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Research paper

Immunohistochemical and genetic characteristics of lung cancer mimicking organizing pneumonia

Tomohiro Ichikawa[a,b,c], Koichi Saruwatari[d], Sachiyo Mimaki[e], Masato Sugano[a], Keiju Aokage[b], Motohiro Kojima[a], Tomoyuki Hishida[b], Satoshi Fujii[a], Junji Yoshida[b], Takeshi Kuwata[a], Atsushi Ochiai[a], Kenji Suzuki[c], Masahiro Tsuboi[b], Koichi Goto[d], Katsuya Tsuchihara[e], Genichiro Ishii[a,*]

[a] Division of Pathology, Exploratory Oncology Research and Clinical Trial Center, National Cancer Center Hospital East, Japan
[b] Division of Thoracic Surgery, National Cancer Center Hospital East, Japan
[c] Department of General Thoracic Surgery, Juntendo University School of Medicine, Japan
[d] Division of Thoracic Oncology, National Cancer Center Hospital East, Japan
[e] Division of Translational Research, Exploratory Oncology Research and Clinical Trial Center, National Cancer Center, Japan

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ABSTRACT

Introduction: Lung cancer mimicking organizing pneumonia (LCOP) is a novel radiological entity of lung adenocarcinoma that could be misdiagnosed as inflammatory lesions. However, the characteristic biological and genetic features of LCOP are not fully clarified.

Materials and methods: We used thin-section CT images to select cases of (LCOP) among surgically resected lung adenocarcinoma patients. We compared the clinicopathological characteristics and the immunophenotypes of LCOP (n = 44) and other lepidic-predominant adenocarcinomas (non-LCOP, n = 56). We also analyzed the genomic mutation features of LCOP (n = 4) by whole-exome sequencing (WES).

Results: All LCOP lesions were lepidic-predominant invasive adenocarcinoma. Patients with LCOP had significantly superior recurrence-free survival, compared to non-LCOP patients (95.5% and 74.4%; P = 0.006, respectively). Vascular invasion and lymph node metastasis were less frequent in LCOP than in non-LCOP patients (P = 0.001 and P = 0.03, respectively). The cancer cell expression levels of aggressiveness-related molecules, including ezrin, ALDH-1, laminin-5 were similar between LCOP and non-LCOP. On the contrary, the number of tumor promoting stromal cells, i.e., podoplanin-positive cancer-associated fibroblasts and CD204-positive tumor associated macrophages, was significantly lower in LOCP (P = 0.021 and P = 0.037, respectively). WES revealed that ABCB1, DNAH3, MSI2, and SLITRK2 were specifically mutated in LCOP.

Conclusions: Our results indicate that LCOP is characterized by fewer tumor-promoting stromal cells, which may contribute to the better prognosis of LCOP patients. Moreover, recognition of specific somatic mutations of LCOP patients may provide information regarding the development and progression of this type of lung cancer.

1. Introduction

With the introduction of computed tomography (CT) screening for lung cancer, the detection rate of early-stage lung cancer has been increasing [1]. There is occasionally lung adenocarcinoma mimicking organizing pneumonia on CT. This entity is characterized by radiological features including air-bronchograms, bubble-like lucencies, and scattered consolidation, which have been reported as predictors of good prognosis [2–5]. We previously reported the first case series of this radiological entity of lung adenocarcinoma, with the term of lung cancer mimicking organizing pneumonia (LCOP). LCOP may be a radiological indicator of an excellent prognosis, as the thirteen patients of LCOP included in that study tended to have favorable outcomes regardless of the large tumor diameter. With respect to pathological findings, most tumors exhibited a central collapse/fibrosis region in which only a small number of cancer cells were found [6].

Several tumor biomarkers are reported to affect prognosis in patient with lung adenocarcinoma. They include ezrin and laminin-5, which

Abbreviations: LCO, Plung cancer mimicking organizing pneumonia; PDPN- CAFs, podoplanin-positive cancer associated fibroblasts; CD204 + TAMs, CD204-positive tumor-associated macrophages

* Corresponding author at: Division of Pathology, Exploratory Oncology Research and Clinical Trial Center, National Cancer Center Hospital East, Japan.
E-mail addresses: gishi@east.ncc.go.jp, ishii691@gmail.com (G. Ishii).

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are functional markers for invasiveness of cancer cells, and ALDH-1, which is a marker for cancer stem-like cells [7–9]. The prognostic significance of stromal cells in cancer cell proliferation, invasiveness, and metastasis is also increasingly recognized [10,11]. We have previously reported that podoplanin-positive cancer associated fibroblasts (PDPN-CAFs), as well as CD204-positive tumor-associated macrophages (CD204 + TAMs), have tumor-promoting functions [12,13].

Certain genetic changes are characteristic of lung adenocarcinomas. Data from molecular genetic analysis have been used to enhance the system of lung cancer classification, whereas certain gene mutations have been found to be responsible for specific histomorphological features of lung cancers. For example, EGFR mutations correlate with the papillary and micropapillary morphology, whereas ALK-rearranged adenocarcinomas exhibit an acinar pattern and extracellular mucus production [14,15]. Molecular study may discover gene mutations specific to LCOP that are responsible for its specific histopathological features.

In this study, we examined larger cohort of patients with LCOP and compared them to those with the other adenocarcinoma subtypes, with special interest in micro-environmental and genomic characteristics of LCOP.

2. Material and methods

2.1. Patient selection and evaluation of CT images

We retrospectively searched our institutional database for patients who had undergone complete tumor resection and complete hilar and mediastinal lymph node dissections for pulmonary adenocarcinoma in our institute between January 2002 and December 2014. Patients who were preoperatively examined with thin-section computed tomography (TSCT) were enrolled. Patients who underwent previous lung surgery or preoperative chemotherapy and/or radiotherapy were excluded from the study. All specimens were collected after obtaining written informed consent from the patient, and hhis study was approved by the institutional review board of the National Cancer Center East Hospital. Each patient was informed that his or her clinical data could be used for various studies, and consent was obtained on that basis. IRB approval numbers of this study are 2016-220 and 2009-196.

All TSCT images were reviewed by one thoracic surgeon (TI) and one thoracic oncologist (KS) with an experience in chest CT interpretation, who were blinded from patients' clinicopathological information. The diagnostic criteria of LCOP, as described in our former study, are as follows: (a) irregular extension of consolidation along the bronchovascular tree toward the hilum, (b) bronchial wall thickening or dilatation, and (c) surrounded by only a few slight peripheral ground-glass opacity areas. Images from 3 patients are shown in Supplemental Fig. 1A–D. Based on these criteria, we radiologically divided the patients into those with LCOP and those with non-LCOP lesions.

2.2. Histological evaluation

All specimens were fixed after infusion of 10% of formalin or methanol through bronchial tree and embedded in paraffin. The tumors were sliced at approximately 5 mm intervals and serial 4 μm sections were stained with hematoxylin-eosin (H-E). The Alcian blue-periodic acid Schiff (AB-PAS) and Verhoeff-van Gieson (VVG) method were performed to visualize cytoplasmic mucin production and elastic fibers, respectively. Vascular invasion and pleural invasion were determined by the VVG staining. Lymphatic permeation was observed in sections stained with H-E. The histological diagnoses were based on the fourth revised World Health Organization histologic classification. Tumors were evaluated for lepidic, acinar, papillary, solid, and micropapillary patterns according to the 2015 IASLC/ATS/ERS classification, and the percentage of each of these subtype were shown in the histology reports; the subtype that largest area was considered to be the predominant subtype. All tumors were staged pathologically using the 7th edition of the TNM classification of lung cancer.

Medical records of each patient were used to extract information with regard to sex, age, smoking status, serum carcinoembryonic antigen (CEA) levels at the time of operation, presence of tumor nodules within the primary lobe, maximum tumor diameter, regional lymph node involvement, lymphatic and vascular permeation, and pleural visceral invasion.

2.3. Immunohistochemical staining

Immunohistochemical staining was performed according to the method as previously reported. [16] For the marker of carcinoma cells, ezrin (1:200, Cell Signaling Technology Inc.), aldehyde dehydrogenase-1 (ALDH-1) (1:400, 44/ALDH, BD Transduction Laboratories), and laminin-5 (1:200, D45B, Chemicon, Temecula, CA) were evaluated. The cleaved caspase-3 (1:300, Cell Signaling Technology Inc.) was used as tumor cell apoptosis maker. For stromal cell markers, CD204 (1:400, SRA-E5, Trans Genic, Japan) was used as tumor associated macrophage marker, and podoplanin (PDPN) (1:50, D2-40, Acris Antibodies) was used as cancer associated fibroblast (CAF) marker.

2.4. Calculation of immunohistochemical scores

Immunostaining scores were the product of staining-intensity score and percentage of positively stained cells, except for CD204 and cleaved caspase-3. Staining intensity scores were 0 (negative; total absence of staining), 1 + (weak staining), 2 + (strong staining); and were multiplied by percentages of immnohistochemically stained tumor cells per section (0–100%), resulting in scores ranging from 0 to 200. The numbers of CD204 positive tumor associated macrophages (TAMs) and cleaved caspase-3 positive tumor cells were counted under a light microscope in high power fields (HPF, 400 x:0.0625 mm^2/field). The average number of positive cells in 3 HPFs was recorded as the scores of CD204 + TAMs and cleaved caspase-3 positive cancer cell. All the immunohistochemical slides were evaluated by two independent pathologists (TI and GI). Both were unaware of clinical and pathological information.

2.5. Whole-exome sequencing

We previously released whole-exome sequencing data from 97 Japanese lung adenocarcinomas as tumor/normal pairs [17]. The sequencing data were deposited into the National Bioscience Database Center (NBDC) under research ID hum0004.v1 and data set ID JGAD00000000001. We used these data to analyze the mutation profile of LCOP.

2.6. Statistical analysis

The cumulative overall survival rates were estimated by the Kaplan-Meier method and compared using the log-rank test. The date of surgical resection was set as the starting point and the date of death or last date of follow-up as the end point. Unpaired *t*-test or chi-square test was used to compare clinicopathological factors between two variables. Mann- Whitney *U* test was used for immunostaining scores. All the statistical calculations were performed by JMP software (version 10.0, SAS Institute, Cary, NC). Statistical analysis was considered significant when the probability value was less than 0.05.

3. Results

3.1. Clinicopathological features of LCOP

The characteristics of the patients with LCOP are presented in