.096$) or *KLK14* ($p=0.078$) was also associated with a negative nodal status. No relationship was observed between *KLK13* or *KLK14* status and histotype, tumor grade, tumor size or stage (data not shown). The relationships were also examined using a descriptive multivariate analysis (multiple correspondence analysis, MCA; Table 3) method. This method is suitable for analyzing results of biological experiments with a small sample size, but high qualitative and quantitative complexity. Relationships between *KLK13* status and positive nodal status were confirmed by the multivariate analysis ($p=0.018$; Table 3). Variations in *KLK14* mRNA expression were associated with histotype ($p=0.020$) and nodal status (p=0.069 and p=0.054, Table 3). Since nodal status indicates a more

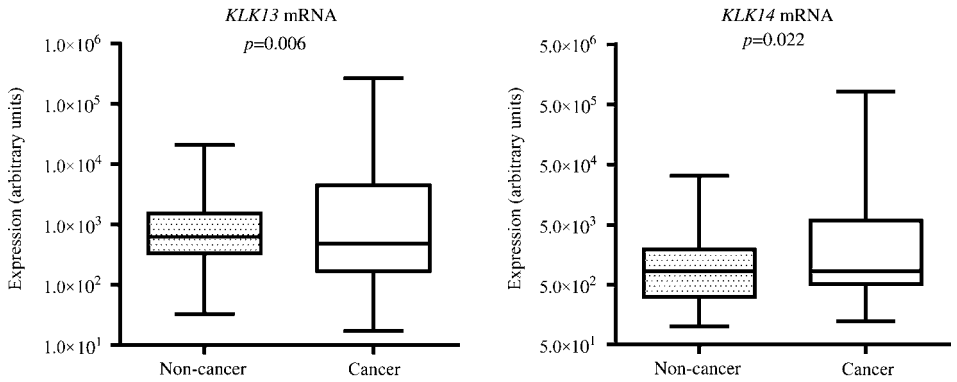

Figure 1 Box-and-whisker plots of *KLK13* and *KLK14* mRNA expression in 57 non-cancerous (Non-cancer) and adjacent cancerous (Cancer) lung tissues.
The horizontal lines in the box denote the 25th, 50th, and 75th percentile values, respectively. The whiskers represent the two lines outside the box that extend to the highest and lowest observations, respectively. Gene expression was normalized to the amount of 18S rRNA and is reported in arbitrary units. The difference between *KLK13* and *KLK14* expression in non-tumoral and tumoral tissues was determined by the non-parametric test with general scores for related samples, using StatXact software.

Table 2 Descriptive statistics for *KLK13* and *KLK14* mRNA expression in NSCLC tissues.

KLK expression	Patients		Raw data (AU)			Fold change in *KLK* expression		
	n	%	Mean±SE	Median	Range	Mean±SE	Median	Range
KLK13 overexpression								
Cancer	18/57	31.6	46225±19693	7477	808–265 484	47.3±21.5	11.5	3–342
Non-cancer			1837±1123	589	46–20 792			
KLK13 underexpression								
Cancer	18/57	31.6	244±83	159	28–1550	-18.1±6.4	-5	-94 to -3
Non-cancer			2169±828	852	85–14 900			
KLK14 overexpression								
Cancer	20/57	35.1	69225±41142	6159	725–818 471	81.2±37.9	8	3–617
Non-cancer			1411±463	351	142–7663			
KLK14 underexpression								
Cancer	10/57	17.5	1324±849	224	122–8752	-8.4±2.8	-5.5	
Non-cancer			5915±3221	1847	392–31 952			-33 to -3

[1]Of the 57 patients (aged 45–83 years, median 65) undergoing surgical treatment for primary non-small-cell lung cancer (NSCLC), 53 had a smoking history. All investigations were carried out in accordance with Helsinki principles and informed consent was obtained from each patient. Total RNA was extracted from matched tumoral and non-tumoral lung samples and reverse-transcribed as previously described (Planque et al., 2005). *KLK13* and *KLK14* expression was determined as mentioned in Table 1 and normalized to the amount of 18S rRNA. A three-fold difference was considered the cutoff in all comparisons. SE, standard error; AU, arbitrary units.

Table 3 Relationships between *KLK13* and *KLK14* mRNA status and other variables.

Variable	Patients		*p*-Value					
	n	%	*KLK13*			*KLK14*		
			C>NC	C<NC	C=NC	C>NC	C<NC	C=NC
Histotype								
Adenocarcinoma	37	64.9	-0.136	+0.459	+0.171	**+0.020**	-0.198	-0.125
Squamous cell carcinoma	20	35.1	+0.136	-0.459	-0.171	-0.020	+0.198	+0.125
Tumor grade								
Poorly differentiated	16	28.1	-0.434	-0.363	+0.494	-0.434	-0.388	+0.290
Differentiated (well+moderately)	41	71.9	+0.434	+0.363	-0.494	+0.434	+0.388	-0.290
Tumor size								
≤3 cm	16	28.1	+0.490	-0.114	+0.334	-0.490	-0.314	-0.384
>3 cm	41	71.9	-0.490	+0.114	-0.334	+0.490	+0.314	+0.384
Nodal status								
N0	39	68.4	-0.018	+0.063	+0.485	-0.069	**+0.054**	+0.445
N1–N2	18	31.6	**+0.018**	-0.063	-0.485	+0.069	-0.054	-0.445
Tumor status								
T1–T2	42	73.7	+0.376	-0.294	+0.425	-0.359	-0.058	+0.321
T3–T4	15	26.3	-0.376	+0.294	-0.425	+0.359	+0.058	-0.321
Stage								
I–II	36	63.2	-0.179	-0.474	+0.303	-0.376	+0.470	-0.418
III–IV	21	36.8	+0.179	+0.474	-0.303	+0.376	-0.470	+0.418

Variations in *KLK13* and *KLK14* expression were categorized and scored from 1 to 3: 1, underexpression in cancerous (C) compared to non-cancerous (NC) lung tissue; 2, overexpression in cancer; 3, same expression in both tissues. Multivariate analysis was then performed according to MCA using SPAD software as previously described (Planque et al., 2006). Briefly, MCA constructs a multidimensional space in which two different categories are close together if they both appear multiple times in the same observation. Finally, statistical relationships between variables are established. A *p*-value of 0.05 or less was considered statistically significant (indicated in bold); owing to the small sample size, a *p*-value of 0.06–0.10 was considered to indicate a strong statistical tendency. +, positive correlation; -, negative correlation.

aggressive course of the disease, we examined the survival outcome of patients displaying dysregulation of *KLK13* or *KLK14* using the Kaplan-Meier method. No significant difference was observed between patients with *KLK13* or *KLK14* overexpression and patients with underexpression (*KLK13*, $p=0.970$; *KLK14*, $p=0.347$; log-rank test).

An optimal cutoff value was identified by χ^2 analysis based on the ability of *KLK13* or *KLK14* to predict the overall survival of the study population. Based on this cutoff (equal to the 70th percentile for *KLK13* and the 42nd percentile for *KLK14*), patients were dichotomized into two groups with low and high expression. No significant associations were found between mRNA expression levels in tumor and clinicopathologic variables (data not shown). Patients with high *KLK13* expression manifested less favorable overall survival rates in comparison to patients with low expression, but the difference was not significant ($p=0.187$, Figure 3). There was no significant correlation between *KLK14* mRNA expression and overall survival ($p=0.524$).

Immunoexpression of KLK13 and KLK14 was observed in the epithelial cells of control tissues (prostate, colon and breast) and in the bronchial epithelium of

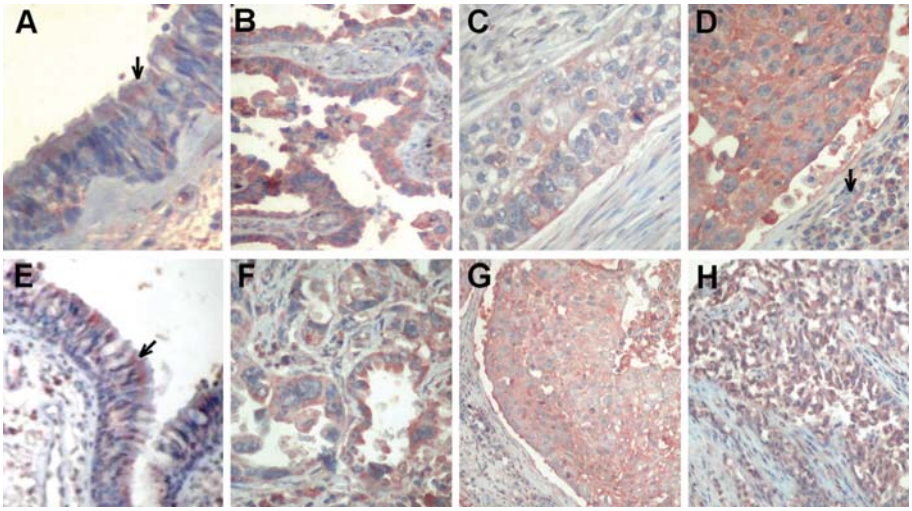

Figure 2 Representative slides showing immunohistochemical staining of KLK13 and KLK14 in lung cancer tissues.
A tissue microarray was created using 56 formalin-fixed lung cancer specimens in triplicate. Fifty of these specimens were included in the mRNA study and diagnosed for adenocarcinoma or squamous cell carcinoma. The additional specimens were from patients with other types of NSCLC (4 large cell carcinoma, 1 carcinoid tumor, 1 mucoepidermoid carcinoma). Control tissues including breast, colon, prostate and normal bronchus were also included in the array. IHC staining was performed according to the protocol of the UltraTech HRP Streptavidin-Biotin Detection System (Beckman Coulter, Brea, CA, USA). A mouse monoclonal antibody (11C1, 1:200) previously described (Petraki et al., 2003) was used for detection of KLK13 (A–D). KLK14 (E–H) was detected using a KLK14-specific rabbit polyclonal (1:1000; Borgono et al., 2003). Moderate expression of KLK13 (A) and KLK14 (E) by epithelium of normal bronchus (arrow); cytoplasmic KLK13 (B) and KLK14 (F) immunostaining in epithelial cells of adenocarcinoma; expression of KLK13 (C) and KLK14 (G) in squamous cell carcinoma; (D) KLK13 immunoexpression in inflammatory cells (arrow, stroma of a squamous cell carcinoma); and (E) KLK14 expression in a specimen from a patient with large cell carcinoma. All original magnifications 250×.

normal lung (Figure 2A,E). These findings are in agreement with previous studies showing that kallikrein-related peptidases are expressed mainly in glandular epithelia (Petraki et al., 2006). Moderate levels of KLK13 and KLK14 were recently quantified by ELISA in a small set of normal lung extracts (Shaw and Diamandis, 2007). Taken together, those data suggest that KLK13 and KLK14 expression represents a physiological feature in normal lung tissue, especially in the bronchial tree. Other members of the kallikrein family, including KLK5, 6, 7, 10, 11 and 12, are expressed in epithelial cells of the normal bronchus (Petraki et al., 2006). Co-expression of KLKs in lung suggests that a KLK enzyme cascade might be operating in the pulmonary tract, similar to that in other tissues (Pampalakis and Sotiropoulou, 2007). The physiological functions of KLKs in the lung remain unknown. We previously hypothesized that bronchial KLKs might be involved in maintenance of the mucociliary function through proteolysis of lumenal components (Planque et al., 2006) and changes in KLK expression might contribute to the development of pathological airway diseases. This idea is supported by a recent study showing that KLK expression in bronchial brushing cells was highly variable in subjects with different pulmonary diseases (Christiansen et al., 2007). KLKs were recently shown to play significant roles in the antimicrobial protection of skin (Yamasaki et al., 2006). Like the skin, airways are in direct contact with pathogens and express a variety of antimicrobial molecules, including defensins and cathelicidins. Interestingly, KLK5 and KLK7 process the proform of cathelicidin hCAP18 (Yamasaki et al., 2006) and are present in lung tissue (Planque et al., 2005; Petraki et al., 2006). Taken together, these observations indicate that KLKs might be involved in the antimicrobial protection of human airways.

Immunohistochemical analysis of KLK13 and KLK14 expression was informative in 88% (44/50) and 84% (42/50) of the cancerous specimens, respectively. Staining for KLK13 was observed in 68.2% (30/44) of the informative specimens, whereas 40 cases (90.9%) were positive for KLK14. Consistent with endoplasmic reticulum localization of KLKs, immunoreactivity of KLK13 and 14 proteins was diffuse in the cytosolic and perinuclear regions of malignant cells (Figure 2). Staining of variable intensity was found in major NSCLC types (adenocarcinoma, squamous cell carcinoma and large cell carcinoma) (Figure 2 and Table 4). In some specimens, inflammatory cells were also stained (Figure 2D). Overall KLK13 staining was significantly higher in adenocarcinoma than in squamous cell carcinoma (6.04 vs. 4.88, $p=0.014$; Table 4), and KLK14 was most frequently detected in tumors of >3 cm in size. Kaplan-Meier survival curves stratified for KLK13 and KLK14 are provided in Figure 3. Although marginally significant, high KLK13 protein expression seems to be associated with poor overall survival. No association was observed for KLK14. Typically, the expression of proteinases is often correlated with cancer progression, and this is mainly attributed to the well-known role of proteolytic enzymes in extracellular matrix (ECM) degradation, which facilitates invasion and metastasis. It was previously shown that KLK13 cleaves the major components of the ECM (collagens I, II and III, fibronectin and laminin) and stimulates migration of ovarian cancer cells through ECM components (Kapadia et al., 2004). However, there is growing evidence indicating a complex contribution of KLKs in

Table 4 Clinicopathologic and immunohistochemical parameters relative to KLK13 and KLK14 expression.

Variable	KLK13				KLK14			
	n (%)	*p*-Value[a]	IHC score	*p*-Value[b]	n (%)	*p*-Value[a]	IHC score	*p*-Value[b]
Histotype								
Adenocarcinoma	19/28 (67.9)	1.000	6.04±0.28	**0.014**	25/27 (92.6)	0.608	5.76±0.18	0.235
Squamous cell carcinoma	11/16 (68.7)		4.88±0.25		13/15 (86.7)		5.43±0.28	
Tumor grade								
Poorly differentiated	9/13 (69.2)	1.000	5.98±0.43	0.341	9/11 (81.8)	0.277	5.68±0.34	0.972
Differentiated	21/31 (67.7)		5.46±0.26		29/31 (93.5)		5.63±0.17	
Tumor size								
≤3 cm	9/12 (75.0)	0.722	5.25±0.44	0.276	8/11 (72.7)	**0.048**	5.81±0.35	0.843
>3 cm	21/32 (65.6)		5.78±0.25		30/31 (96.8)		5.60±0.17	
Nodal status								
N0	18/30 (60.0)	0.163	5.50±0.32	0.351	26/29 (89.7)	1.000	5.58±0.18	0.561
N1–N2	12/14 (85.7)		5.80±0.29		12/13 (92.3)		5.78±0.28	
Tumor status								
T1–T2	21/33 (63.6)	0.456	5.48±0.27	0.352	28/31 (90.3)	1.000	5.70±0.17	0.630
T3–T4	9/11 (81.8)		5.94±0.41		10/11 (90.9)		5.48±0.33	
Stage								
I–II	17/28 (60.7)	0.195	5.46±0.32	0.356	25/27 (92.6)	0.608	5.71±0.18	0.558
III–IV	13/16 (81.2)		5.82±0.31		13/15 (86.7)		5.52±0.28	

To assess KLK13 and KLK14 staining in adenocarcinoma and squamous cell carcinoma, a proportion score and intensity score based on a well-documented system was used (Darling et al., 2006). The overall staining (IHC score, mean±SE) was then expressed as the sum of proportion and intensity scores (0 for negative and 2–8 for positive staining). n, number of positive specimens/number of informative specimens.

[a] *p*-Value calculated using Fisher's exact test; statistically significiant values are indicated in bold.

[b] *p*-Value determined using the Mann-Whitney *U*-test.

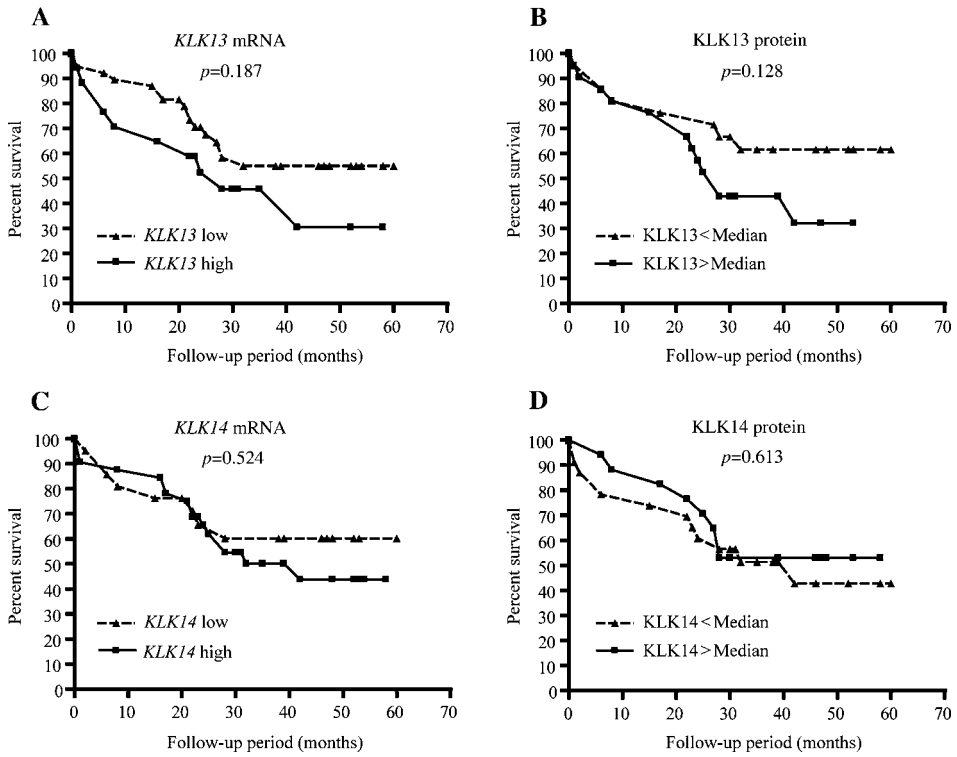

Figure 3 Kaplan-Meier overall survival curves.

Patients were stratified into two groups, according to the level of mRNA expression (A and C) or intratumor protein expression (B and D). The survival rate was calculated using the Kaplan-Meier method and compared between the two groups. Differences between the survival curves were tested for statistical significance using the log-rank test. An optimal cutoff value was identified by χ^2 analysis, based on the ability of *KLK13* or *KLK14* to predict the overall survival of the study population. Based on this cutoff, *KLK13* (2000 in arbitrary units, equal to the 70th percentile) or *KLK14* (685 in arbitrary units, equal to the 42nd percentile) expression was categorized as high or low.

cancer progression with either tumor-promoting or tumor-suppressive actions. For example, high KLK14 expression was associated with poor prognosis in breast and prostate cancers (Borgono et al., 2003; Yousef et al., 2003b) and with a favorable prognosis in ovarian cancer (Yousef et al., 2003a). A recent study with a substrate candidate approach showed that KLK14 might exert both effects interchangeably, depending on the tumor microenvironment (Borgono et al., 2007). Further studies are required to determine the role of KLK13 and KLK14 in NSCLC.

Many members of the kallikrein family are differentially expressed in a wide variety of carcinomas, and several kallikreins are emerging biomarkers for the diagnosis and prognosis of cancer (Paliouras et al., 2007). It was previously reported that KLK13 is an independent marker of favorable prognosis in women with breast or ovarian carcinoma (Chang et al., 2002; Scorilas et al., 2004). KLK14 was also proposed as new biomarker for these two cancers (Borgono et al., 2003; Fritzsche et al., 2006). From the results presented here, it is apparent that KLK13 and KLK14 cannot be considered as specific markers for NSCLC. However, further studies should determine whether KLK13 and KLK14 might be utilized in addition to other markers for NSCLC diagnosis and prognosis.

Acknowledgments

This work was supported by grants from Association pour la Recherche sur le Cancer (ARC grant no. 7771) and Ligue Contre le Cancer (Region Centre). Chris Planque was supported during this work by fellowships from INSERM and the Region Centre.

References

Bhattacharjee, A., Richards, W.G., Staunton, J., Li, C., Monti, S., Vasa, P., Ladd, C., Beheshti, J., Bueno, R., Gillette, M., et al. (2001). Classification of human lung carcinomas by mRNA expression profiling reveals distinct adenocarcinoma subclasses. Proc. Natl. Acad. Sci. USA *98*, 13790–13795.

Borgono, C.A., Grass, L., Soosaipillai, A., Yousef, G.M., Petraki, C.D., Howarth, D.H., Fracchioli, S., Katsaros, D., and Diamandis, E.P. (2003). Human kallikrein 14: a new potential biomarker for ovarian and breast cancer. Cancer Res. *63*, 9032–9041.

Borgono, C.A., Michael, I.P., Shaw, J.L., Luo, L.Y., Ghosh, M.C., Soosaipillai, A., Grass, L., Katsaros, D., and Diamandis, E.P. (2007). Expression and functional characterization of the cancer-related serine protease, human tissue kallikrein 14. J. Biol. Chem. *282*, 2405–2422.

Chang, A., Yousef, G.M., Scorilas, A., Grass, L., Sismondi, P., Ponzone, R., Diamandis, E.P. (2002). Human kallikrein gene 13 (*KLK13*) expression by quantitative RT-PCR: an independent indicator of favourable prognosis in breast cancer. Br. J. Cancer *86*, 1457–1464.

Christiansen, S.C., Delgado, A., Juergens, U., and Zuraw, B.L. (2007). Tissue kallikrein (KLK) expression profile in bronchial epithelial cells of human subject. In: Proceedings of the 2nd International Symposium on Kallikreins and Kallikrein-Related Peptidases, Santorini, Greece, October 16–18, 2007. Abstract P15.

Darling, M.R., Jackson-Boeters, L., Daley, T.D., and Diamandis, E.P. (2006). Human kallikrein 13 expression in salivary gland tumors. Int. J. Biol. Markers *21*, 106–110.

Emami, N. and Diamandis, E.P. (2007). Human tissue kallikreins: a road under construction. Clin. Chim. Acta *381*, 78–84.

Fritzsche, F., Gansukh, T., Borgono, C.A., Burkhardt, M., Pahl, S., Mayordomo, E., Winzer, K.J., Weichert, W., Denkert, C., Jung, K., et al. (2006). Expression of human kallikrein 14 (KLK14) in breast cancer is associated with higher tumour grades and positive nodal status. Br. J. Cancer *94*, 540–547.

Kapadia, C., Ghosh, M.C., Grass, L., and Diamandis, E.P. (2004). Human kallikrein 13 involvement in extracellular matrix degradation. Biochem. Biophys. Res. Commun. *323*, 1084–1090.

Oikonomopoulou, K., Hansen, K.K., Saifeddine, M., Tea, I., Blaber, M., Blaber, S.I., Scarisbrick, I., Andrade-Gordon, P., Cottrell, G.S., Bunnett, N.W., et al. (2006). Proteinase-activated receptors, targets for kallikrein signaling. J. Biol. Chem. *281*, 32095–32112.

Paliouras, M., Borgono, C., and Diamandis, E.P. (2007). Human tissue kallikreins: the cancer biomarker family. Cancer Lett. *249*, 61–79.

Pampalakis, G. and Sotiropoulou, G. (2007). Tissue kallikrein proteolytic cascade pathways in normal physiology and cancer. Biochim. Biophys. Acta *1776*, 22–31.

Petraki, C.D., Karavana, V.N., and Diamandis, E.P. (2003). Human kallikrein 13 expression in normal tissues: an immunohistochemical study. J. Histochem. Cytochem. *51*, 493–501.

Petraki, C.D., Papanastasiou, P.A., Karavana, V.N., and Diamandis, E.P. (2006). Cellular distribution of human tissue kallikreins: immunohistochemical localization. Biol. Chem. *387*, 653–663.

Planque, C., de Monte, M., Guyetant, S., Rollin, J., Desmazes, C., Panel, V., Lemarie, E., and Courty, Y. (2005). KLK5 and KLK7, two members of the human tissue kallikrein family, are differentially expressed in lung cancer. Biochem. Biophys. Res. Commun. *329*, 1260–1266.

Planque, C., Ainciburu, M., Heuze-Vourc'h, N., Regina, S., de Monte, M., and Courty, Y. (2006). Expression of the human kallikrein genes 10 (*KLK10*) and 11 (*KLK11*) in cancerous and non-cancerous lung tissues. Biol. Chem. *387*, 783–788.

Scorilas, A., Borgono, C.A., Harbeck, N., Dorn, J., Schmalfeldt, B., Schmitt, M., and Diamandis, E.P. (2004). Human kallikrein 13 protein in ovarian cancer cytosols: a new favorable prognostic marker. J. Clin. Oncol. *22*, 678–685.

Shaw, J.L. and Diamandis, E.P. (2007). Distribution of 15 human kallikreins in tissues and biological fluids. Clin. Chem. *53*, 1423–1432.

Sher, Y.P., Chou, C.C., Chou, R.H., Wu, H.M., Wayne Chang, W.S., Chen, C.H., Yang, P.C., Wu, C.W., Yu, C.L., and Peck, K. (2006). Human kallikrein 8 protease confers a favorable clinical outcome in non-small cell lung cancer by suppressing tumor cell invasiveness. Cancer Res. *66*, 11763–11770.

Yamasaki, K., Schauber, J., Coda, A., Lin, H., Dorschner, R.A., Schechter, N.M., Bonnart, C., Descargues, P., Hovnanian, A., and Gallo, R.L. (2006). Kallikrein-mediated proteolysis regulates the antimicrobial effects of cathelicidins in skin. FASEB J. *20*, 2068–2080.

Yousef, G.M. and Diamandis, E.P. (2001). The new human tissue kallikrein gene family: structure, function, and association to disease. Endocr. Rev. *22*, 184–204.

Yousef, G.M., Fracchioli, S., Scorilas, A., Borgono, C.A., Iskander, L., Puopolo, M., Massobrio, M., Diamandis, E.P., and Katsaros, D. (2003a). Steroid hormone regulation and prognostic value of the human kallikrein gene 14 in ovarian cancer. Am. J. Clin. Pathol. *119*, 346–355.

Yousef, G.M., Stephan, C., Scorilas, A., Ellatif, M.A., Jung, K., Kristiansen, G., Jung, M., Polymeris, M.E., and Diamandis, E.P. (2003b). Differential expression of the human kallikrein gene 14 (KLK14) in normal and cancerous prostatic tissues. Prostate *56*, 287–292.

Received November 30, 2007; accepted February 15, 2008