Proliferative and Invasive Growth Potentials of Type-C Pulmonary Adenocarcinoma
—The Correlation with Lymph Node Involvement in T1 Cases—

Keiko Henmi, Hiroaki Kurai, Tsutomu Mizota, Qian Rui Zhe and Toshiaki Kunimura

Abstract: To investigate the malignant potential of the T1 sized type-C pulmonary adenocarcinoma (PA), proliferative and invasive growth potentials of the tumor cells were examined. Twenty-one surgically resected cases of type-C PA were selected for this study, each less than 3 cm in the largest dimension and classified as T1. None of these cases had received pre-operative chemotherapy or radiotherapy. Using the Ki-67 labeling index (LI) for tumor cells, which indicates the proliferative growth potential, tumors averaged 6.55 ± 3.52% in the center, and 12.16 ± 4.76% in the periphery. This labeling showed no specific relationship with the tumor dimension or lymph node involvement. The cathepsin D-positive rate, indicative of invasive growth potential, was 42.9% in the center, and 14.3% in the periphery. The cathepsin D-positive rate showed no specific relationship with the tumor dimension, but showed a significant relationship with the level of lymph node involvement. We conclude that the malignant potential of type-C PA depends on cathepsin D expression in tumor cells, rather than the Ki-67 LI.

Key words: pulmonary adenocarcinoma, malignant potential, Ki-67, cathepsin D

Introduction

The incidence of primary pulmonary carcinoma is increasing \(^1,2\), and it is a major cause of death worldwide. Of the four major histological types of primary pulmonary carcinoma (adenocarcinoma, squamous cell carcinoma, small cell carcinoma, and large cell carcinoma), adenocarcinoma accounts for more than 60% and its biological characteristics have been discussed. Pulmonary adenocarcinoma (PA) can be classified into several subtypes \(^3,4\), and each subtype is reported to have unique clinicopathological features. Recently, Noguchi et al classified PA into six subtypes, types A to F, and showed the correlation between each subtype and the prognosis \(^5\).

The present study aimed to clarify the malignant potential of tumor cells in T1 sized (less than 3 cm in the largest dimension) Noguchi's type-C PA, by characterising according to the proliferative and invasive growth potentials. Immunohistochemical methods using anti-sera for Ki-67 and cathepsin D were used to assess the proliferating and the invasive growth potentials of the tumor cells. The relationship between these results and the degree...
of lymph node involvement was used to identify significant malignant factors in type-C PA.

Materials and Methods

Cases and microscopic classification

Twenty-one surgically resected cases of type-C PA were selected for this study, each less than 3cm in the largest dimension and classified as T1. These resected samples were obtained in the First Department of Pathology, Showa University Hospital, between 1997 and 1999. None of these cases had received pre-operative chemotherapy or radiotherapy. The patient age ranged from 37 to 80 years (mean 64.4 ± 12.0 years), the male-to-female ratio was 9:12, and lymph node involvement was observed in 3/21 cases.

Archival tissue samples and immunohistochemistry

All archival tissue samples were obtained from the largest dimension of the tumor mass after fixation in 10% formalin. Paraffin-embedded sections were prepared and deparaffinized sections were autoclaved (120°C, 2 atm., 20 minutes) in 20 mmol/L citrate buffer (pH 6.0) for immunohistochemistry.

Immunohistochemical staining using primary anti-sera for Ki-67 (A0047, DAKO, Kyoto, Japan), and cathepsin D (H908, NICHIREI, Tokyo, Japan) were performed according to the LSAB (labeled streptavidin biotin, K0675, DAKO) method. Colorization was performed by the peroxydase-diaminobenzidine method, and lightly counterstained with hematoxylin for light microscopy. To determine the distribution of proliferative and invasively growing cells, Ki-67 and cathepsin D staining was evaluated in two areas; the center of the tumor, often accompanied by granulation or loose fibrosis, and the peripheral region around the advancing border of the tumor nodule (designated the periphery). The level of Ki-67 expression was evaluated by measuring the percentage of stained nuclei among more than five hundred tumor cells, and this percentage was regarded as the Ki-67 labeling index (LI). The degree of cathepsin D expression was evaluated by measuring the percentage of cells with stained cytoplasm among more than five hundred tumor cells. According to the classification of Ozeki et al, this was negative when less than 50% of tumor cells were stained and positive when more than 50% of tumor cells were stained.

Statistical analysis

Statistical significance was evaluated by means of the t-test or Chi-squared test, with \( p < 0.05 \) as the criterion of statistical significance. The degree of correlation was evaluated by means of the Spearman’s rank correlation coefficient test.

Results

Immunohistochemical study

Ki-67

The proliferative growth potential of the tumor cells was evaluated by determining the Ki-67 LI. Nuclear positivity in the tumor cells was identified in all cases of our series. The Ki-67 LI of the tumor cells in the center ranged from 0.3 to 13.2% (6.55 ± 3.52%), whereas that of cells in the periphery ranged from 6.2 to 22.5% (12.16 ± 4.76%) (Figure 1). This difference in LI was statistically significant (\( p < 0.01 \) by t-test). There was no specific relationship between the tumor dimension and the Ki-67 LI, and the correlation coefficient
between the tumor dimension and the Ki-67 LI was 0.148 ($p > 0.1$) in the center, and was 0.250 ($p > 0.1$) in the periphery (Figure 2).

_Cathepsin D_
The invasive growth potential of the tumor cells was evaluated by determining the cathepsin D expression. Cytoplasmic positivity in the tumor cells was identified in 42.9% (9/21) of the cases, positivity was not observed in the non-tumor cells. The cathepsin D-positive rate of the tumor cells in the center was 42.9% (9/21), whereas that of the periphery was 14.3% (3/21) (Figure 3). This difference in the cathepsin D positive rate was statistically significant ($p<0.05$ by Chi-squared test). The tumor dimension of the cathepsin D-positive cases ranged from 10 to 30 mm (21.0 ± 7.34 mm), whereas that of negative cases ranged from 12 to 30 mm (21.9 ± 6.77 mm). This difference was not statistically significant ($p>0.5$).

**Specific correlation in staining pattern**

**Ki-67 and cathepsin D**

In the center, the Ki-67 LI of the tumor cells in the cathepsin D-positive cases ranged from 2.1 to 11.4% (6.83 ± 3.25%), whereas that in the negative ranged from 0.3 to 13.2% (6.34 ± 3.84%). This difference in LI was not statistically significant ($p>0.7$ by t-test). In the periphery, the Ki-67 LI of the tumor cells in the cathepsin D-positive cases ranged from 10.5 to 15.2% (12.67 ± 2.37%), whereas that in the negative ranged from 4.7 to 22.5% (12.08 ± 5.07%). This difference in LI was not statistically significant ($p>0.5$).

**Specific correlation in staining pattern and lymph node involvement**
**Ki-67 and lymph node involvement**

In the center, the Ki-67 LI of the tumor cells in lymph node involvement cases ranged from 3.3 to 6.7% (4.93 ± 1.70%), whereas in cases with no lymph node involvement it ranged from 0.3 to 13.2% (6.82 ± 3.70%). This difference in LI was not statistically significant (p > 0.4 by t-test). In the periphery, the Ki-67 LI of the tumor cells in lymph node involvement cases ranged from 12.3 to 15.2% (13.8 ± 1.45%), whereas in cases with no lymph node involvement it ranged from 4.7 to 22.5% (11.89 ± 5.06%). This difference in LI was not statistically significant (p > 0.5).

**Cathepsin D and lymph node involvement**

In the center, the cathepsin D-positive rate of the tumor cells in lymph node involvement cases was 100% (3/3), whereas in cases with no lymph node involvement it was 33.3% (6/18). This difference in the cathepsin D-positive rate was statistically significant (p < 0.05 by Chi-squared test). In the periphery, the cathepsin D positive rate of the tumor cells in lymph node metastasis cases was 66.7% (2/3), whereas in cases with no lymph node involvement it was 5.6% (1/18). This difference in the cathepsin D-positive rate was statistically significant (p < 0.01 by Chi-squared test).

**Discussion**

According to the Noguchi's classification of PA[3], type C is a localized bronchio-alveolar carcinoma with fibroblastic foci and invasion, that shows a papillary growth pattern with replacement of alveolar lining cells. As seen in many reports[3,7], type C is the most frequent subtype and accounts for more than 50% of PA. In this study we applied immunohistochemical analysis to identify the significant malignant factors in type-C PA.

The proliferative growth potential is one of the greatest indicators of malignancy in various tumors. This can be estimated using light microscopy by assessing cellular mitotic rate, or by immunohistochemical staining to determine the LI with Ki-67, BrdU (bromodeoxyuridine) or PCNA (proliferating cell nuclear antigen)[7-11]. Fontanini et al[8] and Fujii et al[9] reported an inverse correlation between the PCNA LI of the tumor cells and the prognosis in pulmonary non-small cell carcinoma. Thus, it appears that the proliferative growth potential may influence the malignancy of pulmonary carcinoma. We therefore examined the Ki-67 LI of the tumor cells in type-C PA. The Ki-67 LI of the periphery was significantly higher than that of the center, in accordance with Shoji et al[11] who reported that the proliferative growth potential of cells in individual tumor nodules is heterogeneous. Although the correlation between proliferative growth potential and tumor dimension is observed in many malignant tumors, we found no such correlation for type-C PA. This suggests that the tumor cells in type-C PA maintain their regular proliferative growth potential in the process of replacement growth outward on the alveolar surface[11]. We also found no correlation between the Ki-67 LI both centrally and peripherally, and lymph node involvement[7]. These results suggest that the proliferative growth potential of the tumor cell in type-C PA is independent of tumor dimension and does not influence lymph node involvement.

The invasive growth potential of tumor cells is also an indicator of tumor malignancy which can be shown by the expression of cathepsins. Cathepsins are aspartic lysosomal proteinases that degrade basement membranes, and they appear in many invasive tumors[6,12,13].
In PA, Sukoh et al[12] and Higashiyama et al[14] found a correlation between cathepsin D expression in the tumor tissue and prognosis; cathepsin D expression is considered to be a malignant factor. We found that the cathepsin D-positive rate was significantly lower in the periphery suggesting that the invasive growth potential of the cells in an individual tumor nodule is heterogeneous in type-C PA. Tumor cells in the center are known to retain high malignant potential as ascertained by such criteria as vascular invasiveness[15], although the reason for the discrepancy between the low proliferative growth potential and high invasiveness is not known[11]. We have shown that despite their low proliferative growth potential, indicated by the Ki-67 LI, type-C tumor cells in the center do express cathepsin D, and this may explain the malignant potential of the cells. Whether cathepsin D expression correlates with the tumor dimension is still uncertain, but we found no relationship between them. This suggests that the invasive growth potential of the tumor cell in type-C PA is maintained during the process of outward growth. We found a significant difference in the cathepsin D-positive rate between the cases with lymph node involvement and those without lymph node involvement. Thus, it appears that the invasive growth potential of the tumor cells in type-C PA is independent of the tumor dimension, but strongly associated with the development of lymph node involvement.

In conclusion, the malignant potential of type-C PA tumor cells displaying lymph node involvement depends on the cathepsin D expression of the tumor, rather than the Ki-67 LI[16].

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References


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