Human Tissue Kallikreins As New Serological Biomarkers For Lung Cancer

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ABSTRACT

Tissue kallikreins are a family of 15 genes (*KLK1-KLK15*) co-localized in tandem to chromosome 19q13.4, encoding serine protease enzymes. Previous reports indicate that dysregulated KLKs expression is associated with multiple diseases, primarily cancer. As a consequence, many kallikreins, in addition to KLK3/PSA, used in clinical medicine for the screening, diagnosis and monitoring of prostate cancer, have been identified as promising diagnostic and/or prognostic biomarkers for several hormone related cancers. The aim of the present study was to investigate the expression of several members of the kallikrein family to determine whether any of them have value as serological biomarkers for lung cancer. Sera samples collected by the UCLA Lung Cancer SPORE program from 101 subjects (51 cases and 50 controls) were analyzed blindly, using ELISA assays developed in-house. We identified statistically significant differences in the KLK5, KLK7-8, KLK10-14 protein expression between cases and controls ($p<0.05$), but not for KLK1, PSA, KLK4 and KLK6. In this series of lung carcinoma samples, KLK5, 7, 8, 10 and 12 were generally under-expressed in cancerous sera samples than in controls, while KLK11 and KLK13 were over-expressed in 34% and 26% of the tumour samples at 95% specificity. In total, approximately 67% of patients with lung carcinoma had elevations in at least one of the kallikreins studied. We could not find any correlation between KLK alterations and cancer histotype. However, most elevations in KLKs were seen in patients with stage IV tumours.

In conclusion, these preliminary results indicate that many members of the kallikrein family are dysregulated in lung carcinoma and that KLK11 and KLK13, or panels of many kallikreins, may constitute novel markers for lung cancer, but likely only for late stage disease.

INTRODUCTION

- Despite the development of therapeutic strategies and advances in surgical treatment, lung cancer is the major cancer-related mortality worldwide in both men and women. This cancer is classified into two main histological groups: small cell carcinoma (SCLC) and non-small cell carcinoma (NSCLC). NSCLC accounts for approximately 80% of all cases and comprised of 3 major histological subtypes: adenocarcinoma, squamous cell carcinoma and large cell carcinoma.

- Serum tumor markers are non-invasive diagnostic tools for malignant tumors and they are commonly used for the screening of cancer and as an indicator of the treatment effect. A number of serum tumor antigens have been evaluated as biomarkers for NSCLC, including squamous cell carcinoma antigen (SCC-Ag), carcinoembryonic antigen (CEA), neuron-specific enolase (NSE), cytokeratin 19 fragment (CYFRA 21-1), cancer antigen 125 (CA125) and tissue polypeptide antigen (TPA). However, expression of these antigens does not appear to be sufficiently sensitive and specific enough to be reliable for the diagnosis of the majority of lung malignancies. Therefore, there is a urgent and critical need to discover novel biomarkers for this major cancer.

- Human Tissue Kallikreins are a family of 15 structurally similar serine protease genes that co-localize in tandem to chromosome 19q13.4.

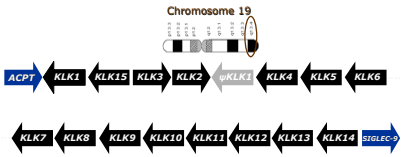

Fig.1. Organization of the tissue kallikrein gene loci in the human genome. *Arrowheads indicate the location of genes and their direction of transcription. Kallikrein pseudogene is represented by grey arrowhead. Blue arrowheads indicate non-kallikrein genes (ACPT and Siglec-9).*

- In addition to KLK3/PSA, many other members of the kallikrein family, dysregulated in cancer, have been identified as promising diagnostic and/or prognostic biomarkers for several cancer types, including ovarian, breast, prostate, and testicular cancers [1,2].

OBJECTIVES

- To investigate the expression of 12 members of the kallikrein family (KLK1, KLK3/PSA, KLK4, KLK5, KLK6, KLK7, KLK8, KLK10, KLK11, KLK12, KLK13 and KLK14) to determine whether any of them have value as serological biomarkers for non-small cell lung cancer.

RESULTS

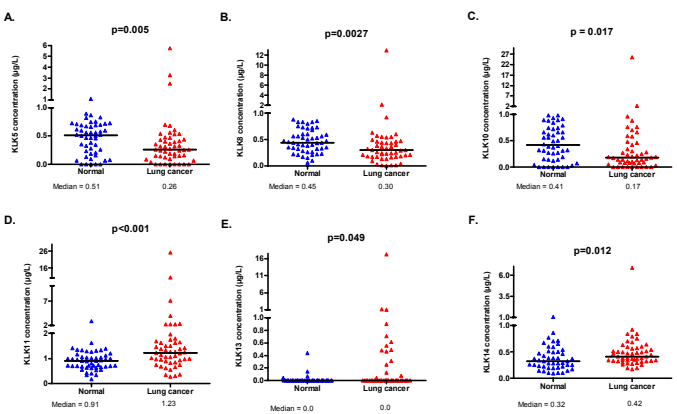

Fig.2. Distribution of different kallikrein levels (µg/L) in the sera of healthy patients (normal) and patients with non-small cell lung cancer. A, **KLK5.** ***B,*** **KLK8.** ***C,*** **KLK10.** ***D,*** **KLK11.** ***E,*** **KLK13.** ***F,*** **KLK14.** *The p value was determined by the Mann-Whitney U-test. Horizontal lines represent the median values.*

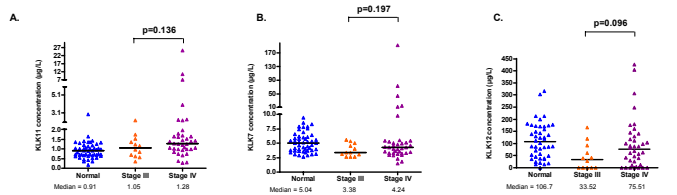

Fig.3. Distribution of different kallikrein levels (µg/L) in the sera of healthy patients (normal) and patients with stages III and IV of non-small cell lung cancer. A, **KLK11.** ***B,*** **KLK7.** ***C,*** **KLK12.** *The p value was determined by the Mann-Whitney U-test to determine differences among cancer stages. Horizontal lines represent the median values.*

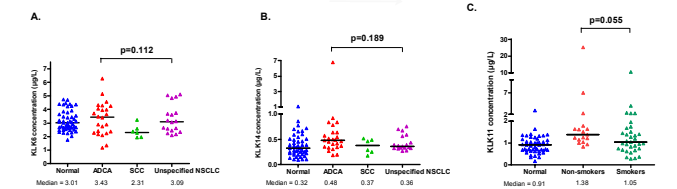

Fig.4. Distribution of different kallikrein levels (µg/L) in the sera of healthy patients (normal) and patients with different NSCLC histotypes (A, **KLK6.** ***B,*** **KLK14***) or with different smoking history (C,* **KLK11***). The p value was determined by the Kruskal-Wallis test (**A. and B.**) or by the Mann-Whitney U-test (**C.**). Horizontal lines represent the median values. ADCA, adenocarcinoma. SCC, squamous cell carcinoma.*

METHODS

Sera samples collection

Sera samples from 101 consented individuals (51 cases and 50 controls) have been collected by the UCLA Lung Cancer SPORE program. Sera samples from diagnosed lung cancer patients have been collected prior to any therapy and surgical procedures, between January 2004 and August 2005.

Table1. Distributions of patients by demographic and clinical characteristics. *ADCA, adenocarcinoma. SCC, squamous cell carcinoma. LCC, large cell carcinoma. BAC, Bronchioloalveolar carcinoma.*

Characteristics	Controls	Cases
Smoking status		
yes	15	34
no	31	17
Age		
< 65	50	25
>= 65	0	26
Gender		
F	20	22
M	30	29
Stage		
III		12
IV		38
x		1
Histology		
ADCA		25
SCC		6
BAC		1
LCC		1
Unsp.NSCLC		18

Immunological assays

For all kallikrein measurements in serum, in house ELISA-type immunofluorometric procedures were employed. The precision of all assays within the dynamic range, cited below (Table 2), was <10%.

Table2. ELISA assays used in the present study.
Mono, monoclonal mouse antibody.
Poly, polyclonal rabbit antibody.

Kallikrein	Coating/Detection Ab	Dynamic range, ng/L	Detection limit, ng/L
KLK1	poly/poly	20,000	100
PSA	mono/mono	5,000	1
KLK4	mono/poly	10,000	100
KLK5	mono/mono	10,000	50
KLK6	mono/mono	20,000	50
KLK7	mono/mono	20,000	200
KLK8	mono/mono	10,000	50
KLK10	mono/mono	10,000	50
KLK11	mono/poly	20,000	200
KLK12	mono/poly	500,000	5,000
KLK13	mono/mono	10,000	50
KLK14	mono/poly	10,000	50

Statistical analysis

Statistical analyses were performed with GraphPad Prism software. Because the distribution of the different KLKs in the NSCLC sera samples was non-gaussian, we used the nonparametric Mann-Whitney U-test to assess differences among cases and controls and, the Kruskal-Wallis test to analyse KLKs expression among NSCLC subtypes. The significant level chosen was $p<0.05$ and all tests were two-sided.

CONCLUSIONS AND DISCUSSION

- Kallikrein dysregulation in Non-small Cell Lung Cancer
  - Down-regulated: KLK5, 7, 8, 10, and 12
  - Up-regulated: KLK11, 13 and 14

- Kallikrein expression and various clinicopathologic variables of lung carcinoma
  - A number of kallikreins, i.e. KLK5, 7, 8, 11, 12, 13, exhibit an elevated level of expression in stages IV of NSCLC
  - No significant correlation was observed with respect to tumour histotype.
  - Smoking in positive cases may affect the expression level of certain kallikreins, e.g. KLK11

- A Mutiparametric panel of Kallikreins are expected to provide a more sensitive/specific screening tool for the screening/diagnosis of NSCLC.

REFERENCES

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