Lung Cancer 81 (2013) 371–376

Contents lists available at SciVerse ScienceDirect

Lung Cancer

journal homepage: www.elsevier.com/locate/lungcan

The utility of the proposed IASLC/ATS/ERS lung adenocarcinoma subtypes for disease prognosis and correlation of driver gene alterations

Koji Tsuta[a,*,1], Mitsumasa Kawago[a,b,1], Eisuke Inoue[c], Akihiko Yoshida[a], Fumiaki Takahashi[c], Hiroyuki Sakurai[b], Shun-ichi Watanabe[b], Masahiro Takeuchi[c], Koh Furuta[a], Hisao Asamura[b], Hitoshi Tsuda[a]

[a] Division of Pathology and Clinical Laboratory Division, National Cancer Center Hospital, Tokyo, Japan
[b] Division of Thoracic Surgery, National Cancer Center Hospital, Tokyo, Japan
[c] Department of Clinical Medicine (Biostatistics), School of Pharmacy, Kitasato University, Tokyo, Japan

ARTICLE INFO

Article history:
Received 19 March 2013
Received in revised form 12 June 2013
Accepted 24 June 2013

Keywords:
IASLC classification
Adenocarcinoma of the lung
ALK
EGFR
KRAS

ABSTRACT

Background: The present study aimed to determine the ability of the revised International Association for the Study of Lung Cancer (IASLC)/American Thoracic Society (ATS)/European Respiratory Society (ERS) classification of lung adenocarcinoma to predict patient survivals and driver gene alterations.
Patients and Methods: A reclassification of 904 surgically resected adenocarcinomas was performed. The results were statistically analyzed to examine the correlation between the classification and overall survival (OS) using Cox regression analyses, and integrated discrimination improvement (IDI) analyses.
Results: The 5-year OS rates for adenocarcinomas in situ (AIS) or minimally invasive adenocarcinoma (MIA) were 98%. Five-year OS rates of Lepidic-, acinar-, papillary-, micropapillary-, and solid-predominant adenocarcinomas was 93%, 67%, 74%, 62%, and 58%, respectively. The IDI estimates revealed that classification of ADC into the 7 subgroups had a higher estimated (0.0175) than did the combined histological grouping (AIS + MIA, lepidic + acinar + papillary, micropapillary + solid + others) (0.0111). Epidermal growth factor receptor mutations, *KRAS* gene mutations, and anaplastic lymphoma kinase gene alterations were statistically prevalent in papillary-predominant ($P = 0.00001$), invasive mucinous ($P = 0.00001$), and micropapillary- and acinar-predominant ($P = 0.00001$) adenocarcinomas, respectively.
Conclusions: The new classification reflects disease prognosis, and was also associated with driver gene alterations.

1. Introduction

Primary lung adenocarcinomas (ADC) have 4 architectural growth patterns: bronchioloalveolar, acinar, papillary, and solid [1,2]. Although approximately 80% of invasive ADCs include 2 or more of these patterns, most cases have been referred to as "mixed adenocarcinoma," based on the latest World Health Organization (WHO) 2004 classification [1,3]. Several current clinicopathological and molecular analyses have revealed that architectural growth patterns of lung ADCs are correlated with both patient survival and the gene mutation profiles of the tumors [3–5]. To improve the clinical utility of histological classification system, the International Association for the Study of Lung Cancer (IASLC), American Thoracic Society (ATS), and European Respiratory Society (ERS) have cooperatively organized an updated classification of lung ADCs [2].

In the IASLC/ATS/ERS classification [2], there are several major changes pertaining to surgically resected tumors: (1) the term bronchioloalveolar carcinoma (BAC) is no longer used and is, instead, referred to as a lepidic pattern; (2) ADC in situ (AIS) describes small (≤3 cm), solitary ADCs with pure lepidic growth, which have not invaded surrounding normal tissue; (3) minimally invasive ADC (MIA) describes small (≤3 cm) predominantly lepidic tumors with ≤0.5 cm of invasion; (4) invasive ADCs are now classified according to their predominant subtype; (5) micropapillary ADC was added as a subtype because of its poor prognosis; (6) the former mucinous BACs are now classified as invasive mucinous ADCs (IMA); and (7) the terms clear-cell and signet-ring ADC were changed from histological subtypes to cytological features. The ADC variants listed in the IASLC/ATS/ERS classification include invasive mucinous, colloid, enteric, and fetal adenocarcinomas.

* Corresponding author at: The Division of Pathology and Clinical Laboratory, National Cancer Center Hospital, 1-1 Tsukiji 5-chome, Chuo-ku, Tokyo 104-0045, Japan. Tel.: +81 3 3542 2511; fax: +81 3 3545 3567.
E-mail address: ktsuta@ncc.go.jp (K. Tsuta).
[1] These authors contributed equally to this study.

0169-5002/$ – see front matter © 2013 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.lungcan.2013.06.012

Recently, 4 research groups analyzed the association between the IASLC/ATS/ERS classification scheme and survival [6–9]. These studies concluded that the IASLC/ATS/ERS classification identified histological subtypes of lung ADCs that were of significant prognostic value. However, one of these reports analyzed only stage I cases, and the population of the other reports was analyzed the 5-year overall survival (OS) rate. In addition, neither report analyzed the association between the IASLC/ATS/ERS classification and the major driver-gene alterations.

In the present study, we analyzed the association between the IASLC/ATS/ERS classification with survival and the ability of this classification scheme to predict gene abnormalities, such as *EGFR*, *KRAS*, or anaplastic lymphoma kinase (*ALK*) gene alterations, using a large series of surgically resected lung ADCs. We also analyzed the relevant T-stage of newly proposed AIS and MIA and describe the possible histological factors affecting the survival in T1 tumors.

2. Materials and methods

2.1. Study population

This study was approved by the Institutional Review Board. Tumor specimens, surgically resected from patients diagnosed with primary lung ADC between January 1998 and December 2002, were retrospectively reviewed. Clinical information was collected regarding the patients' ages, genders, smoking histories, and duration of any tumor recurrences and survival.

2.2. Histological analyses

The pathologic records of the specimens and all available hematoxylin and eosin (HE)-stained tissue sections, in addition to any available sections with special stains or immunohistochemical analyses, were reviewed. Pathological information, including maximum tumor sizes (in cm) and pathologic disease stages (p-stage), was collected. Staging was based on the guidelines of the 7th edition of the TNM classification for lung cancer [10].

All available HE-stained sections, for each case, were examined by 2 researchers blinded to the clinical details of the patient. Histological classification was performed according to the IASLC/ATS/ERS classification of lung ADCs [2]. Lymphovascular invasion was evaluated by examining sections stained with HE and/or the Elastica van Gieson method.

2.3. Analyses of the EGFR and KRAS mutational status, and ALK rearrangement

The mutational status of *EGFR* and *KRAS*, and *ALK* rearrangements were examined. Two common *EGFR* mutations, deletions in exon 19 (DEL) and a point mutation at codon 858 in exon 21 (L858R), and *KRAS* mutations in exons 1 and 2 were detected using high-resolution melting analysis [11]. *ALK* translocations were analyzed by immunohistochemistry, reverse transcription-polymerase chain reaction (RT-PCR), and/or fluorescence in situ hybridization assays, according to previously published methods [12].

2.4. Statistical analyses

Means, medians, ranges, and proportions were used as descriptive statistics. The association between driver gene alterations and ADC subtypes was analyzed by a logistic model adjusted for age, gender, and smoking status. The 5- and 10-year survival rates for each subtype were calculated by the Kaplan–Meier estimator. Among patients with T1 tumors, survival was compared with the clinicopathologic factors using the Cox model.

To examine the significance of each ADC subtype, the Cox models, with and without subtypes, were constructed and likelihood ratio testing of the model was performed. These models were also adjusted by age, gender, stage, and lymphovascular invasions. To assess the improvement of the model performance by adding ADC subtype, the integrated discrimination improvement (IDI) [13] value was calculated and used to evaluate the 10-year risk of death. The IDI value is equivalent to the difference in R2 of the models, with and without subtypes. It is possible to evaluate the gain in predictive ability by adding subtypes to the model. The statistical significance of the IDI values was assessed by examining their 95% confidence intervals, which were calculated by the percentile bootstrap method [13].

All tests were two sided with a 0.05 type I error. *P*-Values were reported without multiple comparison adjustments. Data were analyzed with R version 2.14 [14].

3. Results

3.1. Patient characteristics

Tumor specimens from 956 patients with an original diagnosis of primary lung ADC were reviewed. Patients were excluded if they had received preoperative therapy ($n = 13$), did not have specimens available for review ($n = 9$), had pleural disseminations or malignant effusions ($n = 15$), or were reclassified with lung tumors of histological types other than ADC ($n = 15$). Therefore, the final cohort consisted of 904 patients with lung ADCs.

In this study population, there were 445 (49%) women and 459 (51%) men. The median age was 63 years (range: 23–89-years). All patients had provided documentation of their smoking history; 446 (49.3%) patients were never smokers and 458 (50.7%) were former or present smokers at the time of their diagnoses. Tumor sizes ranged from 0.4 to 17.5 cm (mean, 2.7 cm). Lymphovascular invasions were noted in 440 (48.7%) cases. Five hundred six patients had p-T1 disease, 297 had p-T2, 83 had p-T3 disease, and 18 had p-T4 disease. Lymph node status was recorded in 869 (96.1%) patients, and metastasis was observed in 217 (25%). Five hundred seventy-nine patients had p-stage I disease, 149 had p-stage II disease, and 141 had p-stage III disease.

Among the 904 cases, 69 (8%) cases were AIS and 33 (4%) cases were minimally invasive adenocarcinoma (MIA). Among the 757 cases of invasive adenocarcinoma, the most prevalent variant was papillary predominant ADC (338 cases; 37%; PPA), followed by lepidic-predominant ADC (136 cases; 15%; LPA), solid with mucin production-predominant ADC (124 cases; 14%; SPA), acinar-predominant ADC (98 cases; 11%; APA), and micropapillary-predominant ADC (61 cases; 7%; MPA). There were 45 (5%) cases of IMA, but no cases of mucinous AIS or MIA, or colloid, fetal, or enteric ADC. Clinical and pathological characteristics subdivided by IASLC/ATS/ERS histological subtype were shown in Table 1.

3.2. Survival analysis

Tumor recurrence was observed in 272 (30.1%) of the 904 patients. The mean follow-up time for all 904 patients was 8.2 years (range, 0.19–13.8 years), with 569 (62.9%) patients still alive at the time of the writing of this report. The 5-year OS was 76% for the 904 patients. None of 102 patients with AIS or MIA demonstrated recurrence.

The 5- and 10-year OS rates for AIS and MIA was 98% and 94%, respectively. These OS rates included 6 deaths by other cancers or diseases; therefore, the 5- and 10-year disease-specific survival rates were both 100%. The 5- and 10-year OS rates for the different subtypes, respectively, were 93% and 85% for LPA; 67% and 47% for