Immunohistochemical Studies on Small Cell Lung Cancer

Jin-Bao SHAW and Motohiko ITO

*From the department of thoracic surgery, chest disease research institute, Kyoto University
53, Shogoin Kawahara-cho, Sakyo-ku, Kyoto 606, Japan*

SUMMARY

Seventy cases of pulmonary carcinoma, consisting of 20 cases of squamous cell carcinoma, 22 cases of adenocarcinoma and 28 cases of small cell carcinoma, were examined immunohistochemically using the peroxidase anti-peroxidase method, and antisera raised against carcinoembryonic antigen, keratin, neuron specific enolase, Leu-7, beta-2 microglobulin.

The immunoreactivity of NSE, and of Leu-7 were very different among oat cells, intermediate cells, and combined subtypes of small cell lung cancer, while Leu-7 was rarely seen in adenocarcinoma or squamous cell carcinoma. Keratin was demonstrated not only in non-small cell lung cancer but also in intermediate and combined types of small cell lung cancer.

These results seems to support the "Y" concept which describes the relationship between non-small cell lung cancer and small cell lung cancer.

Besides the WHO classification of 1981, which divides the small cell lung cancer into oat cell, intermediate and combined subtype, the usefulness of a tentative classification of International Association for Study of Lung Cancer (IASLC) which divides them into classic (pure), variant, and combined type was discussed.

INTRODUCTION

For several years, there has been much discussion on the histogenesis, clinical and biological entity of small cell lung cancer (SCLC), and their concepts have undergone major changes. Many investigators including medical and surgical oncologists as well as pathologists are recently concerned about its histological classification, because; 1) the small cell carcinomas comprise 15-25 percent of the lung cancers. 2) Tumors metastasize early and even "limited" disease cases have latent metastases. 3) Cure by local treatment as surgical resection or conventional radiotherapy is difficult, and prognosis is poor (3). 4) The lesion is highly sensitive to many chemotherapeutic agents. Thus, with progress in chemotherapy, improvement of survival and cure rate can be expected in future. 5) Despite its very simple histological structure, its ultrastructure is characterized by the presence of granules analogous to neuroendocrine granules as well as possible differentiation into epithelial cells (4); and 6) biochemically, it shows high activities of L-dopa-decarboxylase, neuron specific enolase and creatinine kinase BB, and polypeptide hormone production, which are characteristic of nerve, neuroendocrine cells, and APUD cells. (5-9)

Many investigations have been made on the histological classification of pulmonary carcinoma using morphological, biological, biochemical and immunohistochemical techniques to establish a further comparative classification that may aid us in predicting the behavior of the tumors and guide the clinician in selecting the most appropriate treatment.

We employed an immunohistochemical technique to investigate the characteristics of the subtypes of SCLC, using the following tumor markers in the cancer cells.

Carcinoembryonic antigen (CEA), one of the most widely studied tumor markers, is detected in the fetal colon during the third trimester of pregnancy. It is found in tissues derived from endoderm, but subsequently has been associated with neoplasms arising from mesoderm and ectoderm. (10) CEA localizes on the tumor cell surface membrane, suggesting that CEA is a peripheral membrane glycoprotein. (11)

Keratin is an epidermal protein which has been shown to localize ultrastructurally to epidermal tonofilaments and is often noted in intimate association with desmosomes. (12)(13) It is present within the basal cells and intermediate cells of the normal trachea, bronchus and in the ducts of the bronchial glands. (14)

Neuron specific enolase (NSE) is a glycolytic enzyme, present in the cytoplasm of the neuroendocine cell. It was first reported as a valuable tumor marker of SCLC in 1982 by Carney. (15) Recently, SCLC has been found to contain NSE, which exhibits neuroendocrine properties. (16)

Anti Leu-7 antibody was initially found to react with natural killer cells (NK cell), and subsequently with myelinated nerves of the peripheral system and central nervous system. (17) Leu-7 is also a marker of neuroendocrine properties of SCLC. (18)

Beta-2 microglobulin (Beta-2 M) is a component of transplantation antigens present in the surface membrane of mammalian cells. Recently, Beta-2 M has been suggested to be associated with tumor specific antigens suggested to be modified transplantation antigens. It has been found to be structurally related to immunoglobulin G and to the common portion fragment of HL-antigen molecules and produced by a variety of nucleated cells. The Beta-2 M concentration is fully related to the stage of differentiation of the tumors, the most differentiated tumors contain the highest concentration of Beta-2 M, and poorly differentiated tumors contain a lower concentration. (21)

In this report we tried to clarify the difference in subtypes of small cell lung cancer by an immunohistology of some tumor markers.

MATERIALS AND METHODS

Histological section of 70 cases of pulmonary carcinoma were obtained from surgical specimens. Twenty eight small cell carcinoma, including 8 cases of oat cell subtype, 16 intermediate subtype, and 4 combined subtype, 22 adenocarcinoma, 20 squamous cell carcinoma were investigated after the histological study, according to the World Health Organization (WHO) classification. (Table-1) (20)

The immunohistochemical technique was performed on 5 μm thick paraffin-embedded sections using the immunoperoxidase method (peroxidase anti-peroxidase). (1)

Sections were sequentially incubated for 30 minutes at room temperature with each of the

Table 1.   Histopathologic classification of small cell lung cancer

WHO 1967. (19)	WHO 1981. (20)	Tentative.
1. Fusiform.	1. Oat-cell.	1. Pure (classic).
2. Polygonal.	2. Intermediate.	2. Varient.
3. Lymphocyte-like (oat-cell).	3. Combined of SCC.	3. Combined.
4. Other.		

following reagents : 1. Methanolic hydrogen peroxide. 2. Non-specific background staining was minimized by using normal swine serum (1:20) for 30 minutes following methanolic peroxide treatment.   3. Rabbit anti-human CEA, Beta-2 M, keratin (DAKO INC. DENMARK), NSE (DAKO PAP kit), anti Leu-7 antibody (Becton Dickinson Inc.).   4. Swine anti-rabbit serum IgG. (1:20. DAKO).   Demonstration of Leu-7 was performed by the ABC kit.   (Vector Lab, Inc.)   5. Horseradish peroxidase-rabbit anti-horseradish peroxidase soluble complexes. (1:50. DAKO).

This buffer (0. 1 M PH : 7. 6) was used for all dilutions. Following each incubation, slides were washed thoroughly with this buffer (0. 1 M PH : 7. 6) for 20 minutes.

Antigen localization was determined on the basis of peroxidase activity, stained by a 5 minute incubation of the sections with a freshly prepared solution containing 3, 3'diaminobenzidine tetrahydrochloride, (Nakarai Chemical Ltd.) and hydrogen peroxide. Slides were washed with water, counterstained with Meyer's hematoxylin, dehydrated and mounted in Permount.

Normal rabbit serum or normal mouse serum was used as a control.

RESULTS

Table 2 summarizes the results of immunohistochemical studies in various human lung cancers.

The CEA immunoreactivity was detected in 5 of the 8 cases of the oat cell subtype, 12 of the 16 cases of the intermediate subtype, 3 of the 4 cases of combined subtype of SCLC (Fig-1), 16 of the 22 cases of adenocarcinoma, and 12 of the 20 cases of squamous cell carcinoma, although the intensity of staining ranged from weak to strong. (Fig-2) Thus, there was no significant difference in the positive rate of CEA among squamous cell carcinoma, adenocarcinoma and small cell carcinoma of lung.

Many of the intermediate type cases of small cell carcinoma were positive for keratin

Table 2.   Results of the immunohistochemical studies on pulmonary carcinomas.
according to WHO classification

	CEA.	Keratin.	NSE.	Leu-7.	Beta-2 M.
Small cell carcinoma.					
Oat cell type.	62. 5%	0%	75%	62. 5%	0%
Intermediate subtype.	75%	81. 2%	81. 2%	68. 7%	6. 2%
Combined subtype.	75%	75%	25%	50%	0%
Adenocarcinoma.	73%	77%	59%	18%	50%
Squamous cell carcinoma.	60%	85%	35%	0%	40%
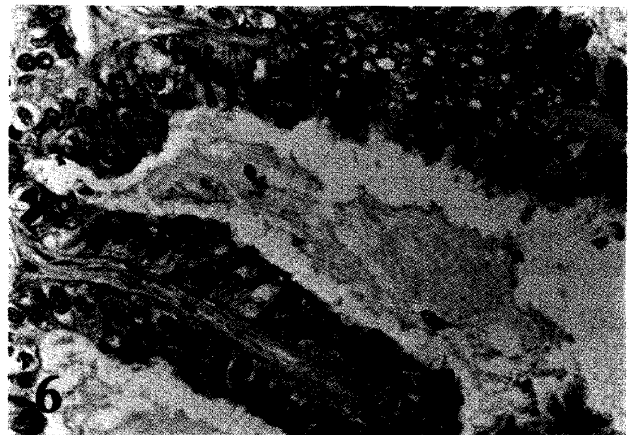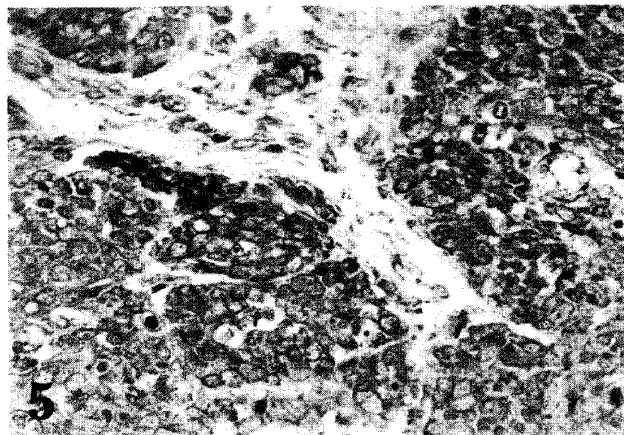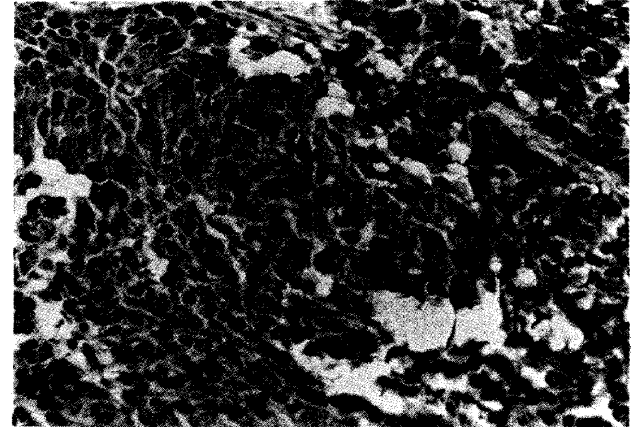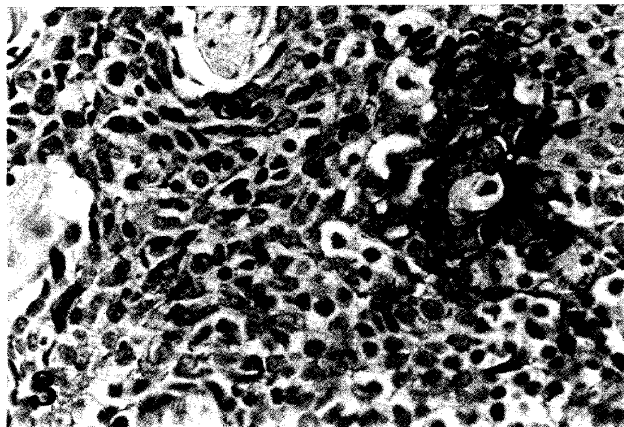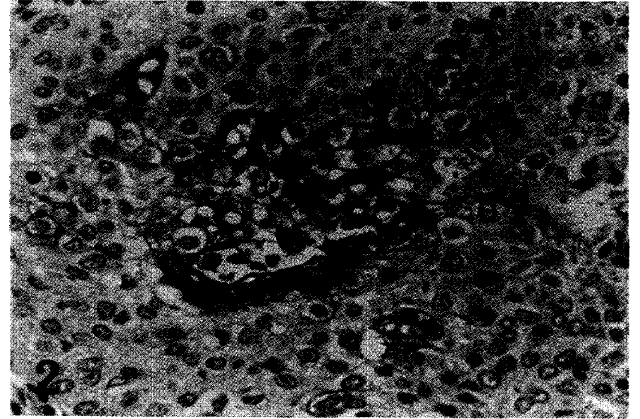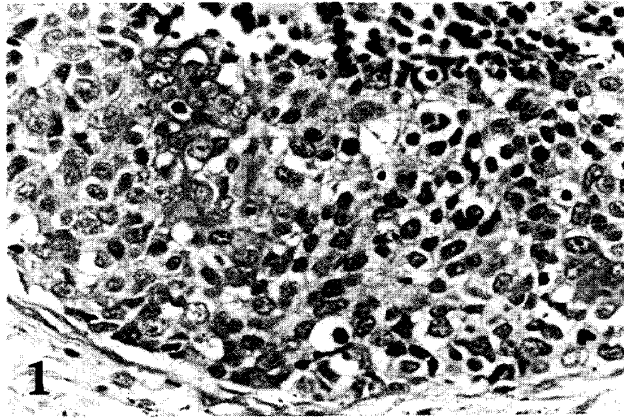
Fig. 1. Combined subtype of SCLC stained for CEA by the immunoperoxidase method. Note the positive staining on tumor cell surface membrane. (X2000)

Fig. 2. Squamous cell carcinoma of lung. Strong staining for intracellular CEA is observed in tumor cells. (X2000)

Fig. 3. Immunoperoxidase positivities of keratin in intermediate subtype of SCLC. But not in oat cell type. (X2000)

Fig. 4. Oat cell subtype of SCLC stained for NSE by the immunoperoxidase method. Note the marked cytoplasmic staining of tumor cells. (X200)

Fig. 5. Oat cell subtype of SCLC stained for Leu-7 by the immunoperoxidase method. Positive staining is observed in many tumor cells. (X200)

Fig. 6. Adenocarcinoma of lung stained for Beta-2 M by the immunoperoxidase method. Positive staining is observed in most differentiated tumor cells, but negative in small cell carcinoma of lung. (X200)

(Fig-3), but, none of the oat cell subtype cases were. Furthermore, keratin was demonstrated frequently in the combined subtype. Strongly positive staining was observed in the squamous cell carcinoma and adenocarcinoma cells.

In our study, NSE immunoreactivity was present in a high percentage of the oat cell and intermediate subtype cells (Fig-4), but in only 1 of the 4 cases of the combined subtype. NSE was demonstrated in 35% of the squamous cell carcinomas and 59% of the adenocarcinomas.

Squamous cell carcinomas were negative for Leu-7, and only 4 of the 22 adenocarcinomas were positive for Leu-7. However, 5 of the 8 oat cell subtype cases, 11 of the 16 intermediate subtype cases, and 2 of the 4 combined subtype cases of the SCLC were positive for Leu-7 (Fig-5), which was focally positive in small clusters or in occasional solitary cells. This pattern of reactivity was similar to the immunohistological findings of NSE.

In contrast, 27 of the 28 cases of the small cell carcinoma were non-reactive with anti-Beta-2 M, but 11 of the 22 cases in adenocarcinoma, and 8 of the 20 cases in squamous cell carcinoma were positive. (Fig-6)

DISCUSSION

SCLC has been thought to be an APUDoma of the lung derived from Kultschitzky cells of the bronchus, and differs from other types of lung cancers. This hypothesis was proposed by Pearse in 1971, (2) and has been supported by a number of investigators. (24) Because of its rapid growth and high sensitivity to chemotherapy, SCLC has generally been considered an "undifferentiated tumor." However, this concept has been re-examined because some findings have indicated that SCLS is derived from the same pulmonary endodermal precursor as other lung cancers and differs only in its degree and direction of differentiation. (25)

Detailed examination revealed that approximately 6% of the SCLC contained components of NSCLC, and patients with the initial diagnosis of SCLC showed the coexistence of other histological types or a complete change to the diagnosis of NSCLC, at autopsy.   (26) Electron-microscopically, some SCLC contain cytoskeletal components such as tonofilaments and desmosomes in the cytoplasm. (27)

The SCLC cell line transforms into a NSCLC cell line (mainly into large cell carcinoma) during serial passage, losing the APUD features ; and slight enzyme activities indicative of the APUD features are observed in adenocarcinoma and squamous cell carcinoma. In addition, SCLC cell lines and xenografts almost always express the findings of the intermediate subtype and this subtype has been postulated to be the true morphological expression of SCLC while the oat cell subtype probably represents a degenerative form or artifact. (33) (35) Cell culture studies indicated that SCLC cell lines can be divided into three distinct subclasses : Classic cell lines had morphological features of the intermediate subtype of SCLC, expressing the entire range of SCLC biochemical feature. Variant cell lines could further be divided into two subclasses : SCLC biochemical variant cell lines (SCLC-BV) and SCLC morphological variant cell lines (SCLC-MV). SCLC-MV cell lines and their xenografts had some or all of the morphological features of large cell undifferentiated carcinoma.   (28) These findings led Gazdar et al. to support the "unitarian theory" of the histogenesis proposed by Yesner and Carter in 1982. (29)

This unitarian theory explains the mixture of NSCLC in the tumor tissue of SCLC and

transformation of SCLC into NSCLC during the therapeutic course in some cases. Therefore, SCLC and NSCLC can be of "common stem cell origin" and SCLC is a tumor that has undergone a "neuroendocrine programmed" differentiation. (30)

According to the WHO histological classification of SCLC, (Table-1), the polygonal and fusiform variants are classified as an intermediate type and a new combined type category was introduced to put the SCLC together with other histologic types. Recently, some studies have suggested that the various SCLC subtypes have different responses to therapy or survival, (31) but others found no significant difference in the clinical behavior, response to therapy or survival among these subtypes. (32) By light microscopy, the oat cell carcinomas are characterized by uniform small cells with a very sparse cytoplasm, the intermediate type of small cell carcinoma is very similar to the oat cell type, but in some cells the cytoplasm is more abundant, and epidermoid differentiation is not discernible at the light microscopic level. Almost 10% of the SCLC are difficult to interprete by light microscopy particularly when the tumors are undergoing transition to large cell carcinoma and they have squamous cell differentiation and/or glandular components. It is important that the small cell elements of these tumors be recognized for appropriate classification. (33) (34) In our studies the keratin positive rate was high in the non-oat cell type, whereas, keratin was not demonstrated at all in the oat cell type. Thus, the concentration of keratin seems to be useful to distinguish oat cell type from intermediate cell type. As mentioned above, however, the question is whether the WHO classification of 1981 is not practical use, because this classification does not reflect the clinical feature.

Recently, a new tentative classification was introduced : classic (pure), variant, and combined subtypes, according to morphological feature, and histogenetical characterizations which manifested on the immunohistochemical profile. (22)

When observed by this classification there was no difference with CEA immunoreactivity among the three SCLC subtypes. (Table-3) Classic cell types retained higher concentration of NSE and Leu-7, but had significantly lower concentrations of Beta-2 M. Keratin was found in 43.7% of classic cell types. The variant cell types of SCLC presented almost higher reactivities with CEA, NSE, keratin and Leu-7. However Beta-2 M was also negatively stained in the classic type. This suggests that the variant type cell shows an epidermoid differentiation as does squamous cell carcinoma, although tumor combinations of SCLC with squamous cell carcinoma or adenocarcinoma are classified as "combined SCLC" which present a weakly positive immunoreaction with NSE and Leu-7.

The fact that keratin was found in 43.7% of classic cell subtype but in none of oat cell subtype, suggest that the WHO classification is more practical than the new tentative classification from the biological point of view, and that SCLC seems to be differentiated from

Table 3. Results of the immunohistochemical studies on small cell lung cancer according to tentative classification of IASLC

	CEA	Keratin	NSE.	Leu-7.	Bete-2 M.
pure subtype (classic).	62.5%	43.7%	87.5%	75%	0%
variant subtype.	87.5%	75%	62.5%	50%	12.5%
combined subtype.	75%	75%	25%	50%	0%

"common stem cell" as NSCLC, and also seems to support the "Y" concept (29) which describes the relationship between SCLC and NSCLC. We expect this "unitarian theory" on the histogenesis of lung cancer may be more consistent and useful in subclassifying the SCLC. Further biological and morphological investigation is necessary to study the more adequate classification of SCLC in future.

REFERENCES

1) Sternberger, L. A.: The unlabelled antibody peroxidase-antiperoxidase(PAP) method. Immunocyto-chemistry, 2nd ed. (ed by Sternberger) pp. 104-169, John Wiley & Sons. New York, 1979.

2) Pearce, A. G. E. & Polak, J. M.: Neural crest origin of the endocrine polypeptide(APUD) cells of the gastrointestinal tract and pancreas. Gut 12: 783-788, 1971.

3) Shimosato, Y.: Lung Cancer. Pr. Soc. Pathol. Jpn. 72: 29-57, 1982.

4) Kameya, T. et al.: Ultrastructure of small cell carcinoma (oat and intermediate cell type) in relation to histogenesis and to carcinoid tumor. Morphogenesis of lung cancer. Vol. 2. pp. 15-43, CRC Press, Boca Raton, 1982.

5) Gazdar, A. F. et al.: The biology of endocrine tumors of the lung. The endocrine lung in health and disease(ed by Gazdar and Becker), pp. 448-459, Saunders, Philadelphia, 1984.

6) Gazdar, A. F. et al.: Establishment of continuous clonable culture of small cell carcinoma of the lung which have amine precursor uptake and decarboxilation cell properties. Cancer Res. 40: 3502-3507, 1980.

7) Carney, D. N.: Endocrine properties of small cell carcinoma of lung. The endocrine lung in health and disease(ed by Gazdar and Backer), pp. 501-508, Saunders, Philadelphia, 1984.

8) Baylin, S. B. et al.: Activities of L-dopa decarboxylase as a marker for small(oat) cell carcinoma in cell culture. Cancer Res. 40: 1990-1994, 1984.

9) Gazdar, A. F. et al.: Levels of creatinine kinase and its BB isoenzymes in lung cancer specimens and cultures. Cancer Res. 40: 1990-1994, 1980.

10) Goldnberg, D. M. et al.: CEA in histopathology. J Natl. Cancer Inst. 57: 11-22, 1976.

11) Rosenthal, K. L. et al.: Antibody-induced redistribution of CEA on the cell surface: Utilization in separation of CEA and isoantigen A. J. Immunol. 115: 1049-1052, 1975.

12) Gray, R. H. et al.: Immunocytochemical localization of a protein in tonofilament as a morphologic marker for epidermal differentiation. J. Histochem. et Cytochem. 25: 1127-1132, 1977.

13) Caselitz, Ih. L. & Otto, H. F.: Immunohistochemical demonstration of keratin in an autopsy series. Virch. Arch. Pathol. Anat. 392: 7-20, 1981.

14) Schegel, R. et al.: Immunoperoxidase localization of keratin in human neoplasms. Am. J. Pathol. 101: 41-50, 1980.

15) Carney, D. N. et al.: A marker for disease extent and response to therapy of small cell lung cancer. Lancet 1. 583-585, 1982.

16) Ariyoshi, Y. et al.: Neuron specific enolase as a new tumor marker. J. Cancer Chem. 10: 1744-1753, 1983.

17) Abo, T. & Balch, C. M.: A differentiation antigen of human NK and K cells identified by mono-clonal antibody (HNK).

18) Paul, A. et al.: Small cell cancer, endocrine cells of the foetal bronchus and other neuroendocrine cells express the Leu-7 antigenic determinant present on natural killer cells. Blood 65: 764-768, 1985.

19) Histological typing of lung tumors. World Health Organization. Geneva, 1967.

20) Histological typing of lung tumors (2nd ed). World Health Organization. Geneva, 1981.

21) Shuster, J. et al.: Beta-2 microgloburin levels in cancerous and other disese states. Clin. Chim. Acta. 67: 307-313, 1976.

22) Yesner, R.: Classification of lung cancer histology. New Engl. J. Med. 312: 652-653, 1985.

23) Wharton, J. et al.: Neuron specific enolase as an immunocytochemical marker for the diffuse neuroendo-crine system in human fetal lung. J. Histochem. Cytochem. 29: 1359-1364, 1981.

24) Welbourn, R. B. et al.: The APUD cells of the alimentary tract in health and disease. Med Clin. North Am. 58: 1359-1374, 1974.

25) Baylin, S. B. et al.: Variable content histamine, L-dopa and calcitonin in small cell carcinoma of the

lung. Biological and clinlcal implications. N. Engl. J. Med. 299 : 105-110, 1978.

26) Fer, M. F. et al.: Biology and management of lung cancer. Lung Cancer(ed by Greco et al), pp. 109-124, The Hague, Martinus Nijhoff Publishers, Boston, 1983.

27) Blobel, G. A., et al : Coexpression of neuroendocrine markers and epithelial cytoskeletal proteins in bronchopulmonary neuroendocrine neoplasms. Lab. Invest. 52 : 39-51, 1985.

28) Gazdar, A. F.: Characterization of variant subclasses of cell lines derived from SCLC having distinctive biochemical morphological and growth properties. Cancer Res. 45 : 2924-2930, 1985.

29) Yesner, R. & Carter, D.: Pathology of carcinoma of the lung ; changing patterns. Clin. Chest Med. 3 : 257-289, 1982.

30) Gazdar, A. F. et al.: Small cell carcinoma of the lung ; cellular origin and relationship to other pulmonary tumors. Small cell lung cancer(ed by Greco, et al.), pp. 145-175, Grune and Stratton Inc. New York, 1981.

31) Davis, S. et al.: Small cell carcinoma of the lung—Survival according to histologic subtype. Cancer 47 : 1863-1866, 1981.

32) Carney D. N.: Influence of histologic subtype of SCLC on clinical presentation, response to therapy and survival. J. Natl. Cancer Inst. 65 : 1225-1230, 1980.

33) Gazdar, A. F.: The pathology of endocrine tumors of the lung. An overview. The endocrine lung in health and disease(ed by Becker & Gazdar) pp. 364-372, 1984.

34) Fred, R : Histopathologic classification of small cell lung cancer. Cancer 50 : 1360-1366, 1982.

35) Matthews, M. J. & Gazdar, A. F.: Pathology of small cell carcinoma of the lung and its subtypes : A clinicopathologic correlation. Lung Cancer (ed by Livingston), pp. 283-306, The Hague, Martinus Nijhoff Publishers, Boston, 1981.