Original Article

Nuclear Morphometry of Lung Squamous Cell Carcinomas in Cytologic Study

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Background: The nuclei of most cancer cells in histopathologic preparations differ from normal nuclei and vary individually in size, shape, and chromatin pattern. Although the cytologic characteristics of squamous cell carcinoma (SCC) of the lung have been described, quantification of the cytologic features has not been established.

Methods: Cytologic investigations were performed on bronchial brushings or washings, or fine-needle aspirates. We analyzed the nuclear area (NA) of 50 tumor cells in 32 patients with SCC of the lung and 50 bronchial epithelial cells in 20 patients with no evidence of malignancy including inflammatory lesions.

Results: The NA of tumor cells (102.4 ± 26.2 μm^2) was significantly larger than that of bronchial epithelial cells (64.1 ± 16.9 μm^2) ($P = 0.001$). The receiver operating characteristic (ROC) curve analysis showed that an NA cutoff level of 86 μm^2 had a sensitivity of 75% and specificity of 88% to detect malignant components.

Conclusion: We conducted quantitative analyses of NA in SCC using cytologic specimen, NA was a useful parameter for evaluation of differential diagnosis between SCC and non-malignancies even in cytologic specimens.

Keywords: morphometry, nuclear, squamous cell carcinoma, cytology, bronchial epithelial cell

Introduction

In cancer cells, the tightly coordinated cytologic function is interrupted and the nuclei of most solid tumor cells differ in size, shape, and chromatin pattern, from normal nuclei.[1] In 1954, the potential role of cytologic diagnosis was reported by Papanicolaou.[2] In summary, exfoliative cytology was found to be a valuable diagnostic tool based on sound morphologic principles, and its proper use is as a supplement and not as a substitute for biopsy. However, this study depends on morphology and the quantitative determination of cytologic proliferation is difficult. Therefore, we used nuclear morphometry as a method for quantitative measurement of cytologic changes in the appearance of stained cell nuclei. Nuclear morphometry of cytologic specimens has not been performed in lung squamous cell carcinoma (SCC). In this study, we used NanoZoomer Digital Pathology System and examined the nuclear size, the area of nucleus, of SCC cells in comparison to that of bronchial epithelium cells including inflammatory lesions.

Materials and Methods

Patients and histologic typing

A total of 67 patients underwent biopsy at the Gunma Prefectural Cancer Center Hospital during the period

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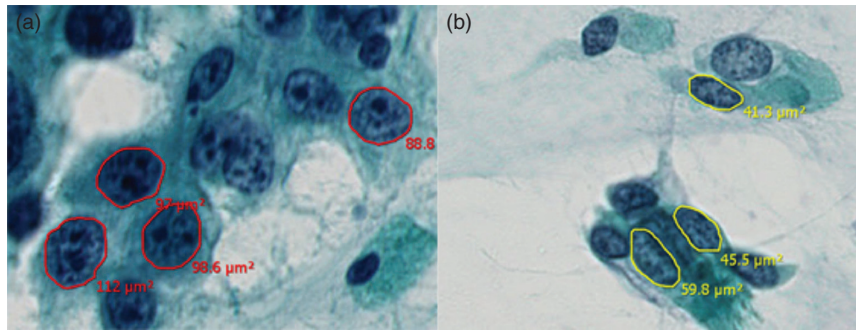[3]*Division of Clinical Laboratory, Gunma Prefectural Cancer Center, Gunma, Ota, Japan*

Fig. 1 Morphometric analysis of cytologic specimen. Papanicolaou stain, original magnification ×630. (**a**) The red area represents the nuclei of SCC. (**b**) The yellow area represents the nuclei of bronchial epithelial cells. SCC: squamous cell carcinoma

from January 2011 to April 2014 were enrolled. The study focused on a series of 52 patients, excluding 15 patients because of double cancer, mycosis, and connective tissue disease. In all, 32 patients were diagnosed with primary pulmonary SCC and 20 patients with no evidence of malignancy (inflammatory disease, metaplasia, and normal cells). Investigation of the cytology was performed using bronchial brushings or washings, or fine-needle aspirates preoperatively obtained from the patients. We analyzed the nuclear area (NA) of 50 tumor cells without keratinized cells and 50 bronchial epithelial cells that we randomly selected from cytologic specimens.

Morphometric procedure

The cytologic characteristics of SCCs and bronchial epithelial cells were analyzed using the NanoZoomer Digital Pathology System (Hamamatsu Photonics, Shizuoka, Japan). After calibration of the instrument with a micrometer slide, all measurements were performed at a magnification of ×630 digital ocular on the monitor. On examination, tumor cells from the most cellular area of the specimen were sought. Overlapping nuclei that cannot be measured were omitted. Ten nuclei of the tumor cells in each specimen were measured. The images captured from cytologic specimens were manipulated on the computer monitor. Then, the nuclei were identified and measurements of each NA were assessed by tracing the nuclear membrane using the computer mouse (**Fig. 1**) using the computer software (NDP View Ver.1.1.8, Hamamatsu Photonics, Shizuoka, Japan).

Statistical analysis

Clinicopathologic features including nuclear size were statistically analyzed using Student's t-test and Tukey's test. The cutoff value of nuclear size was determined using receiver operating characteristic (ROC) analysis. Cutoff value for malignant component was identified from the ROC curves by dividing the area under the curve (AUC) by the individual nuclear sizes. These statistical analyses were performed using SPSS v 12.0 (SPSS Inc., Chicago, IL, USA).

Results

Clinical and histologic findings

The most relevant clinicopathologic features are listed in **Table 1**. The 52 cases consisted of 32 SCCs and 20 non-malignancies including inflammatory lesions and metaplasia. The tumor location of more than two-thirds of the SCC was central.

Morphometric analysis and outcome

We performed morphometric analysis on the 52 cytologic specimens. Mean NA in tumor cells was $102.4 \pm 26.2 \ \mu m^2$ (range: 56–184). On the other hand, the mean NA in bronchial epithelial cells was $64.1 \pm 16.9 \ \mu m^2$ (range: 35–102). During the bronchial epithelial cells, mean NA in normal epithelial cell group (10 cases) was $52.2 \pm 14.6 \ \mu m^2$ (range: 35–68), and that in inflammatory epithelial cell group (eight cases) was $70.8 \pm 20.8 \ \mu m^2$ (range: 48–102). The NA of tumor cells was significantly larger than that of bronchial epithelial cells (**Fig. 2**, $P < 0.001$). The ROC curve analysis showed that an NA cutoff level of $86 \ \mu m^2$ had a sensitivity of 75% and specificity of 88%, and AUC of 0.898 (**Fig. 3**). Patients with NA of $86 \ \mu m^2$ and more was diagnosed as malignancy compared to those of less than $86 \ \mu m^2$ ($P < 0.001$, 95% confidential interval = 0.850–0.947).

Table 1 Patient characteristics

	SCC	Non-malignancy
Number of patients	32	20
Gender (male/female)	24/8	15/5
Age (range)	72.4 ± 12.2 (47–85 y)	69.1 ± 6.9 (61–78 y)
Tumor location (central/peripheral)	22/10	0/20
Chief complaint		
Hemosputum	14	0
Cough	8	0
X-ray positive	10	20
Biopsy method (TBB/TBLB)	22/10	0/20
Histology		
Normal epithelium	–	10
Metaplasia	–	2
Inflammatory lesion	–	8
SCC	32	
Pathological grade of SCC (G1/2/3/4)	8/20/4/0	
Tumor size of SCC	4.3 ± 2.2 (2.2–12.8 cm)	
Stage of SCC (I/II/III/IV)	0/2/22/8	
Therapy		
Chemotherapy	20	–
Chemoradiotherapy	8	–
Surgery	4	–
Observation	–	6
Antibiotics	–	4

SCC: squamous cell carcinoma; y: year; TBLB: trans-bronchial lung biopsy; TBB: trans-bronchial biopsy
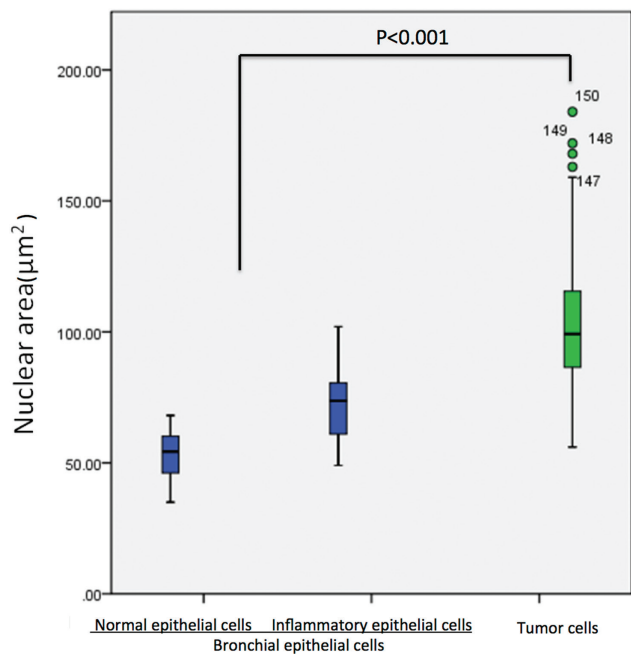

Fig. 2 Comparison of NA between tumor cells and bronchial epithelial cells. NA: nuclear area
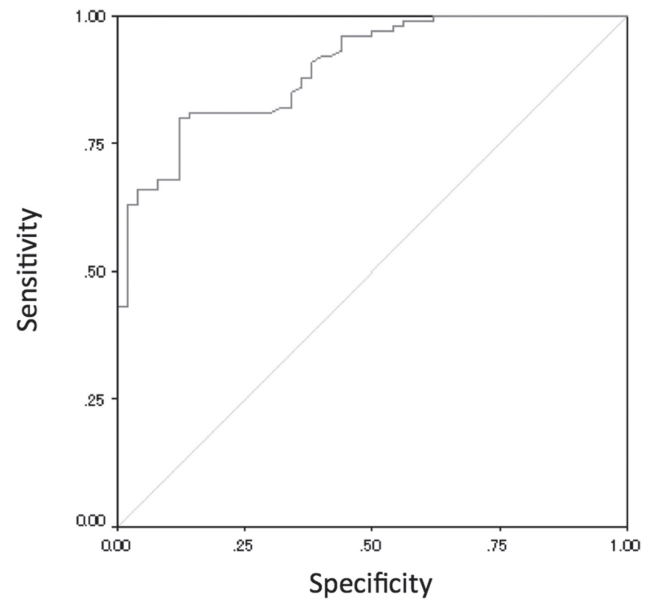

Fig. 3 ROC curve of NA. ROC: receiver operating characteristic; NA: nuclear area

Table 2   Morphometry of nuclear size reported in lung cancer

	No. of patients	Materials	Morphology	No. of tumor cells	Analysis
Kurita et al.[6]	–	Adenocarcinoma, stage I	NA, NP	–	Prognosis
Marchevsky et al.[7]	13	NSCLC and SCLC	Nuclear/cytoplasmic ratio	29	
Burns et al.[8]	10	LCC and adenocarcinoma	Nucleolar/nuclear ratio	–	
Morishita et al.[9]	193	Adenocarcinoma	NA	–	Noguchi's classification

NSCLC: non-small-cell lung cancer; SCLC: small-cell lung cancer; LCC: large-cell carcinoma; NA: nuclear area; NP: nuclear perimeter

Discussion

In the present study, we conducted quantitative analyses and revealed that NA of SCCs was larger than that of non-malignant epithelial cells, and the cutoff value of NA between SCCs and non-malignant cells was 86 μm^2.

Morphometry has been studied extensively, and the measurement of NA has been performed with cells of many organs. In 1987, Paplanus et al.[3] reported that the morphometry had a potent role in surgical pathology. Some studies have indicated that these assessments may provide clinically relevant information on the degree of progression and malignant potential of various cancers.[3–5] **Table 2** shows that the quantitative assessment of nuclear morphometry has been used to enhance the diagnostic and prognostic efforts of pathologists in cytologic studies of pulmonary malignant tumors.[6–9] Morishita[9] and colleagues reported a study using large number of specimen of adenocarcinoma. Nuclear size, variations in nuclear size, appearance of nucleolus, and nuclear atypia of the adenocarcinoma cells were found to be different during the various histologic subtypes of adenocarcinoma. The NA (86.7 ± 32.7 μm^2) of advanced adenocarcinoma was significantly larger than that of early adenocarcinoma (58.4 ± 14.0 μm^2). The nuclei of advanced adenocarcinomas were generally larger and showed more variation in size compared to those of early adenocarcinomas. However, the information of nuclei in SCC has not been reported. In the present study, the cutoff value of 86 μm^2 in NA of SCC was similar to that of average NA in advanced adenocarcinomas, which is larger than that of early adenocarcinomas.

Although oncogenesis and its genetic change occur independent of histologic types of lung cancer, its frequency and timing of occurrence for cancer progression in SCC likely differ from those of adenocarcinoma. Furthermore, a number of genetic and epigenetic differences have been identified in SCCs that arise from bronchial epithelial cells through a squamous metaplasia/dysplasia process.[10,11] In the present study, we showed that NA of SCCs was larger than that of non-malignant epithelial cells including metaplasia using quantitative analysis. Because there were small number of cases and we measured only 50 cells per specimen, we were not able to detect significance between SCC and epithelial cells in inflammatory lesions. However, NA of SCC was consistently larger than that of cells from inflammatory lesions.

Limitations of our study are, as described above, the small population of subjects and its design as a single institution report. Additional studies are necessary.

Conclusion

It was proven quantitatively that nuclei of neoplastic cells of SCC grew remarkably large. The ROC curve analysis showed that an NA cutoff value of 86 μm^2 was a sensitive parameter for detecting a malignant structure of SCC.

Disclosure Statement

None was declared.

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