Lung Cancer 118 (2018) 30–35

Contents lists available at ScienceDirect

Lung Cancer

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

The ratio of cancer cells to stroma within the invasive area is a histologic prognostic parameter of lung adenocarcinoma

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ARTICLE INFO

Keywords:
Lung adenocarcinoma
Prognosis
Elastic fiber
Cancer cell ratio
Invasive area

ABSTRACT

Objectives: This study evaluated whether the proportion of cancer cells to non-cancerous stroma within the invasive area is associated with the prognosis of patients with lung adenocarcinoma.
Materials and methods: A total of 127 patients with lung adenocarcinomas with tumors larger than 3 cm in total size were enrolled in this study. We classified the tumors according to the ratio of area occupied by cancer cells within the invasive area (Type A: more than 50% of the invasive area, Type B: 10–50%, and Type C: less than 10%) and analyzed the clinicopathological differences between Types A, B, and C.
Results: The invasive size of Type A tumors (n = 35) was significantly larger than those of the other two tumor types; however, there was no significant difference in the invasive size between Types B (n = 65) and C (n = 25) tumors. The recurrence-free survival time of patients with Type C tumors was significantly longer than those of patients with Type A and B ($P < .001$) tumors. Multivariate analysis revealed that Type C tumor was an independent favorable prognostic factor ($P = .037$) but that invasive size was not. The invasive area of Type C tumor was composed of a significantly higher proportion of collapsed elastic fibers than the invasive areas of Type A and B tumors ($P < .001$).
Conclusion: A lower cancer cell to stroma ratio within the invasive area could be a significant prognostic factor in lung adenocarcinoma, suggesting that not only the invasive size but also the invasive character might be an important histologic prognostic parameter.

1. Introduction

The prognostic significance of the tumor size in lung cancer has been confirmed by a number of investigators [1–3]. Therefore, the TNM staging system for lung cancer has been based on the total tumor size [4,5]. However, recent studies have revealed pathological invasive size to be a better predictor of survival than total tumor size in lung adenocarcinoma [6,7]. In the 8th edition of the TNM classification of lung cancer, only the invasive area, which is determined according to the area excluding the lepidic component, is used as a descriptor of the T categories [8]. However, there are substantial differences in the outcomes of patients with the same stage lung adenocarcinomas following surgical resection. Therefore, the identification of prognostic indicators beyond the TNM stage is of great interest.

Invasive tumor area is composed of not only cancer cells but also of various kinds of non-cancerous components including stromal cells, namely fibroblasts, vascular-constituent cells, immune cells, and extracellular matrix fiber, including collagen and elastic fibers. Certain kinds of stromal cells influence cancer cell proliferation, invasiveness, and metastasis [9–13]. Therefore, it is important to identify prognostic factors to establish quantitative and/or qualitative analysis of stromal cells.

We reported that one entity of lung adenocarcinoma had a large central invasive area comprising a low number of tumor cells and a large amount of collapsed elastic fibers. This type of adenocarcinoma also had significantly better outcomes [14,15]. These findings suggest

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https://doi.org/10.1016/j.lungcan.2018.01.023
Received 25 October 2017; Received in revised form 25 January 2018; Accepted 29 January 2018

that an invasive area with fewer tumor cells and a large amount of Verhoeff-Van Gieson (VVG)-positive elastic fibers may be an important histologic parameter for predicting outcome.

Based on these results, we hypothesized that the character of the invasive area also plays an important role in the prognosis of patients with larger lung adenocarcinoma. To address this hypothesis, we retrospectively classified patients into three groups according to the proportion of cancer cells to non-cancerous stroma within the invasive area and investigated the relationship between the proposed classification and prognosis. In addition, to evaluate the invasive character from the viewpoint of the non-cancerous component, we measured the proportion of VVG-positive elastic fibers within the invasive area and explored the relationship between the proportion of elastic fiber and patient prognosis.

2. Materials and methods

2.1. Patient selection

We retrospectively searched our institute's database for participants who had undergone complete tumor resection and complete hilar and mediastinal lymph node dissection for lung adenocarcinoma more than 3 cm in size between January 1999 and December 2003. Patients who had previously received lung surgery or preoperative therapy, including chemotherapy and radiotherapy, were excluded from the study. A total of 127 patients were included in this study. This study was approved by the institutional review board of the National Cancer Center (approval numbers 2016–220). Each patient was informed that his or her clinical data could be used for various studies and comprehensive informed consent was obtained on that basis.

2.2. Histological evaluation

All specimens were fixed via infusion through the bronchial tree with 10% formalin or methanol 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). Alcian blue-periodic acid Schiff and VVG staining was performed to visualize cytoplasmic mucin production and elastic fibers, respectively. All slides, each containing the maximum surface area of the tumor from each case, were coded and masked for identifiable information and reviewed by two pathologists (T.I. and G.I.). Vascular and pleural invasion were determined by VVG staining. Lymphatic permeation was determined in sections stained with H-E. Histological diagnoses were based on the 4th revised World Health Organization (WHO) histologic classifications. Specimens were classified as lepidic, acinar, papillary, solid, or micropapillary according to the 4th edition of the WHO Classification of Tumours of the Lung, Pleura, Thymus and Heart; the percentages of each subtype were shown in the histology reports, with the subtype with the largest area considered the predominant subtype. The invasive component was clearly defined according to the International Association for the Study of Lung Cancer (IASLC)/American Thoracic Society/European Respiratory Society consensus report as follows: (1) histological subtypes other than a lepidic pattern (i.e., acinar, papillary, micropapillary, and/or solid) or (2) tumor cells infiltrating myofibroblastic stroma. The areas of invasion were measured at 20× or 40× magnification. When the tumor was large and the size of invasion could not be measured on a single slide we calculated total size of invasion under the microscope across the two or more slides. All tumors were staged pathologically using the 7th edition of the TNM classification of lung cancer published by the IASLC.

2.3. Grading and classification of the proportion of cancer cells to the invasive area

The invasive size was defined as the maximum dimension of the invasive component, excluding the lepidic growth component. Each case was categorized by the proposed invasive categorical classification according to the percentage of tumor cells, as follows: Type A: More than 50% of the invasive area occupied by cancer cells (Fig. 1, upper panel), Type B: Cancer cells occupied 10–50% of the invasive area (Fig. 1, middle panel), and Type C: Cancer cells occupied less than 10% of the invasive area and more than 90% of the invasive area was occupied by the stromal component (Fig. 1, lower panel).

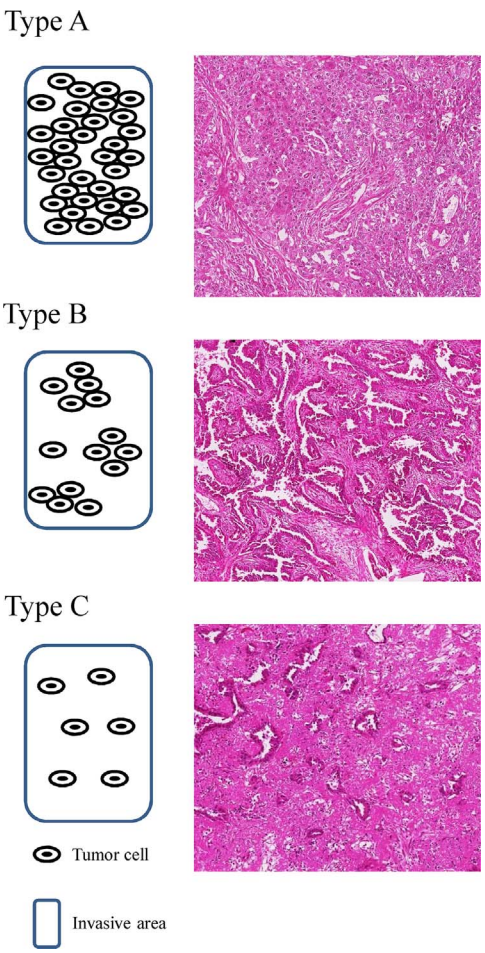

Fig. 1. *Classification of adenocarcinoma based on the proportion of cancer cells to the invasive area.* Type A: More than 50% of the invasive area was occupied by cancer cells (upper panel). Type B: Cancer cells occupied 10–50% of the invasive area (middle panel). Type C: Cancer cells occupied less than 10% of the invasive area and more than 90% of the invasive area was occupied by stromal component (lower panel).

2.4. Measurement of the VVG-positive elastic fiber component within the invasive area

We identified the invasive area on VVG-stained slides and captured digital photographic images by ×20 magnification. The area of VVG-positive elastic fibers was then measured in these images using Image J image analysis software (NIH, Bethesda, MD). The proportion of elastic fibers in the invasive area was represented as the percentage of the maximum invasive area.

2.5. Statistical analysis

Unpaired *t*-test, chi-square test, or the Fisher's exact test was used as appropriate to compare clinicopathological factors between two variables. Differences between the three groups were assessed using one-way analysis of variance (ANOVA) or chi-squared tests. Posthoc tests after ANOVA were performed using Bonferroni correction. The