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Pentraxin-3 Is a Novel Biomarker of Lung CarcinomaEleftherios P. Diamandis¹, Lee Goodglick³, Chris Planque², and Mark D. Thornquist⁴**Abstract**

Purpose: Our objective was to validate the performance of three new candidate lung cancer biomarkers, pentraxin-3 (PTX3), human kallikrein 11 (KLK11), and progranulin.

Experimental Design: We analyzed by commercial ELISA, and with a blinded protocol, 422 samples from 203 patients with lung carcinoma, 180 individuals with high risk for lung cancer (heavy smokers), and 43 individuals with cancers other than lung. All samples were obtained from the Early Detection Research Network (Reference set A).

Results: We found that progranulin and KLK11 were not informative lung cancer biomarkers, with areas under the receiver operating characteristic curve (AUC; ROC), close to 0.50. However, PTX3 was an informative lung cancer biomarker, with considerable ability to separate lung cancer patients from high-risk controls. At 90% and 80% specificity, the sensitivities versus the high-risk control group were 37% and 48%, respectively. The discriminatory ability of PTX3 was about the same with all major subtypes and histotypes of lung cancer. The AUC of the ROC curves increased according to the disease stage, from 0.64 (stage I) to 0.72 (stage IV).

Conclusion: PTX3, but not KLK11 or progranulin, is a new serum biomarker for lung carcinoma. Its diagnostic sensitivity and specificity is similar to other clinically used lung cancer biomarkers. More studies are needed to establish if PTX3 has clinical utility for lung cancer diagnosis and management. *Clin Cancer Res*; 17(8); 2395–9. ©2011 AACR.

Introduction

Lung cancer is the leading cause of cancer-related mortality in both men and women (1). Lung cancer survival and therapy mainly depend on the histology and stage of the disease at diagnosis (2). Lung cancers are largely classified into 2 histologically distinct subtypes (according to the World Health Organization), based on the size and appearance of the malignant cells: small cell (SCLC) and non-small cell lung carcinoma (NSCLC). NSCLC is the most common and represents approximately 80% of all lung cancers, which can be further subdivided into adeno-

carcinomas, squamous cell carcinomas (SCC), and large cell carcinomas (3).

The overall 5-year survival rate of lung cancer is less than 15%. This is mainly because most lung cancer patients are diagnosed at advanced stages. Prognosis is more favorable if the lung cancer is detected early (4), by using either computed tomography and chest X-rays, or diagnostic biomarkers. The most widely-used lung cancer biomarkers to date are carcinoembryonic antigen (CEA), SCC antigen, neuron-specific enolase, tissue polypeptide antigen, CYFRA 21-1 (cytokeratine 19 fragment), and progastrin-releasing peptide (5). Although these markers are elevated in serum of a proportion of patients with lung cancer, they are not sensitive or specific enough, alone or in combination, to reliably diagnose asymptomatic patients with lung cancer. Recently, Molina and colleagues (5) reported optimal combinations of currently available lung cancer biomarkers for differentiating between NSCLC and SCLC.

We have recently undertaken a proteomic approach for identifying novel lung cancer biomarkers (6). Proteomic analysis of condition media of 4 lung carcinoma cell lines identified thousands of proteins. This approach (7, 8) was capable in identifying most of the currently known lung cancer biomarkers (6), thus validating its effectiveness. By using bioinformatics and other selection criteria, we were able to identify 5 novel candidate lung cancer biomarkers, namely ADAM-17, osteoprotegerin, pentraxin-3 (PTX3), follistatin, and tumor necrosis factor receptor superfamily member 1A. Analysis of these

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Translational Relevance

We report for the first time that pentraxin-3, a molecule that is involved with innate immunity and inflammation, is a new biomarker for lung carcinoma. In a blinded validation study, with serum samples obtained from the Early Detection Research Network (Reference set A), serum elevations of this marker were found to occur in approximately 50% of patients of all histological types and the positivity correlated with disease stage. This biomarker could be used either alone, or in combination with other biomarkers, for diagnosis and prognosis of lung carcinoma.

biomarkers in small numbers of serum samples indicated that they may have value for discriminating lung cancer patients from healthy controls (6).

The Early Detection Research Network (EDRN; <http://edrn.nci.nih.gov>) is an organization that promotes discovery and validation of cancer biomarkers. One mandate of EDRN is to blindly validate new candidate biomarkers by using serum samples collected by its investigators from various sites, under controlled conditions. We obtained approval to use serum Reference Set A for lung carcinoma, in order to validate 3 biomarker candidates: PTX3; human kallikrein 11 (KLK11), which was previously shown to have some value for lung cancer diagnosis (9); and progranulin, another candidate biomarker identified recently by our group (8). The limited amount of serum provided (100 μ L) did not allow for validating the other 4 aforementioned biomarkers. In this paper, we report that with this blinded validation protocol, PTX3 has been shown to be a promising new biomarker for lung carcinoma.

Materials and Methods

Clinical samples

A total of 426 samples from 203 patients diagnosed with lung carcinoma (please see below), 180 individuals at high risk for lung cancer due to a history of cigarette smoking, and 43 individuals with cancers other than lung (25 breast cancer, 18 colon cancer) were enrolled in this study. The lung cancer cases and high-risk controls were at least 40 years old, and the high-risk controls had a cigarette smoking history of at least 30 pack-years. Cases and high-risk controls were frequency matched for age, cigarette smoking history, and center where the specimens were collected. The specimens tested in this study represented a copy of the lung cancer "Reference Set A" (for more details see: <http://edrn.jpl.nasa.gov/resources/samples-reference-sets/LCBG%20protocol%20July%202009.pdf>) created by EDRN. Specimens in this reference set were contributed by 4 institutions (MD Anderson Cancer Center; New York University; University of California, Los Angeles; and Vanderbilt University), from archived samples previously collected and stored at -80°C . One aliquot (100 μ L of

serum) was shipped to the laboratory of Dr. E.P. Diamandis on dry ice. Samples were labeled with a number and they were blinded to the investigators participating in this study. The code was broken only after ELISA analysis was completed and the data submitted to the statistician (Dr. M.D. Thornquist).

ELISA for pentraxin-3, KLK11, and progranulin

In all serum samples, PTX3, KLK11, and progranulin were quantified by using ELISA methodologies. The ELISA for KLK11 was developed in-house and is described elsewhere (10). The ELISA kit for progranulin was purchased from R&D Systems and was used according to the manufacturer's recommendations. Because KLK11 and progranulin were found in this study to be noninformative biomarkers for lung carcinoma, no further details will be provided on either the analytical methods or the clinical data.

PTX3 ELISA kits were purchased from R&D Systems. The assay is based on 2 antibodies, 1 used for capture (monoclonal mouse antibody) and 1 used for detection (biotinylated goat polyclonal antibody). Standardization was achieved by using recombinant, purified PTX3 provided by the manufacturer. The manufacturer's recommendations and protocol were used and serum samples were diluted 3-fold with a 6% bovine serum albumin solution before analysis. The calibration curve was linear from 200 to 20,000 pg/mL and the precision in this range was $<10\%$. All assays were performed in duplicate.

Statistical methods

To evaluate PTX3 for disease screening and diagnosis, we used receiver operating characteristic (ROC) curves, which is the most commonly used summary of classification accuracy. An ROC curve is a plot of the true positive fraction (sensitivity) versus the false positive fraction (one minus the specificity). ROC curves were constructed for the whole group of patients and controls, as well as for case subgroups stratified by histology type and stage and control subgroups stratified by control type (high-risk versus other cancers). The area under the ROC curve (AUC) and the sensitivity of PTX3 at selected specificity cutpoints were also calculated and confidence intervals for these quantities were calculated by bootstrap. Not all patients had complete clinicopathological information and, as deemed necessary, subgroups were combined to increase the statistical power of the calculations. All analyses were performed using Stata version 11 and the *pcvsuite* of basic ROC analysis commands created by Dr. M. Pepe (11, 12).

Results

The ROC curves for progranulin and KLK11 for the whole patient group versus all controls, or only the high-risk controls, were not informative (the AUCs were close to 0.50 and were not statistically significant; data not

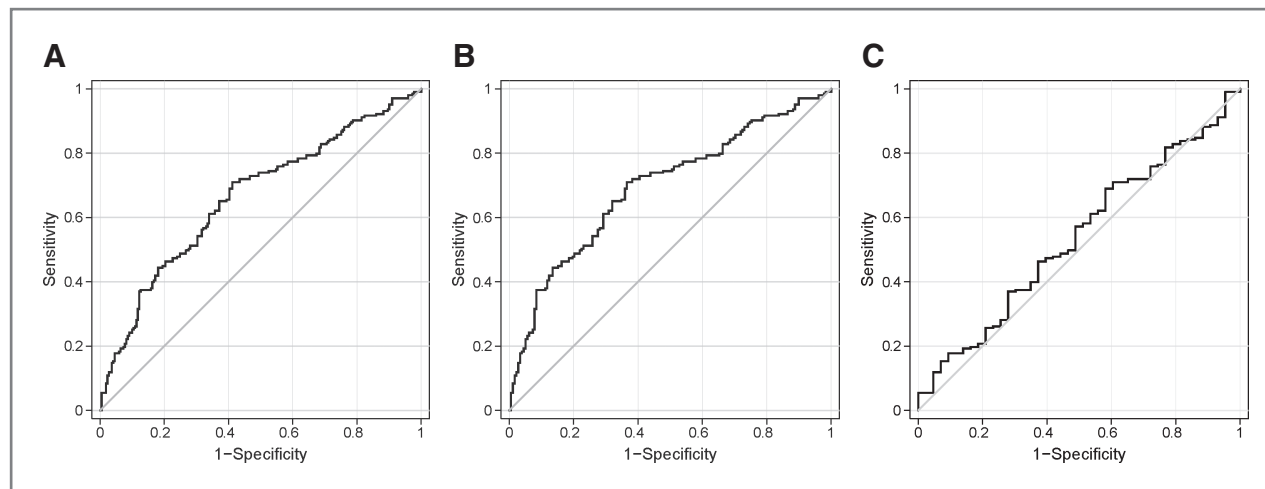


Figure 1. A, ROC curve for pentraxin-3 comparing all cases ($N = 203$) and all controls ($N = 223$). AUC = 0.66, 95% CI, 0.61 to 0.71. B, ROC curve for pentraxin-3 comparing all cases ($N = 203$) and high-risk controls ($N = 180$). AUC = 0.69, 95% CI, 0.64 to 0.75. C, ROC curve for pentraxin-3 comparing all cases ($N = 203$) and other cancer controls ($N = 43$). AUC = 0.54, 95% CI, 0.44 to 0.63.

shown). For this reason, further statistical analyses for these 2 biomarkers were not performed.

The ROC curve for PTX3 for all cases ($N = 203$) versus all controls ($N = 223$), all cases versus high-risk controls ($N = 180$) and all cases versus other cancer controls ($N = 43$) are shown in Figure 1 (A, B, C, respectively). PTX3 has significant discriminatory value, especially when comparing all cases versus high-risk controls (B; AUC = 0.69), which is the most relevant group for population screening purposes.

We further calculated the sensitivity of PTX3 versus high-risk controls and all controls at various specificity cut-offs (Table 1). At 90% and 80% specificity, the sensitivities versus the high-risk controls were 37% and 48%, respectively.

We further performed ROC curve analysis in subgroups of patients, stratified by histology (when available) against

the high-risk control group. The ROC curves for these subgroups are shown in Supplementary Figure S1. A summary of the AUCs for each of the subgroups is shown in Table 2. The ROC curves and associated AUCs are generally similar with all subgroups. Thus, we concluded that PTX3 has similar discriminating ability for all of the major subtypes and histotypes of lung cancer.

There were only 44 patients with known pathological stage: 29 in stage I, 3 in stage II, 8 in stage III, and 4 in stage IV. The small number of patients per stage precludes meaningful analysis but we noticed an increase in AUC (patients versus high-risk controls) from stage I to stage IV, as follows: AUC 0.62 for stage I, 0.64 for stage II, 0.69 for stage III, and 0.72 for stage IV disease (Supplementary Figure S2). For some patients, either the pathological or clinical stage was known. When we analyzed the data

Table 1. Sensitivity and specificity of pentraxin-3 in lung cancer patients

Specificity	Sensitivity			
	Versus high-risk controls ^a		Versus all controls ^a	
	Estimate	95% CI	Estimate	95% CI
0.99	0.054	(0.000–0.158)	0.054	(0.000–0.133)
0.95	0.192	(0.094–0.296)	0.177	(0.118–0.281)
0.90	0.374	(0.212–0.478)	0.251	(0.163–0.399)
0.80	0.478	(0.394–0.557)	0.448	(0.365–0.542)
0.70	0.611	(0.493–0.734)	0.512	(0.419–0.611)
0.60	0.719	(0.621–0.783)	0.655	(0.542–0.734)
0.50	0.744	(0.670–0.798)	0.739	(0.680–0.808)

^aPlease see text for descriptions.

Table 2. Area under the ROC curve per lung cancer type

Lung cancer type	N ^a	AUC ^b	95% CI
All types	120	0.67	0.60–0.74
NSCLC	90	0.65	0.58–0.72
SCLC	13	0.67	0.47–0.83
NSCLC			
Adenocarcinoma	57	0.65	0.56–0.76
Squamous	30	0.63	0.52–0.75

^aNumber of lung cancer patients.

^bComparison to 119 high-risk controls; some patients do not have complete information. AUC, area under the curve.

according to combined stage (either pathological or clinical stage), we found the following: AUC = 0.61 (stage I; *N* = 45), AUC = 0.67 (stage II; *N* = 11), AUC = 0.68 (stage III; *N* = 16), and AUC = 0.61 (stage IV; *N* = 10; Supplementary Figure S3).

Discussion

There is currently a plethora of tumor markers for lung cancer. Such markers include CA125, CA19.9, CA15.3, TAG-72.3, CEA, CYFRA21-1, SCC, and NSE (5). None of these markers is suitable for population screening or diagnosis, because of their low sensitivity and specificity, especially in patients with renal failure and liver diseases. Nevertheless, there are significant differences in the performance of these biomarkers in various subtypes of lung carcinoma. For example, CEA, SCC, CA125, CA15.3, and TAG-72.3 are more elevated in NSCLC than in SCLC. In squamous tumors, SCC is more elevated than the other biomarkers, in comparison to adenocarcinomas. On the other hand, NSE is elevated in a much higher proportion of SCLC patients, in comparison to NSCLC patients. Combinations of these biomarkers can aid in the differential diagnosis of NSCLC and SCLC, and some of these biomarkers are also useful for disease monitoring during therapeutic interventions (13). There is still a need for discovering additional lung cancer biomarkers for screening, diagnosis, prognosis, and monitoring response to therapy.

Recently, we and others have used proteomic approaches to identify biomarkers for various cancer types (14–20). In our own proteomic effort, we identified 5 candidate markers that appear to be elevated in serum of lung cancer patients, in comparison to control subjects (6). One of these markers, PTX3, was also identified as a candidate prostate cancer biomarker, by using similar principles (21). In this investigation, we further validated PTX3, along with another 2 candidate biomarkers, KLK11 and progranulin, in a relatively large cohort of patients and controls, obtained from

EDRN. The limited serum sample availability precluded us from validating the other 4 candidate lung cancer biomarkers.

Although KLK11 and progranulin were not informative biomarkers in this cohort of lung cancer patients, PTX3 was able to discriminate lung cancer patients from high-risk controls with moderate sensitivity (37% at 90% specificity and 48% at 80% specificity). Such sensitivities and specificities are similar to many other lung cancer biomarkers that are currently used in the clinic (5). It will be interesting, in the future, to investigate if PTX3 can be incorporated into a multiparametric panel with other lung cancer biomarkers. Our data further indicate that PTX3 is equally effective in NSCLC and SCLC, as well as with adenocarcinomas and squamous cell carcinomas. The serum levels of PTX3 appear to correlate positively with tumor stage.

Pentraxins are a superfamily of proteins that are characterized by a structural motif, the pentraxin domain, and are divided into short and long pentraxins. PTX3 is a member of the long pentraxin superfamily and appears to play a role in mediating resistance to fungal pathogens (22). PTX3 is overexpressed in some malignancies, including liposarcomas (23). A role for pentraxin in clearance of apoptotic cells has also been suggested (24). PTX3 expression appears to be regulated by various cytokines, including tumor necrosis factor α (TNF α) and IL1 β (25). Under conditions of tissue damage (e.g., myocardial infarction) or infection, PTX3 levels increase more rapidly than C-reactive protein. PTX3 alterations have been seen in sepsis, *Aspergillus fumigatus* infections, tuberculosis, and dengue.

The elevations of PTX3 in serum of patients with lung cancer are not well understood, but its known overexpression by endothelial cells and macrophages in response to inflammatory signals, as well as its role in clearance of cells undergoing apoptosis (24), suggests that PTX3 may act as a biomarker for lung carcinoma, through its elevation in the inflammatory microenvironment of the tumor and in the clearance of apoptotic cells.

We conclude that PTX3 is a novel biomarker for lung carcinoma, which displays comparable sensitivity and specificity to other currently used lung cancer biomarkers. It appears that the observed serum elevations of PTX3 are associated with inflammation and cancer cell apoptosis around the tumor microenvironment. More studies will be necessary to establish if PTX3 has clinical utility in lung cancer, either alone or as a member of a biomarker panel. In such studies, inclusion of patients with benign lung diseases, in order to further assess the specificity of these biomarkers, would be important. As mentioned earlier, PTX3 may also be elevated in other malignancies such as prostate cancer (21) and liposarcomas (23).

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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