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■ ORIGINAL ARTICLE ■

Morphologic Analysis of Pulmonary Neuroendocrine Tumors

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Background: Few studies on how to diagnose pulmonary neuroendocrine tumors through morphometric analysis have been reported. In this study, we measured and analyzed the characteristic parameters of pulmonary neuroendocrine tumors using an image analyzer to aid in diagnosis. **Methods:** Sixteen cases of typical carcinoid tumor, 5 cases of atypical carcinoid tumor, 15 cases of small cell carcinoma, and 51 cases of large cell neuroendocrine carcinoma were analyzed. Using an image analyzer, we measured the nuclear area, perimeter, and the major and minor axes. **Results:** The mean nuclear area was 0.318 ± 0.101 μm^2 in typical carcinoid tumors, 0.326 ± 0.119 μm^2 in atypical carcinoid tumors, 0.314 ± 0.107 μm^2 in small cell carcinomas, and 0.446 ± 0.145 μm^2 in large cell neuroendocrine carcinomas. The mean nuclear circumference was 2.268 ± 0.600 μm in typical carcinoid tumors, 2.408 ± 0.680 μm in atypical carcinoid tumors, 2.158 ± 0.438 μm in small cell carcinomas, and 3.247 ± 1.276 μm in large cell neuroendocrine carcinomas. All parameters were useful in distinguishing large cell neuroendocrine carcinoma from other tumors ($p = 0.001$) and in particular, nuclear circumference was the most effective ($p = 0.001$). **Conclusions:** Pulmonary neuroendocrine tumors showed nuclear morphology differences by subtype. Therefore, evaluation of quantitative nuclear parameters improves the accuracy and reliability of diagnosis.

Key Words: Typical carcinoid tumor; Atypical carcinoid tumor; Carcinoma, small cell; Carcinoma, large cell; Carcinoma, neuroendocrine; Pulmonary neuroendocrine tumor

Neuroendocrine tumors originate in neural crest cells and can be found in anywhere in the body including the gastrointestinal tract, pancreas, and lung.[1] Pulmonary neuroendocrine tumors represent approximately 20% of all primary neoplasms of the lung. Neuroendocrine tumors are classified according to four subtypes in the lung: 1) typical carcinoid tumor (TC), 2) atypical carcinoid tumor (AC), 3) small cell carcinoma (SCC), and 4) large cell neuroendocrine carcinoma (LCNEC).[1-3]

Due to different therapies and prognoses according to the aforementioned subtypes, many studies have reported the diagnostic clues of these pulmonary neuroendocrine tumors.[4-6] TC is low-grade, AC is intermediate-grade, and SCC and LCNEC are high-grade malignancies. Franks and Galvin[4] reviewed cases that had been previously diagnosed as pulmonary neuroendocrine tumors for identification of the different histological features of each subtypes. They found that TC and AC have similar morphologies but are different in mitotic activity and necrosis. According to Siddiqui[5] who studied the cytologic findings of pulmonary neuroendocrine tumors, AC cells are round, ovoid or spindle-shaped and have a moderate and homogeneous cytoplasm. Different from TC, necrosis and inflammation are common findings in AC. Tumor cells of SCC are round, ovoid or spindle-shaped and necrosis is easily found. The size of SCC tumor cells is up to three-fold smaller than a mature lymphocyte. These tumor cells have a high nucleus to cytoplasm ratio, high mitotic rates, and are commonly necrotic. Cytologic features of LCNEC, such as abundant mitosis and necrosis, are similar to those of SCC. Mitotic activity is high with a mean mitotic rate of 60 per high power field (HPF). The mitotic rate in LCNEC is more than 11 per HPF, but is usually 60 per HPF on average.

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Reactivity for immunohistochemical neuroendocrine markers (synaptophysin, chromogranin, and CD56) is used for discrimination from other carcinomas.

Although SCC is more sensitive to chemotherapy and radiation therapy than any other tumors, due to rapid growth and early metastasis, prognosis is not good. No standard chemotherapy has been established for patients with LCNEC.[6] Therefore, patients with LCNEC receive chemotherapy for treatment of non-small cell carcinoma or SCC, but its response to chemotherapy is still debatable.[6] Although subtyping of neuroendocrine tumors is dependent upon the morphologic features and the amount of mitotic activity, reproducibility rates among pathologists are relatively low.[7,8] Therefore, a critical need exists to identify further diagnostic clues.

In this study, we measured and analyzed the characteristic nuclear parameters of pulmonary neuroendocrine tumors using an image analyzer to aid in diagnosis and to distinguish SCC from LCNEC.

MATERIALS AND METHODS

Materials

Pulmonary neuroendocrine tumors of 146 cases diagnosed as TC, AC, SCC, and LCNEC were reviewed. All specimens were obtained by lobectomy or wedge resection in either Samsung Medical Center of Sungkyunkwan University, Dong-A University Hospital, or Gachon University Gil Medical Center between 1995 and 2010. Of the 146 cases, 59 cases were excluded because evaluation by image analysis could not be performed. The reasons for exclusion were poor sample quality, extensive necrosis, and squeezing artifacts. A total of 87 cases consisting of TC (n = 16), AC (n = 5), SCC (n = 15), and LCNEC (n = 51) were enrolled in this study.

Methods

Image and analysis

All 87 cases were obtained by formalin-fixed paraffin-embedded tissues and stained with hematoxylin and eosin. One or two representative slides were selected for each case. For morphologic analysis, five to ten pictures were selected. Pictures were taken using a DP70 digital camera (Olympus, Tokyo, Japan). The pictures were captured in a high power magnification (×400) using BX51 microscope (Olympus). Areas of dry or squeezing artifact were excluded.

Tumor cells of each case were measured in area, perimeter, and major and minor axes of nucleus using i-Solution ver. 8.4 (IMT i-Solution, Coquitlam, BC, Canada), the image analyzer software package. The major and minor axes of the nucleus are the longest and shortest nuclear diameters, respectively. Each picture contained 10-20 nuclei that were measured and their mean values were calculated. Only cells with clear cytoplasmic and nuclear boundaries, which did not overlap with other cells, were selected (Fig. 1).

Statistical analysis

SPSS ver. 17.0 (SPSS Inc., Chicago, IL, USA) was used for all statistical analyses. Differences were considered significant when the p-value was less than 0.05. Analysis of variance was used to compare differences between pulmonary neuroendocrine tumor subtypes. The Bonferroni correction was used for post-hoc comparisons.

RESULTS

Clinical characteristics

The mean age of patients with TC was 57.4 years (range, 50 to 67 years) and male to female (M : F) ratio was 3 : 1. The mean age of patients with AC was 38.5 years (range, 36 to 41 years) and M : F ratio was 1 : 4. The mean age of patients with SCC was 66.3 years (range, 42 to 85 years) and M : F ratio was 7 : 1. The mean age of patients with LCNEC was 69.3 years (range, 31 to 89 years) and M : F ratio was 5 : 1 (Table 1).

The sites of the lung tumors were equally distributed, however the right upper and left upper lobes were common sites of tumor involvement.

Morphological analysis of the nucleus

Nuclear area

The mean nuclear area was 0.318 ± 0.101 μm^2 in TC, 0.326 ± 0.119 μm^2 in AC, 0.314 ± 0.107 μm^2 in SCC, and 0.446 ± 0.145 μm^2 in LCNEC (Table 2). The nuclear areas were significantly different between subtypes. The nuclear area of LCNEC compared with those of other neuroendocrine tumors was largest (p = 0.00l). TC area was not statistically different from AC or SCC.

Nuclear perimeter

The mean nuclear circumference was 2.268 ± 0.600 μm in TC, 2.408 ± 0.680 μm in AC, 2.158 ± 0.438 μm in SCC, and 3.247 ± 1.276 μm in LCNEC (Table 2). All four subtypes were shown significantly different from each other in nuclear perimeter (p = 0.04 to 0.001) (Table 3).

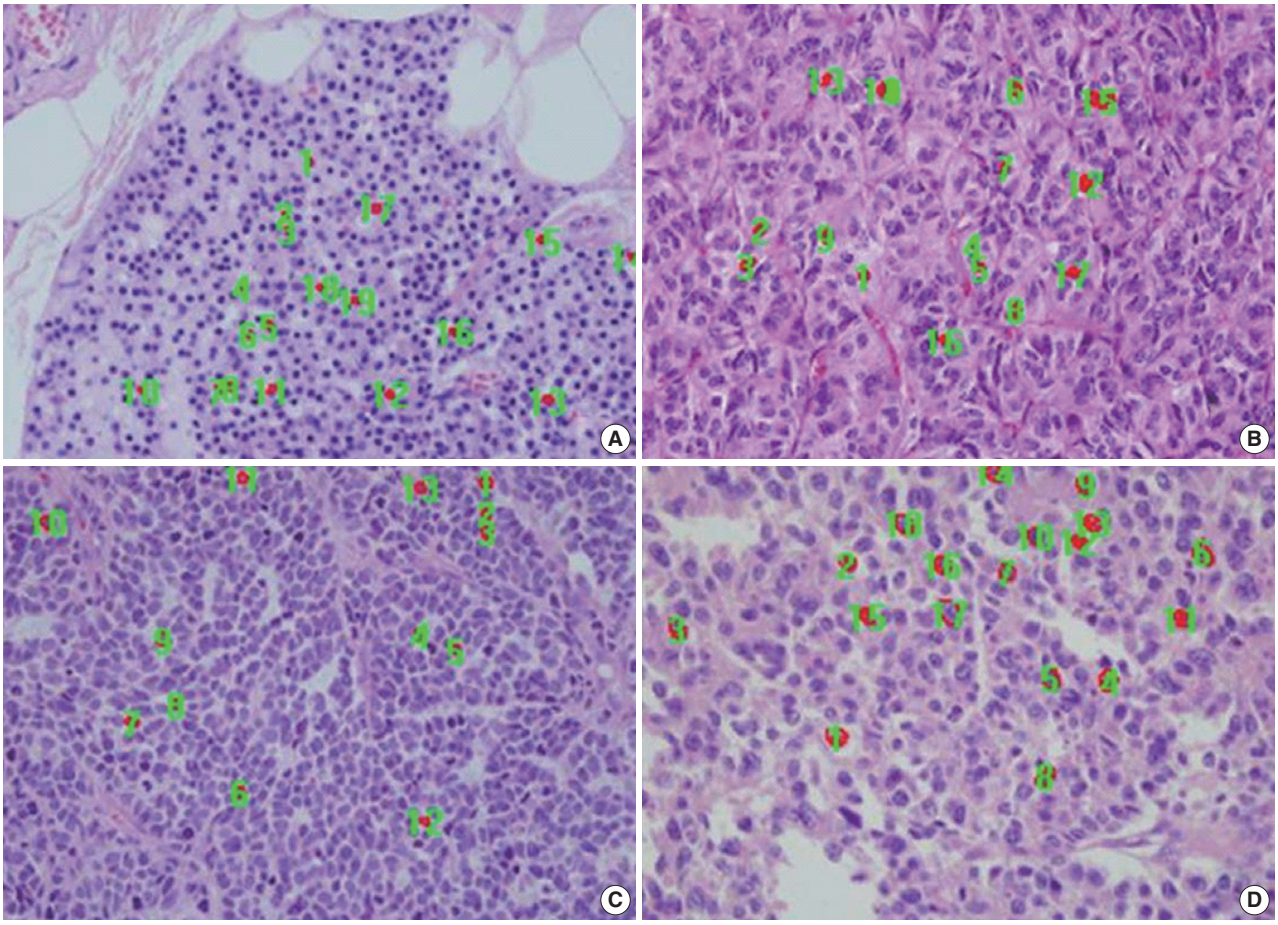

Fig. 1. Measurement of the area, perimeter, and major and minor axes of 10 to 20 nuclei per picture in typical carcinoid tumor (A), atypical carcinoid (B), small cell carcinoma (C), and large cell neuroendocrine carcinoma (D).

Table 1. Clinical characteristics and materials of neuroendocrine tumors of the lungs

		TC	AC	SCC	LCNEC
Age (mean)		57.4	38.5	66.3	69.3
Sex	Male	12	1	13	43
	Female	4	4	2	8
No. of total slides		16	5	15	51
No. of total measured cells		1,339	794	3,643	8,840

TC, typical carcinoid tumor; AC, atypical carcinoid tumor; SCC, small cell carcinoma; LCNEC, large cell neuroendocrine carcinoma.

Table 3. p-values of nuclear circumferences for pulmonary neuroendocrine tumors

	TC	AC	SCC	LCNEC
TC	–	0.004	0.002	0.000
AC	–	–	0.000	0.000
SCC	–	–	–	0.000
LCNEC	–	–	–	–

TC, typical carcinoid tumor; AC, atypical carcinoid tumor; SCC, small cell carcinoma; LCNEC, large cell neuroendocrine carcinoma.

Table 2. Mean morphologic measurement values of the nucleus in pulmonary neuroendocrine tumors

	TC	AC	SCC	LCNEC	p-value[a]
Area (μm^2)	0.318 ± 0.101	0.326 ± 0.119	0.314 ± 0.107	0.446 ± 0.145	0.001
Circumference (μm)	2.268 ± 0.600	2.408 ± 0.680	2.158 ± 0.438	3.247 ± 1.276	0.001
Major axis (μm)	0.670 ± 0.119	0.727 ± 0.144	0.735 ± 0.154	0.855 ± 0.158	0.001
Minor axis (μm)	0.571 ± 0.091	0.569 ± 0.108	0.538 ± 0.099	0.671 ± 0.113	0.001

TC, typical carcinoid tumor; AC, atypical carcinoid tumor; SCC, small cell carcinoma; LCNEC, large cell neuroendocrine carcinoma.
[a]Statistically significance are tested by oneway analysis of variance among groups.

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Major axis of the nucleus

The mean major axis of nucleus was 0.670±0.119 μm in TC, 0.727±0.144 μm in AC, 0.735±0.154 μm in SCC, and 0.855±0.158 μm in LCNEC (Table 2). Statistically significant differences in the sizes of the nuclear major axes were observed among the four subtypes. TC and LCNEC were larger compared to the other tumors (p=0.001). AC and SCC were not significantly different.

Minor axis of the nucleus

The mean minor axis of nucleus was 0.571±0.091 μm in TC, 0.569±0.108 μm in AC, 0.538±0.099 μm in SCC, and 0.671±0.113 μm in LCNEC (Table 2). Statistically significant differences in the sizes of the minor axes were observed for TC, AC, SCC, and LCNEC. SCC and LCNEC were significantly larger than the other tumors (p=0.001). TC and AC were not significantly different.

DISCUSSION

Worldwide, lung cancer is the most common cause of cancer death.[3] Histologic confirmation is important for treatment and prognosis determination.[6,9] Although the assessment of neuroendocrine features is possible by immunohistochemical studies, confirmation of the subtype can only be made by light microscopic findings. Characteristic histological findings according to each of the subtypes are sometimes ambiguous, therefore, interobserver variability is common.[7,8] In this study, we measured and analyzed the nuclear areas, perimeters, and major and minor axes of pulmonary neuroendocrine tumors using an image analyzer.

We evaluated the measured nuclear morphological characteristics and determined the statistical significance of these values. LCNEC had a significantly larger nuclear area and circumference than all other tumors (0.446±0.145 μm^2 and 3.247±1.276 μm). For the other tumors the nuclear areas and circumferences, in the decreasing order, were AC (0.326±0.119 μm^2 and 2.408±0.680 μm), TC (0.318±0.101 μm^2 and 2.268±0.600 μm), and SCC (0.314±0.107 μm^2 and 2.158±0.438 μm; p<0.001).

The difference between major and minor axes of every nucleus was calculated. SCC (0.197 μm) showed the greatest difference in value followed by LCNEC (0.184 μm), AC (0.158 μm), and TC (0.099 μm), in decreasing order. In addition, the proportion of major axis and minor axis within tumors was evaluated. This ratio was 1:0.73 for SCC, 1:0.78 for LCNEC, 1:0.78 for AC, and 1:0.85 for TC. SCC showed the greatest difference in proportion. Although SCC showed the smallest values in nuclear area and circumference, it was larger than any other tumors in the proportion and difference between major and minor axes. Squeezing artifacts were common in SCC compared with other tumors. Therefore, diagnosing pulmonary neuroendocrine tumors on the basis of only one parameter of the nucleus alone is sometimes difficult. The SCC was actually as small as name on befitting, however, due to the presence of squeezing artifacts, parameters of nuclear size on the slides were not always the smallest value. In addition, TC had a minimal difference and proportion between major and minor axes, which is accordant with its nuclear morphology.

Among the four parameters measured, the perimeter was most effective for identifying the pulmonary neuroendocrine tumor subtype. The remaining parameters were only statistically significant for distinguishing LCNEC from the other subtypes. The nuclear perimeter in LCNEC was 33.5% larger than that in SCC, the smallest one. Nuclear area was an effective parameter for distinguishing between LCNEC and the other subtypes. The difference between LCNEC and SCC was 29.6%. The major axis was only effective for distinguishing between LCNEC and TC and the minor axis was effective for distinguishing between SCC and LCNEC. The major axis in LCNEC was 21.6% larger than that in TC, which had the smallest major axis value. The minor axis value difference between LCNEC and SCC was minimal at 19.8%.

Kim *et al.*[10] measured the nuclear area, perimeter, circularity, and density in SCC and LCNEC. According to their results, the mean area of the nucleus was 0.31±0.12 μm^2 in SCC and 0.45±0.20 μm^2 in LCNEC. The mean perimeter of the nucleus was 2.54±0.62 μm in SCC and 3.16±0.82 μm in LCNEC. Their results are similar to those of our study. In particular, the mean area of the nucleus in SCC and LCNEC were very similar.

The nucleus is assumed to be a complete ellipsis and the nuclear perimeter is proportional to the sum of the major and minor axes. In addition, the nuclear area is proportional to the product of the major and minor axes. Based on this assumption, we found characteristic nuclear morphometric features. The nuclear area value divided into the major and minor axes products (area/major axis×minor axis) was similar: 0.831 in TC, 0.789 in AC, 0.794 in SCC, and 0.777 in LCNEC. If the nucleus were completely elliptical or circular in shape, these values should be 0.79. However, differences by subtype were observed in the nuclear perimeter value when divided into the sum of the major and minor axes (perimeter/[major axis+minor axis]); 2.128 in LCNEC, 1.847 in AC, 1.828 in TC, and 1.695 in SCC, in descending

order. If the nucleus was completely elliptical in shape, these values should be 1.57. Although it might be due to an irregular nuclear membrane boundary by each subtype of pulmonary neuroendocrine tumor, the significance was not demonstrated in our study or in other literature.[10]

In this study, we observed that each subtype of neuroendocrine tumor has a different nuclear morphology with the nuclear perimeter being the most effective factor for distinguishing neuroendocrine tumor subtype. Although measurement of these nuclear parameters is difficult, these nuclear values could be referred to improvement of diagnosis.

Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

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