Comprehensive analysis of marker gene detection and computed tomography for the diagnosis of human lung cancer

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Abstract. Non-small cell lung cancer (NSCLC) is one of leading causes of cancer-associated mortality, with a high number of cases caused by metastasis. The early diagnosis of cancer contributes to the successful treatment of patients with lung cancer. The aim of the present study was to analyze the efficacy of marker gene detection and computed tomography (CT) in diagnosing human lung cancer. Lung cancer marker genes, including carcinoembryonic antigen (CEA), cancer antigen 125 (CA125), tissue polypeptide antigen (TPA), pro-gastrin-releasing peptide (ProGRB), cytokeratin fragment 21-1 (Cyfra21-1) and neuron-specific enolase (NSE), were analyzed in patients with lung cancer. The tumor size was evaluated using CT, and the association between lung serum levels of marker gene protein expression and tumor size was investigated. A total of 328 patients with lung cancer were identified, including 204 adenocarcinoma, 75 large cell carcinoma and 49 squamous cell carcinoma cases. All patients were indicated to have a high serum level of CEA, CA125, TPA, ProGRB, Cyfra21-1 and NSE, compared with the normal range. Immunohistochemistry demonstrated higher expression levels of CEA, CA125, TPA, ProGRB, Cyfra21-1 and NSE in lung tumor tissues, compared with the normal range. Results indicated that CT was able to diagnose tumor size for patients with lung cancer. The CEA and CA125 expression levels were associated with CT-diagnosed adenocarcinoma tumor size. Large cell carcinoma tumor size was associated with serum levels of CEA, TPA and ProGRB. Results indicated that Cyfra21-1 and NSE were associated with the squamous cell carcinoma cases, as demonstrated using CT. In conclusion, these results indicated that comprehensive analysis of marker gene detection and CT results may be used to diagnose human lung cancer.

Introduction

Non-small cell lung cancer (NSCLC) is one of the most common types of human cancer, and makes up >80% of all lung cancer cases (1,2). Data analyses indicate that the 5-year survival rate is <5% for the majority of patients with NSCLC who have suffered from distant metastasis (3). NSCLC includes adenocarcinoma, large cell carcinoma and squamous cell carcinoma (4-6). Although various strategies exist for the treatment of NSCLC, the survival rate remains poor for affected patients (7); therefore an early diagnosis of NSCLC is beneficial for patients with cancer, so that they can receive the most appropriate therapeutic regimens.

An early diagnosis enables early treatment of lung cancer (8-10). Detection of carcinoembryonic antigen (CEA) in the serum is a sensitive panel for the potential molecular diagnosis of NSCLC (11). Expression of cancer antigen 125 (CA125) is associated with cisplatin susceptibility in human lung cancer cells, which may aid the diagnostic and therapeutic approaches (12). A previous study indicated that tissue polypeptide antigen (TPA) and cytokeratin fragment 21-1 (Cyfra21-1) are serum markers in patients with NSCLC that may be beneficial for the recognition of tumor relapse (13). In addition, pro-gastrin-releasing peptide (ProGRB) is a valuable marker with a high specificity for the diagnosis of lung cancer, with a similar diagnostic accuracy to pathological diagnosis (14). Furthermore, neuron-specific enolase (NSE) is a potential target in the diagnosis and treatment of lung cancer (15). These data indicate that expression levels of CEA, CA125, TPA, ProGRB, Cyfra21-1 and NSE are associated with the progression of lung cancer.

The purpose of the present study was to analyze the efficacy of marker gene detection and computed tomography (CT) in diagnosing human lung cancer. The association between lung cancer marker genes (CEA, CA125, TPA, ProGRB and Cyfra21-1) and tumor size in patients with lung cancer was investigated.

Materials and methods

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Key words: gene detection, computed tomography, lung cancer, tumor size

Ethics statement. The clinical study design was approved by the Ethics Committee of the China Japan Union Hospital (Changchun, China; approval no. 20130701XA) and all
patients provided written informed consent prior to the examinations.

Patients and healthy individuals. A total of 328 patients with NSCLC (186 males and 142 females; median age, 57 years old) and 328 healthy individuals (168 males and 160 females; median age, 57 years old) were enrolled between July 2013 and May 2017 at the China Japan Union Hospital. All patients with NSCLC who received any chemotherapy or radiotherapy prior to surgery were excluded from the study. All subjects in the present study underwent CT diagnosis (16). All subjects in the present study underwent imaging with a trimodality CT system. The characteristics of the patients with NSCLC are summarized in Table I.

CT scan protocol. The CT system consisted of a full-ring time-of-flight 64-slice positron emission tomography (PET)/CT scanner (Discovery PET/CT 690 VCT; GE Healthcare, Chicago, IL, USA). The CT diagnosis system was used to analyze NSCLC tumor size using the preprogrammed setting. The preprogrammed setting was optimized, as described previously (17) to achieve the best image formation. The lungs in all patients underwent contrast-enhanced CT according to the manufacturer’s protocols (Discovery PET/CT 690 VCT). The Tumor-Node-Metastasis (TNM) stage (seventh edition) (18) was assigned based on intraboard consensus decision.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Total RNA was extracted from cancer tissues using an RNAeasy Mini kit (Qiagen GmbH, Hilden, Germany). RNA was reverse transcribed to cDNA using SuperScript™ III One-Step RT-PCR System with Platinum™ Taq High Fidelity DNA Polymerase (Thermo Fisher Scientific, Inc.), according to manufacturer’s protocol. All forward and reverse primers were synthesized by Invitrogen (Thermo Fisher Scientific, Inc.; Table II). The thermocycling conditions were as follows: Initial denaturation at 95˚C for 1 min, followed by 45 cycles of 95˚C for 30 sec, 56˚C for 30 sec and 72˚C for 15 sec. The reaction volume (50 µl) contained 50 ng genomic cDNA, 200 µM dNTPs, 200 µM primers, 2.5 U Taq DNA polymerase (Thermo Fisher Scientific, Inc.) and 2.5 U SYBR®-Green (Thermo Fisher Scientific, Inc.). Relative mRNA expression levels were calculated by the 2-ΔΔCq method (19).

ELISA. A total of 10 ml blood samples were collected from all patients and serum was obtained from centrifugation at 4,500 x g for 10 min at 4˚C. Serum levels of CEA (catalog no. EHCEA; Thermo Fisher Scientific, Inc., Waltham, MA, USA), CA125 (EHMUC16; Thermo Fisher Scientific, Inc.), TPA (BMS258-2; Thermo Fisher Scientific, Inc.), ProGRB (DY7847-05; Bio-Rad Laboratories, Inc., Hercules, CA, USA), Cyfra21-1 (EHCYF211; Thermo Fisher Scientific, Inc.) or NSE (DENL20; Bio-Rad Laboratories, Inc.) were detected in subjects using ELISA kits according to the manufacturer’s protocols. Finally, the serum concentrations of CEA, CA125, TPA, ProGRB, Cyfra21-1 and NSE were measured using an enzyme microplate reader at 450 nm (Bio-Rad Laboratories, Inc.).

Immunohistochemistry staining. Cancer tissues from 328 patients who underwent surgery for NSCLC were collected in the present study. The 328 tissue samples were fixed used 10% paraformaldehyde for 15 min at 37˚C and stained with hematoxylin and eosin for 30 min at 37˚C, and final pathological diagnoses were confirmed. Immunohistochemical analysis was used to identify expression levels of NSCLC tumor markers CEA, CA125, TPA, ProGRB, Cyfra21-1 and NSE. Paraffin-embedded tissue sections (4-µm thick) were prepared and dewaxed in xylene for 12 h at 37˚C, followed by rehydrating in a gradient concentration of ethanol (100, 95 and 85%). The paraffin sections were immersed in 3% H2O2, at room temperature for 5 min at 37˚C and antigen retrieval was performed at 95˚C for 30 min using antigen retrieval buffer [10 mM citrate acid, 0.05% Tween-20 (pH 6.0)]. Following incubation with PBS containing 10% bovine serum albumin (Beijing Solarbio Science & Technology Co., Ltd., Beijing, China) for 2 h at 37˚C, the tissue sections were incubated at 4˚C overnight with the following anti-human antibodies: CEA (1:1,000; cat. no. ab133633; Abcam, Cambridge, UK), CA125 (1:1,000; cat. no. ab10033; Abcam), TPA (1:1,000; cat. no. ab157469; Abcam), ProGRB (1:1,000; cat. no. ab158593; Abcam), Cyfra21-1 (1:1,000; cat. no. ab25831; Abcam) or NSE (1:1,000; cat. no. ab757575; Abcam), followed by further incubation with horseradish peroxidase-conjugated IgG monoclonal antibody (cat. no. PV-6001, OriGene Technologies, Inc., Beijing, China) at 4˚C overnight with diaminobenzidine-peroxidase substrate (Gene Tech, Biotechnology Co., Ltd., Shanghai, China). Slides were finally counterstained with hematoxylin for 2 h at 37˚C and mounted with coverslips, and images were captured using a MicroChemi 4.2 instrument (Eastwin, Shenzhen, China) at x40, magnification.

Table I. Characteristics of patients with NSCLC.

| Characteristics | NSCLC patients | Healthy patients |
|-----------------|----------------|-----------------|
| Total no., n    | 328            | 328             |
| Males, n        | 186            | 168             |
| Females, n      | 164            | 160             |
| Age range (mean), years | 36-68 (57) | 36-68 (57) |

NSCLC, non-small cell lung cancer.

Regression analysis. The serum levels of CEA, CA125, TPA, ProGRB, Cyfra21-1 and NSE in the detective data (Y) were analyzed by regression analysis in various tumor sizes in patients with NSCLC using the least square convergence (20). Serum levels of CEA, CA125, TPA, ProGRB, Cyfra21-1 and NSE were predicted according to tumor size. The predicted curve that results in the lowest sum of squares is the best fit using Spearman’s rank correlation analysis. If the fit is robust, then the parameters of the observed curve may be inferred from those of the predicted curve.

Statistical analysis. All data are presented as the mean ± standard error. All data were analyzed using SPSS Statistics (version 19.0; IBM Corp., Armonk, NY, USA). Student’s t-test were used to make comparisons between groups. P<0.05 was considered to indicate a statistically significant difference.
Results

Serum levels of tumor markers in patients with lung cancer. In the present study, the serum levels of tumor markers were analyzed in 328 patients with NSCLC and age-matched healthy volunteers. The results demonstrated that serum levels of CEA, CA125, TPA, ProGRB, Cyfra21-1 and NSE were increased in patients with NSCLC compared with those in the healthy volunteers (Table III). These results indicated that tumor markers of CEA, CA125, TPA, ProGRB, Cyfra21-1 and NSE are associated with the progression of NSCLC.

Marker gene expression in NSCLC tissues. The expression of CEA, CA125, TPA, ProGRB, Cyfra21-1 and NSE marker genes was examined in NSCLC tissues. It was indicated that gene expression levels of CEA, CA125, TPA, ProGRB, Cyfra21-1 and NSE were upregulated in NSCLC tissues compared with those in the corresponding adjacent non-tumor tissues (Table IV). Immunohistochemistry demonstrated that expression levels of CEA, CA125, TPA, ProGRB, Cyfra21-1 and NSE were notably upregulated in NSCLC tissues compared with those in the corresponding adjacent non-tumor tissues (Fig. 2). These results indicated that NSCLC tissues...
These results indicated that comprehensive analysis of marker gene detection and CT for NSCLC diagnosis was comprehensively analyzed. It was demonstrated that serum levels of CEA, CA125, TPA, ProGRB, Cyfra21-1 and NSE were significantly upregulated in patients with NSCLC. Results of the present study also indicated that marker gene expression levels were correlated with tumor size determined by CT for patients with NSCLC, according to Spearman's rank correlation analysis.

Currently, there are numerous cancer diagnostic methods, including ultrasound, X-ray screening, CT, magnetic resonance imaging and immunological detection (24-26). CT (fluorodeoxyglucose PET/CT) is frequently performed in hilar and mediastinal lymph node staging of NSCLC (27). The present study demonstrated that CT could approximately measure the NSCLC tumor size. A previous study indicated that PET/CT has become feasible for fast imaging and may be used for cancer staging in patients with a malignant condition (28). In addition, three-dimensional CT measurement may be useful for diagnosing nodal metastases (29). Notably, the present study determined that single CT diagnosis is insufficient in the diagnosis of patients with NSCLC.

The clinical value of a cancer diagnosis with tumor marker determination has been identified in lung cancer (30,31). The present study demonstrated that tumor marker expression combined with CT is an efficient method of diagnosing patients with NSCLC. A previous study demonstrated the clinical value of CEA and CA125 to evaluate the relapse, metastasis and prognosis of patients with resectable NSCLC (32). The present study demonstrated that serum levels of CEA and CA125 levels were associated with CT-diagnosed adenocarcinoma tumor size. In addition, Wang et al (33) indicated that the serum concentrations of Cyfra21-1, NSE and CEA may be diagnostic indicators of meningeal carcinomatosis of lung cancer. Results of the present study indicated that Cyfra21-1 and NSE were associated with the squamous cell carcinoma cases. A previous meta-analysis indicated that the serum level of ProGRB is a promising biomarker for SCLC diagnosis (34). In the present study, it was indicated that the large cell carcinoma tumor size was associated with serum levels of CEA and ProGRB. These data have identified that the tumor markers CEA, CA125, TPA, ProGRB, Cyfra21-1 and NSE are useful in determining tumor size.

Discussion

Lung cancer is a respiratory disease that is a major cause of cancer-associated mortality resulting from air contamination caused by industrial pollution globally (21). It has been indicated that the early diagnosis of patients with NSCLC is beneficial for the treatment of cancer (22). Serum tumor markers have been demonstrated to possess the ability to predict the efficacy of pemetrexed-based chemotherapy (23). In the present study, the efficacy of marker gene detection and CT for human lung cancer diagnosis was comprehensively analyzed. It was demonstrated that serum levels of CEA, CA125, TPA, ProGRB, Cyfra21-1 and NSE were significantly upregulated in patients with NSCLC. Results of the present study also indicated that marker gene expression levels were correlated with tumor size determined by CT for patients with NSCLC.

These results indicated that comprehensive analysis of marker gene detection and CT is an efficient method for the diagnosis of patients with NSCLC.

Efficacy of comprehensive analysis of marker gene detection and CT for NSCLC diagnosis. Finally, the diagnostic efficacy of marker gene detection combined with CT was investigated in a total of 328 patients with NSCLC. It was demonstrated that comprehensive analysis of marker gene detection and CT was able to diagnose 320 patients with NSCLC, whereas CT alone diagnosed 256 patients with NSCLC and marker gene detection diagnosed 270 patients with NSCLC (Table VI). It was determined that only 8 patients with NSCLC could not be diagnosed using marker gene detection combined with CT. These results indicated that comprehensive analysis of marker gene detection and CT is an efficient method for the diagnosis of patients with NSCLC.

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In conclusion, the present study investigated the effects of tumor marker genes combined with CT in diagnosing patients with NSCLC. The results demonstrated that tumor markers CEA, CA125, TPA, ProGRB, Cyfra21-1 and NSE were significantly upregulated in NSCLC tissues. Furthermore, it was determined that tumor marker serum levels were associated with tumor size.
Figure 2. Expression levels of CEA, CA125, TPA, ProGRB, Cyfra21-1 and NSE in NSCLC tissues compared with those in corresponding adjacent non-tumor tissues, as determined by immunohistochemistry. Magnification, x40. CEA, carcinoembryonic antigen; CA125, cancer antigen 125; TPA, tissue polypeptide antigen; ProGRB, pro-gastrin-releasing peptide; Cyfra21-1, cytokeratin fragment 21-1; NSE, neuron-specific enolase; NSCLC, non-small cell lung cancer.

Figure 3. Representative images of tumor size diagnosed by computed tomography in patients with NSCLC. NSCLC, non-small cell lung cancer.

Figure 4. Analysis of the association between marker gene detection and tumor size. (A) Serum levels of CEA and CA125 are associated with CT-diagnosed adenocarcinoma tumor size. (B) Serum levels of CEA, TPA and ProGRB are associated with large cell carcinoma tumor size. (C) Serum levels of Cyfra21-1 and NSE are associated with CT-diagnosed squamous cell carcinoma tumor size. CEA, carcinoembryonic antigen; CA, cancer antigen 125; TPA, tissue polypeptide antigen; ProGRB, pro-gastrin-releasing peptide; Cyfra21-1, cytokeratin fragment 21-1; NSE, neuron-specific enolase; CT, computed tomography.
Importantly, tumor markers combined with CT may contribute to diagnosing the progression and treatment of NSCLC cancer.

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Availability of data and materials

The analyzed data sets generated during the study are available from the corresponding author on reasonable request.

Authors' contributions

MC conducted major experiments in this study. XS, GL, KC and ZL conducted a small number of experiments. CS and DX analyzed the experimental data in the present study. LL designed all experiments in the present study.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of the China Japan Union Hospital (Changchun, China; approval no. 20130701XA).

Patient consent for publication

All patients provided written informed consent prior to the examinations.

Competing interests

The authors declare that they have no competing interests.

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