Virchows Arch [Pathol Anat] (1983) 400:245–257

Virchows Archiv A

Peptide hormone production by adenocarcinomas of the lung; Its morphologic basis and histogenetic considerations*

Toru Kameya[1], Yukio Shimosato[1], Tetsuro Kodama[1], Masaru Tsumuraya[1], Tsutomu Koide[1], Ken Yamaguchi[2], and Kaoru Abe[2]

[1] Pathology Division and [2] Endocrinology Division, National Cancer Center Research Institute, Tokyo, Japan

Summary. The cell source of peptide hormone production and the morphological differentiation were investigated in 18 adenocarcinomas of the lung by immunohistochemistry and/or by electron microscopy. These tumors were found by radioimmunoassay of tumor extracts to contain either one or more of 7 peptide hormones, i.e. adrenocorticotropin (ACTH), β- and γ-melanocyte stimulating hormones (MSH), somatostatin (SS), vasoactive intestinal polypeptide (VIP), gastrin releasing peptide (GRP) and calcitonin (CT). In a combined adeno- and small cell carcinoma, a considerable number of small tumor cells were positively stained for ACTH, β- and γ-MSHs and GRP. In a poorly differentiated adenocarcinoma with mucin and CT production, these products were localized in some single cells. Electron microscopy revealed secretory granules indistinguishable from exocrine or endocrine types. In another mucin-positive adenocarcinoma with high SS and CT contents, some tumor cells were stained for SS and/or CT. Two distinct exocrine and endocrine type secretory granules were found in the same cells. In tumors with 100 ng or less of the peptides/g tissue, most tumor cells were not stained for the peptides but a small number showed morphological endocrine differentiation. In conclusion, a considerable proportion of the adenocarcinomas of the lung may show heterogeneous differentiation in both endocrine and exocrine directions.

Key words: Lung cancer – Adenocarcinoma – Peptide hormone – Immunostaining

Offprint requests to: T. Kameya, Pathology Division, National Cancer Center Research Institute, 5-1-1, Tsukiji, Chuo-ku, Tokyo 104, Japan

* This work was supported in part by Grants-in-Aid for Research from the Ministry of Health and Welfare (No. 54-12, 56-6) and from the Ministry of Education, Science and Culture (No. 401034, 401066, 57010082 and 57440036)

T.K.: Head, 3rd Histopathology Section; Y.S.: Chief; Kodama, Tsumuraya and Koide: Research Members, 3rd Histopathology Section; K.Y.: Research Member; K.A.: Chief

Introduction

Significant amounts of adrenocorticotropic hormone (ACTH) and calcitonin (CT) were reported to be present in a high proportion of lung carcinomas including tumors classified by conventional histology as adenocarcinomas, squamous cell carcinomas and undifferentiated small cell carcinomas (Gewirtz and Yalow 1974; Bloomfield et al. 1977; Abe et al. 1977). Gewirtz and Yalow (1974) showed the presence of immunoreactive ACTH in almost all lung tumors examined. To our knowledge, however, there has been little morphological evidence that the tumor cells of lung cancer can produce and/or store peptide hormones, although circumstantial evidence based on the detection of argyrophil cells and dense-core small secretory granules in tumor cells of oat cell carcinomas and carcinoids has been presented (Bensch et al. 1968; Hattori et al. 1972). A previous study by two of the authors (T.K. and Y.S.) provided direct evidence of CT production by tumor cells in a case of small cell carcinoma (Kameya et al. 1977). The cell source of the production of peptide hormones by adenocarcinomas of the lung has never been investigated. This study reports direct evidence of peptide storage in tumor cells of adenocarcinomas of the lung and their morphological characterization at histochemical and ultrastructural levels.

Materials and methods

Eighteen tumors of 30 histologically proven adenocarcinomas of the lung were found by radioimmunoassay (RIA) to contain either one or more of 7 immunoreactive peptide hormones, i.e. adrenocorticotropin (ACTH), β- and γ-melanocyte stimulating hormones (β- and γ-MSHs), somatostatin (SS), vasoactive intestinal polypeptide (VIP), gastrin releasing peptide (GRP) and calcitonin (CT). Of these 18 adenocarcinomas 17 were resected surgically and 1 was obtained at autopsy. Details of the methods of extraction and RIA for the measurement of content of the peptide hormones have been described previously (Abe et al. 1977; Yanaihara et al. 1977; Yamaguchi et al. 1980a and b) and detailed results are presented in another paper (Yamaguchi et al. 1983 in press).

Tumor sections cut out from more than one routinely formalin-fixed paraffin embedded block were stained with haematoxylin-eosin, Alcian blue alone or combined with periodic acid-Schiff (PAS), Grimelius silver (Grimelius 1969), and immunohistochemistry for hormone localization by an unlabeled peroxidase-antiperoxidase procedure (Sternberger 1979).

Anti-ACTH, -β-MSH, and -CT were prepared in our laboratory (Abe et al. 1977). Anti-γ-MSH (Tanaka et al. 1980) was a gift from Dr. H. Imura, Kyoto University, Kyoto, and anti-SS, VIP (Yanaihara et al. 1977), and anti-GRP (Yanaihara et al. 1981) from Dr. N. Yanaihara, Shizuoka College of Pharmacy, Shizuoka. A batch of antiserum to secretory component of immunoglobulin A, an antigenically distinct portion of the secretory immunoglobulin A found in external secretions, was purchased from Dakopatts, Ltd., Copenhagen, Denmark (Code No. A187). The second layer of anti-rabbit immunoglobulin and the third layer of peroxidase-antiperoxidase complex were also purchased from the same company and used in 1:50 and 1:200 dilutions, respectively. Specificity was confirmed by the immunostaining of normal tissues of known localization and an absorption test for each antiserum. In immunohistochemical analysis of adjacent paired sections for simultaneous localization of two hormones, each antiserum of working dilution containing excess counterpart antigen (more than 100 μg/ml diluted antiserum) was used to ensure the absence of possible cross-reaction with each other. Nuclear counterstaining was performed as needed by veronal-acetate buffered methylgreen (Barka 1962).

Tumors from 16 of the 18 cases were fixed in 2.5% glutaraldehyde followed by 1% osmium tetroxide and processed for routine electron microscopy. Two or three epon-embedded sections from each tumor were observed by an electron microscope 100U, JEOL Ltd., or H-600, Hitachi Ltd., Japan.

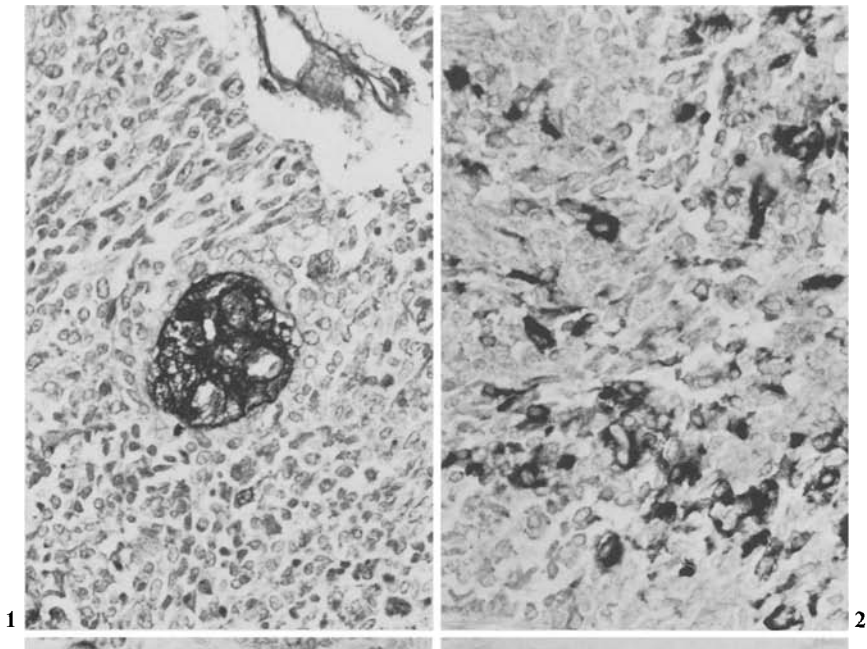
In Case 3, the original tumor was transplanted to nude mice (BALB/c nu/nu) (Shimosato et al. 1976) and passaged up to the 3rd generation. Tumors of every passage were observed by light and electron microscopes.

Results

The 18 tumors consisted of one combined oat cell carcinoma, and 5 well differentiated, 5 moderately differentiated and 7 poorly differentiated adenocarcinomas according to the revised WHO histologic typing (WHO 1981). The evaluation of differentiation was based on the degree of tubular and papillary configuration. In poorly differentiated cases, mucin revealed by Alcian blue-PAS (AB-PAS) was also a hallmark of adenocarcinoma, which could thereby be differentiated from undifferentiated large cell carcinoma. No small cell or carcinoid tumor component was found by routine histological examination in any case except the single combined oat cell carcinoma (Case 1 of this paper).

Tumor cells of 7 cases were immunohistochemically positively stained for either one or more peptide hormones, but only 4 tumors which were found by RIA to contain more than 100 ng of the peptides/g wet tissue showed numerous positive cells. The findings of 3 of the 4 cases are described in some detail. Aspects of Cases 1 and 2 were described briefly elsewhere (Kameya et al. 1982a and b).

Case 1. The patient showed typical Cushing's syndrome with a tumor of the right upper lobe bronchus. The histological sections of the lung tumor and metastatic foci obtained at autopsy (kindly supplied by Dr. S. Yoda, Yokohama City Hospital, Yokohama) showed undifferentiated small cell carcinoma of oat cell type accompanied by numerous tubules. The tubules were formed predominantly by cuboidal cells with vesicular nuclei, cytologically different from oat cells and occasional mucin in cytoplasm and rarely mixed with oat cells (Fig. 1). Their tubule lumina were filled with mucin stained by AB-PAS. ACTH, β-, and γ-MSH-, GRP-immunoreactive cells were present in the tumor. Positive cells were often found in foci and clusters (Figs. 2 and 3), while sparsely scattered elsewhere. Punctate and fibrillar staining was considered due to characteristic cytoplasmic processes of tumor cells, as shown in electronmicrographs of small cell carcinomas (Bensch et al. 1968; Hattori et al. 1972; Shimosato et al. 1979a; Kameya et al. 1982a). Grimelius silver stain revealed a similar positive pattern. Paired adjacent immunohistochemical sections occasionally revealed the presence of immunoreactivity to both anti-ACTH and anti-β-MSH (or anti-γ-MSH) in identical cells (Fig. 4). No cross-reaction was shown in paired sections. Positive reaction was rarely seen in tubule-forming cells. No CT- or VIP-cells were found. Coexistence of mucin and the immunoreactive peptides in ident-



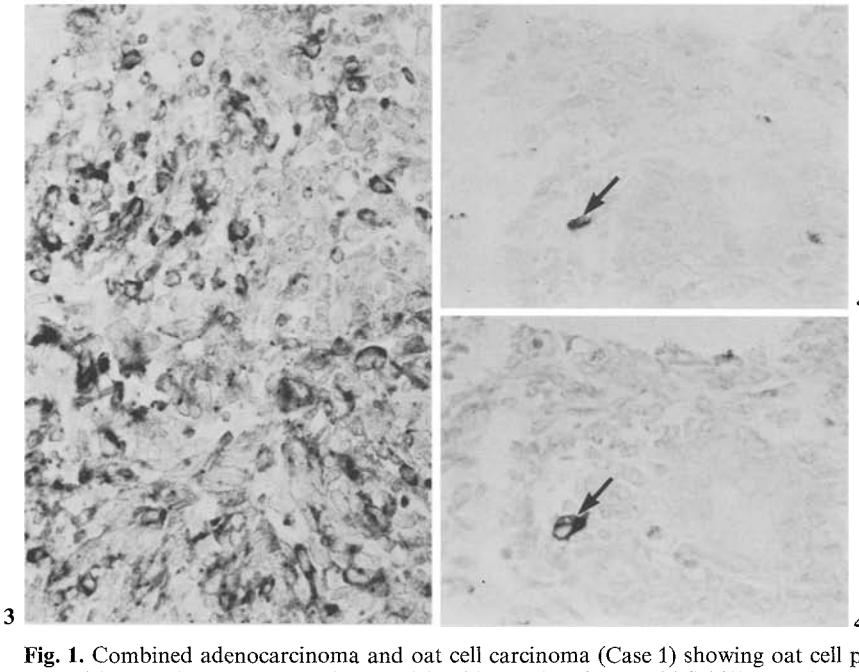


Fig. 1. Combined adenocarcinoma and oat cell carcinoma (Case 1) showing oat cell proliferation with a mucin-containing tubule. (Alcian blue and periodic acid-Schiff, × 300)

Fig. 2. Combined adenocarcinoma and oat cell carcinoma (Case 1) showing numerous cells positive for ACTH. (Immunoperoxidase stain for ACTH. × 300.)

Fig. 3. Combined adenocarcinoma and oat cell carcinoma (Case 1) showing numerous cells positive for GRP. (Immunoperoxidase stain for GRP. × 300)

Fig. 4a, b. Combined adenocarcinoma and oat cell carcinoma (Case 1) showing the same cell (*arrow*) positively stained with both anti-ACTH and anti-γ-MSH. (**a**) and (**b**) are from adjacent sections, showing the same area. (Immunoperoxidase stain for ACTH, **a** γ-MSH, **b** × 1,500)

Table 1. Tumor peptide hormones in Case 1 (combined adenocarcinoma and oat cell carcinoma)

	ACTH	β-MSH	γ-MSH	β-EP	GRP	CT	VIP
Primary tumor	1,714	3,370	n.m.	812	n.m.	n.d.	n.d.
LN metastasis 1	696	1,920	10,800	287	101	n.d.	n.d.
LN metastasis 2	32,000	26,000	115,000	9,870	241	n.d.	n.d.

Values shown as ng/wet g tissue measured by RIA, LN: lymph node, ACTH: adrenocorticotropin, MSH: melanocyte stimulating hormone, EP: endorphin, GRP: gastrin releasing peptide, CT: calcitonin, VIP: vasoactive intestinal polypeptide, n.m.: not measured, n.d.: not detectable

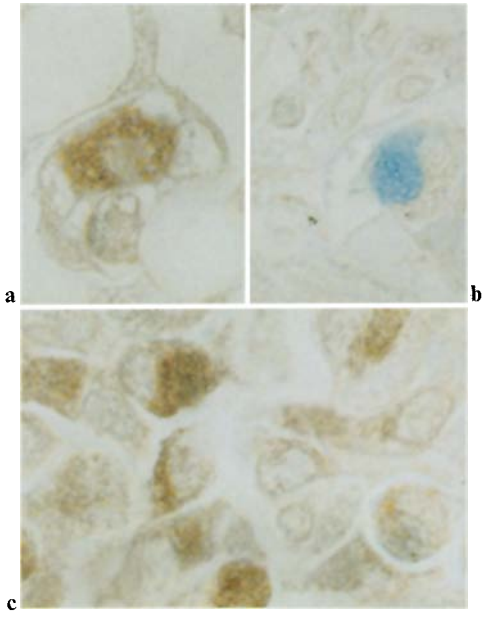


Fig. 5a–c. Poorly differentiated adenocarcinoma (Case 2) showing the presence of CT (*brown* in **a**) and mucin (*blue* in **b**) in two tumor cells and the coexistence of both substances in some other cells (*mixtures of brown and blue* in **c**). (Double staining by immunoperoxidase for CT and Alcian blue for mucin. ×1,000)

ical cells was not found. Electronmicrographs of the autopsy material revealed numerous endocrine type granules (250+40 nm) in some tumor cells. RIA data of tumor extracts shown in Table 1 confirms an approximate coincidence of peptide hormone contents with immunohistochemical data.

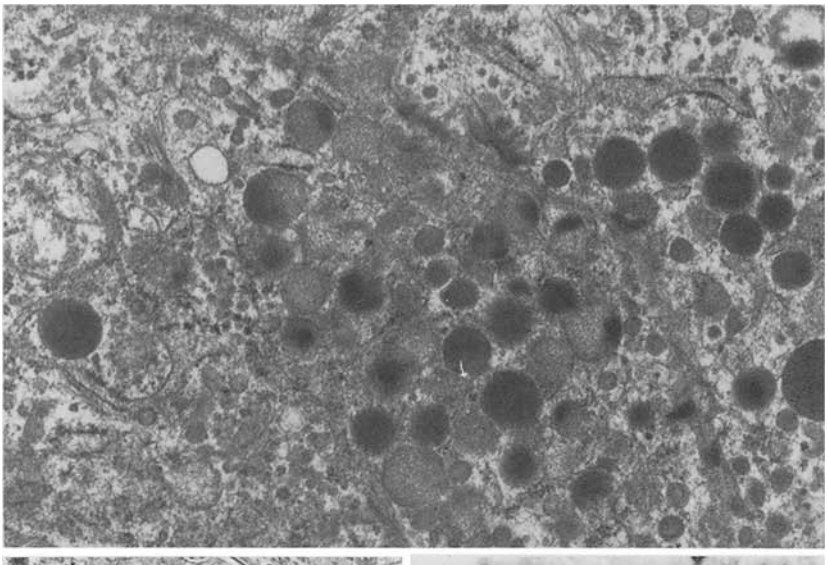
Case. 2. This tumor, which was diagnosed as poorly differentiated adenocarcinoma, was found to possess a high content of CT (166 ng/g wet tissue), which was comparable to that of medullary carcinoma of the thyroid (Kameya et al. 1977). Immunoreactive CT and AB-positive cells were scattered in the tumor (Fig. 5). Argyrophil cells were occasionally revealed by Grimelius stain. In some areas, CT-positive cells were predominant while, in other areas, mucin-positive cells were more frequent. However, double staining of immunoperoxidase and mucin showed the coexistence of both substances in some tumor cells (Fig. 5). No tumor cells stained for other peptide hor-

mones. Electron microscopy revealed that tumor cells contained a variable number of secretory granules measuring 150 to 400 nm in diameter on one side of some cells, and showing hetrogeneous matrix (Fig. 6). These granules probably contained mucin. No definite endocrine type cell containing small dense-core granules and thus resembling neoplastic C-cells of the throid was seen. Direct submicroscopic evidence of what cell type contained mucin and/or CT was not available.

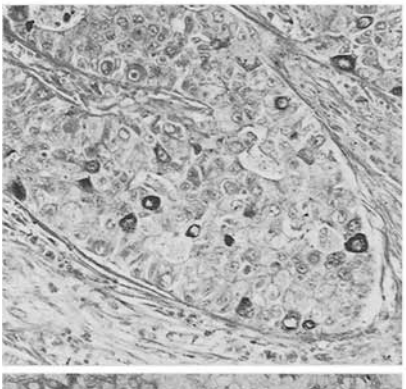
Case 3. This was a poorly differentiated adenocarcinoma with cribriform and solid pattern, possessing occasional mucin and argyrophil cells (Fig. 7). Antiserum against secretory component (SC) without crossreaction to IgA also stained some tumor cells (Fig. 8). Preliminary studies disclosed that most endocrine cells did not stain for SC, while various types of exocrine cells are known to produce SC. Numerous cells were immunohistochemically positive for CT and SS. In some areas, practically no positive cells were encountered. In one area, CT-cells were far more numerous than SS-cells, and almost all the SS-cells were CT-positive, while not all the CT-cells were positive for SS as confirmed by paired adjacent sections (Fig. 9). In other areas, there was a mixture of cells positive for either of the two hormones. Most cells positive for the hormones were argyrophilic. Very few cells were found to be positive for ACTH and β-MSH. Some cells were revealed by the use of paired adjacent sections to be positive for both substances. Although EM specimens of the original tumor were inappropriate for examination, mouse-transplanted tumors were studied. Many tumor cells possessed both exocrine and endocrine type secretory granules. The former were membrane-bound, electron-dense and homogeneous. They measured from 280 to 700 nm (mean: $490+90$ nm) in diameter. The latter were smaller granules, resembling endocrine type granules measuring from 100 to 350 nm (mean: $160+30$ nm) in diameter (Fig. 10). Some cells possessed well-formed microvilli on their apical surface. However, the evidence that the larger exocrine type granules were not of lysosomal nature remains to be determined.

In spite of intensive immunohistochemical studies of the other 14 adenocarcinoma cases, in which any one or more peptides measured by RIA were detected but less than 100 ng/g, only a very few cells in 3 cases and none in 11 cases were positively stained for the peptide hormones examined. Positive results detected by immunohistochemistry and RIA did not coincide in 2 cases. For example, a very few cells were positive by immunostaining for VIP in a case of well differentiated adenocarcinoma, while the hormone was not detected by RIA. However, the hormones were, in general, more frequently detected by RIA than by immunohistochemistry. Cells which located in lung parenchyma outside the tumor were invariably negative for the hormones in this series.

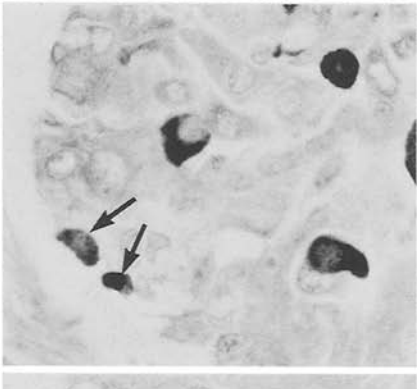
In electron microscopic examinations of 11 cases in which the peptide contents were less than 100 ng/g, occasional clusters of either tumor cells or non-neoplastic cells, which might have been incorporated into tumor, possessed small dense granules similar to endocrine type granules (Fig. 11 a), while most tumor cells possessed larger exocrine type granules (Fig. 11 b).



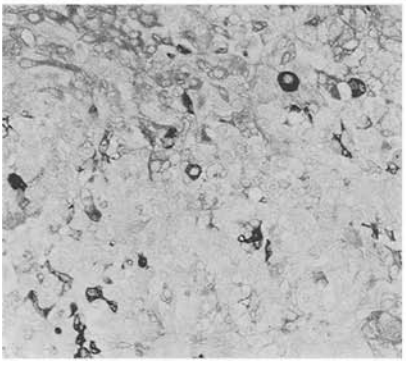
6



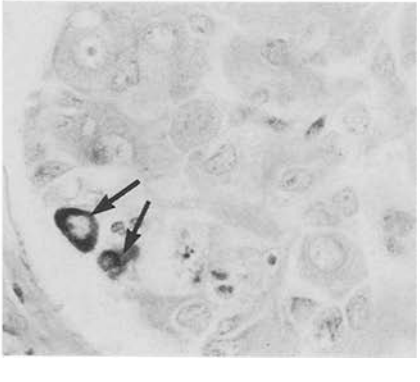
7



9a



8



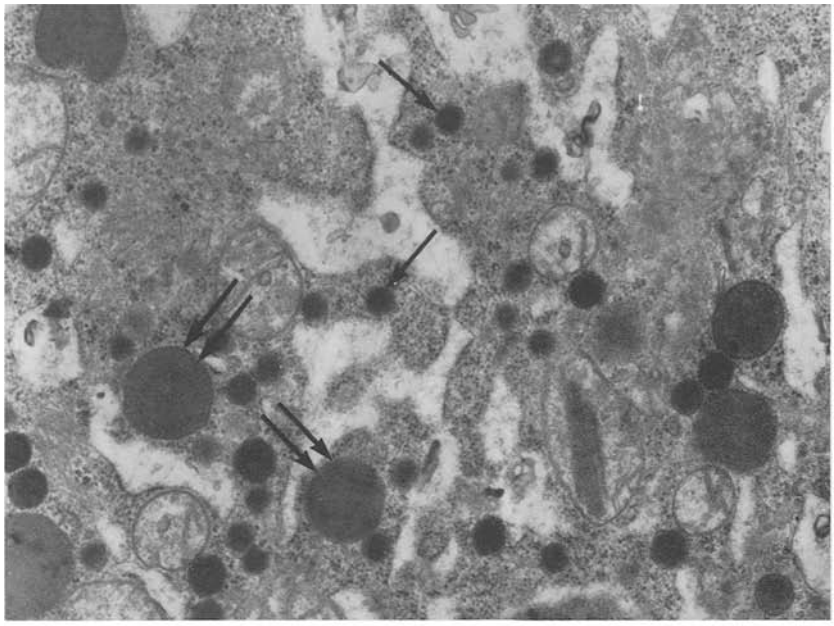
9b

Fig. 6. Electron micrograph of a poorly differentiated adenocarcinoma (Case 2) showing a cluster of secretory granules on one side of a tumor cell. (× 22,500)

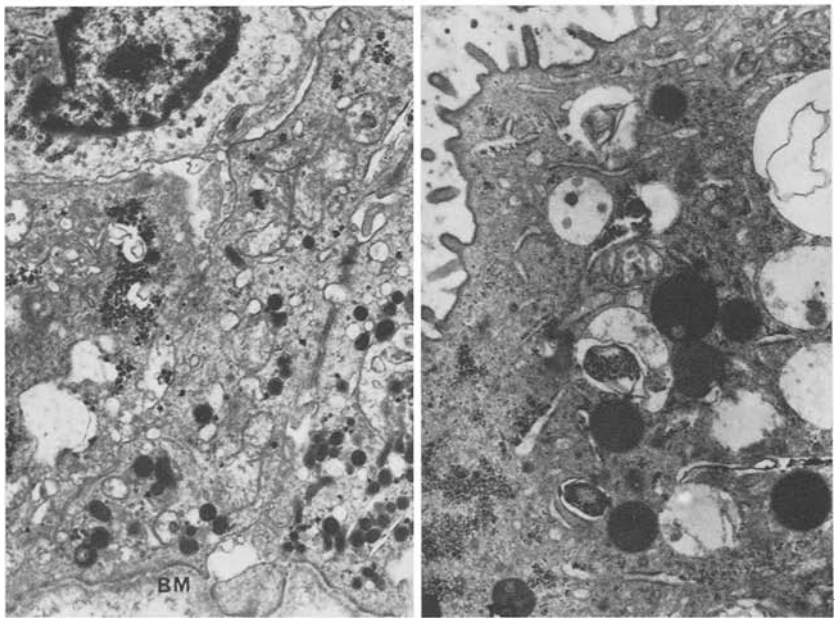
Fig. 7. Poorly differentiated adenocarcinoma (Case 3) showing argyrophil cells. (Grimelius silver stain. × 150)

Fig. 8. Poorly differentiated adenocarcinoma (Case 3) showing clustered or solitary cells positively stained for secretory component. (Immunoperoxidase stain for secretory component. × 150)

Fig. 9. CT (**a**) – and SS (**b**) – positive cells in two identical cells (*arrow*) of two adjacent sections of Case 3. (Immunoperoxidase stain for CT and SS. × 450)



10



11a 11b

Fig. 10. The electron micrograph of a transplant of the poorly differentiated adenocarcinoma of Case 3 to a nude mouse showing two types of small (↑) and large (↑↑) secretory granules. (×22,500)

Fig. 11. Well differentiated papillary adenocarcinoma, showing two cells containing endocrine type dense granules in the basal region (**a**) and cells containing exocrine type granules in the apical region (**b**) of the papillary configuration. BM indicates basement membrane. (×11,300)

Discussion

The present study disclosed that cells of adenocarcinoma of the lung were capable of producting peptide hormones. In Case 1, although it was a rare case of combined oat cell carcinoma, immunohistochemical demonstration of ACTH, β-MSH, γ-MSH, and GRP in tumor cells indicated that tumor cells were the source of hormone production. The evidence that immunoreactivity to ACTH, β-MSH and γ-MSH was present in the same tumor cells was consistent with the evidence that these hormones are processed from a common big precursor of about 30,000 molecular weight in the normal pituitary gland (Nakanishi et al. 1979), and that these hormones were located in the same cells in the normal mammalian pituitary glands (Phifer et al. 1974; Bugnon et al. 1974; Saint-Guillain et al. 1974). A newly recognized peptide hormone, gastrin releasing peptide (GRP) with 27 amino acid residues (McDonald et al. 1979; Yanaihara et al. 1981) is a counterpart of amphibian bombesin, a peptide with 14 amino acid residues, and possessing a striking homology with GRP in the carboxyl terminal region, which was originally isolated from the skin of a frog *Bombina bombina* (Erspamer et al. 1972). It was noteworthy that immunoreactive GRP was localized in many tumor cells of Case 1 with a high content of the hormone because bombesin-like immunoreactivity has been found in the epithelium of the human fetal lung (Wharton et al. 1978) and small cell carcinomas of the lung (Moody et al. 1981; Wood et al. 1981).

In our Case 2, simultaneous storage of mucin and CT in some same cells indicates bidirectional differentiation in function of individual cells.

In Case 3, non-small cell and non-carcinoid tumor was demonstrated to harbor numerous endocrine type cells and morphologically "mixed" or "hybrid" (Sidhu 1979) type cells showing differentiation in both endocrine and exocrine directions as revealed by ultrastructure and the presence of polypeptide hormones CT and SS and the secretory component (SC) of IgA which is characteristically produced by exocrine cells such as nongoblet cells of the gastrointestinal tract, salivary gland ducts and breast lobules and ducts (Rossen et al. 1968; Brandtzaeg 1974). However the distinction of endocrine from exocrine differentiation in terms of morphology alone is, in reality, often arbitrary and presumptive. Simultaneous synthesis and storage of CT and SS by single C-cells of the thyroid has been reported (Noorden et al. 1977). An analogy was demonstrated with our Case 3 tumor and with a case of medullary carcinoma of the thyroid (Sano et al. 1980). Interpreptation of this phenomenon remains to be established at the molecular level since CT and SS do not possess homologous amino acid chains.

In a study of 7 non-small and non-carcinoid type malignant peripheral lung tumors that were diagnosed by light microscopy as large cell carcinomas or as epidermoid or adenocarcinomas (McDowell et al. 1981a), tumor cells were demonstrated to possess dense-core granules in 7, mucrosubstances in 4, argyrophilia in 5 and serotonin in 6 cases. Almost concurrently with the above report, two adenocarcinomas of the lung were reported to contain high levels of CT and dopa decarboxylase (Berger et al. 1981),

the latter being an enzyme known to be elevated in small cell carcinomas of the lung (Baylin et al. 1980) and supposed to be a key enzyme of the APUD cell system (Pearse et al. 1977). These tumors reported by the two groups seemed to resemble our cases 2 and 3.

The present study based on detection of some specific substances such as peptide hormones, mucin and SC, rather than on morphology, confirmed multidirectional and heterogenous cell differentiation in adenocarcinomas. This has already been elucidated and discussed by many pathologists in regard to histogenesis of tumors of the lung (Azzopardi 1959; Willis 1961; Matthews 1976; Yesner 1979; Sidhu 1979; Shimosato et al. 1979a and b; McDowell and Trump 1981a; Kameya et al. 1982) as well as of other organs, especially in the gastrointestinal tract (Bates and Belter 1967; Hernandez and Reid 1969; Klein 1970; Goldenberg and Fisher 1970; Tahara et al. 1975; Ratzenhofer 1977; Sidhu 1979; Matsuyama et al. 1979; Gould et al. 1981; Lyss et al. 1981). The coexistence of cells with endocrine, exocrine and/or epidermoid differentiation severely challenges the conventional concepts on the cell origin of each histological type of lung cancer as well as neoplasms of other organs.

For example, small cell carcinomas and carcinoid tumors of the lung have been considered to derive from K-cells (or presumptive endocrine cells) based on electron microscopic findings and endocrine nature such as argyrophilia, serotonin and/or peptide hormone production in both these tumors and K-cells of normal bronchial epithelium (Bensch et al. 1968; Hattori et al. 1972). However, the presence of "mixed" or "hybrid" cell tumors poses questions the tumor histogenesis previously proposed. Also, there is no evidence that neoplastic transformation can occur in most fully differentiated cells such as K-cells. Therefore, the following recently proposed hypothesis (McDowell and Trump 1981a; McDowell et al. 1981b; Gazdar et al. 1981) seems more attractive; that is that all pulmonary tumors, including tumors with endocrine characteristics can arise from "indifferent" cells or "stem" cells, which can arise, under appropriate stimuli, from divisions of any cell in the normal adult epithelium, that is, basal cells, mucous cells, and possibly also endocrine cells. They may multiply, leading to malignant transformation into various histological types. Were this the case, mixed forms of cancer could naturally occur. Needless to say, however, no definite evidence of the cell origin of any given neoplasm has been presented.

Discrepancies in the detection of peptide hormones by RIA and immunostaining in the present series of 18 adenocarcinomas of the lung may be explained by 1) difference in detection thresholds inherent in each method, 2) sources of tissue materials examined, suggested by markedly heterogeneous or uneven distribution of immunostained cells for hormones in a given tumor, 3) possible differences in the antigenic sites recognized by antisera used. However, it must be stressed that all tumors containing more than 100 ng of the hormones/g tissue measured by RIA revealed numerous immunostained cells on routine paraffin sections. In tumors which contained lesser amounts of immunoreactive hormones and were weakly reactive to antisera on paraffin sections, another possibility exists, that such tumor cells have receptors for peptide hormones and that they are capable of adsorbing the hormones which are of pituitary or other endocrine cell origin, rather than being responsible for their synthesis. However, we have not obtained any specific immunostaining of normal target cells for any peptide

hormone by using routinely processed tissue specimens and the same antisera as in the present study; for example, adrenal cortical cells for ACTH and gastric G cells for GRP.

The demonstration of significant amounts of ACTH in the non-tumor lung tissue which correlated with tumor levels led to speculation (Gewirtz and Yalow 1974; Bloomfield et al. 1977) that such ACTH synthesis could represent a "field" neoplastic or dysplastic change of endocrine cells, which are known to be present throughout the normal lung (Bensch et al. 1968; Hage 1972; Tateishi 1972). However, in the present series, "normal" or non-neoplastic areas around tumor did not contain any appreciable number of hormone-producing cells.

Acknowledgements. The authors would like to acknowledge the supply of antisera by Dr. N. Yanaihara, Shizuoka College of Pharmacy, Shizuoka and by Dr. H. Imura, Kyoto University, Kyoto, the technical assistance by Ms. R. Imagiire and Mr. M. Ogawa and the clerical work by Ms. K. Sugino. We also thank Associate Professor J.P. Barron, St. Marianne University School of Medicine for revision of the English manuscript.

References

Abe K, Adachi I, Miyakawa S, Tanaka M, Yamaguchi K, Tanaka N, Kameya T, Shimosato Y (1977) Production of calcitonin, adrenocorticotropic hormone, and β-melanocyte stimulating hormone in tumors derived from amine precursor uptake and decarboxylation. Cancer Res 37:4190–4194

Azzopardi JG (1959) Oat-cell carcinoma of the bronchus. J Pathol Bacteriol 78:513–519

Barka T, Anderson PJ (1962) Histochemical methods for acid phosphatase using hexazonium pararosanilin as coupler. J Histochem Cytochem 10:741–753

Bates HR Jr, Belter LF (1967) Composite carcinoid tumor (argentaffinoma-adenocarcinoma) of the colon; report of two cases. Dis Colon Rectum 10:467–470

Baylin SB, Abeloff MD, Goodwin G, Carney DN, Gazdar AF (1980) Activities of L-dopa decarboxylase and diamine oxidase (histaminase) in human lung cancers and decarboxylase as a marker for small (oat) cell cancer in cell culture. Cancer Res 40:1990–1994

Bensch KG, Corrin B, Pariente R, Spencer H (1968) Oat-cell carcinoma of the lung; its origin and relationship to bronchial carcinoid. Cancer 22:1163–1172

Berger CL, Goodwin G, Mendelsohn G, Eggleston JC, Abeloff MD, Aisiner S, Baylin SB (1981) Endocrine-related biochemistry in the spectrum of human lung carcinoma. J Clin Endocrinol Metab 53:422–429

Bloomfield GS, Holdaway IM, Corrin B, Ratcliffe JG, Rees GM, Ellison M, Rees LM (1977) Lung tumours and ACTH production. Clin Endocrinol (Oxf) 6:95–104

Brandtzaeg P (1974) Mucosal and glandular distribution of immunoglobulin components – Immunohistochemistry with a cold ethanol-fixation technique. Immunology 26:1101–1114

Bugnon C, Lenys D, Herlant M, Dessy C (1974) Caractérisation de diverses cellules de l'adénohypophyse du Renard par immunofluorescence sur coupes semi-fines et superposition des données de microscopie électronique. C R Séances Acad Sc 278 (Sér D): 2185–2188

Erspamer V, Falconieri-Erspamer G, Inservini M, Negri L (1972) Occurrence of bombesin and alytesin in extracts of the skin of three European discoglossid frogs and pharmacological actions of bombesin on extravascular smooth muscle. Br J Pharmacol 45:333–348

Gazdar AF, Carney DN, Guccion JG, Baylin SB (1981) Small cell carcinoma of the lung: cellular origin and relationship to other pulmonary tumors. In: Greco FA, Oldham RK, Bunn PA Jr (eds). Small cell lung cancer. Grune & Stratton, New York, pp 145–175

Gewirtz G, Yalow RS (1974) Ectopic ACTH production in carcinoma of the lung. J Clin Invest 53:1022–1032

Goldenberg DM, Fisher ER (1970) Histogenetic relationship between carcinoids and mucin-secreting carcinomas of colon as revealed by heterotransplantation. Br J Cancer 24:610–614

Gould VE, Memoli VA, Dardi LE (1981) Multidirectional differentiation in human epithelial cancers. J Submicrosc Cytol 13:97–115

Grimelius L (1969) Studies of adult human pancreatic islet cells with a new silver nitrate stain. Acta Univ Upsal (Abstract from Upsala Dissertations in Medicine 62)

Hage E (1972) Endocrine cells in the bronchial mucosa of human foetuses. Acta Pathol Microbiol Scand [A] 80:225–234

Hattori S, Matsuda H, Tateishi R, Nishihara H, Horai T (1972) Oat cell carcinoma of the lung; clinical and morphological studies in relation to its histogenesis. Cancer 30:1014–1024

Hernandez FJ, Reid JD (1969) Mixed carcinoid and mucus-secreting intestinal tumors. Arch Pathol Lab Med 88:489–496

Kameya T, Shimosato Y, Hayashi H, Tsumuraya M (1977a) Growth and differentiation of hormone-producing human tumors in nude mice. In: Nomura T, Ohsawa N, Tamaoki N, Fujiwara K (eds). Proceedings of the Second International Workshop on Nude Mice. University of Tokyo Press/Gustav Fischer Verlag, Tokyo/Stuttgart, pp 405–416

Kameya T, Shimosato Y, Adachi I, Abe K, Kasai N, Kimura K, Baba K (1977b) Immunohistochemical and ultrastructural analysis of medullary carcinoma of the thyroid in relation to hormone production. Am J Pathol 89:555–574

Kameya T, Kodama T, Shimosato Y (1982a) Ultrastructure of small cell carcinoma (oat and intermediate cell type) in relation to histogenesis and to carcinoid tumor. In: Shimosato Y, Melamed M, Nettesheim P (eds). Morphogenesis of lung cancer, Vol. II, CRC Press, Boca Raton, Florida, pp 15–43

Kameya T, Kodama T, Shimosato Y (1982b) Morphology of lung cancer in relation to its function. In: Shimosato Y, Melamed M, Nettesheim P (eds). Morphogenesis of Lung Cancer, Vol. II, CRC Press, Boca Raton, Florida, pp 107–129

Klein HZ (1970) Mucinous carcinoid tumor of the vermiform appendix. Cancer 33:770–777

Lyss AP, Thompson JJ, Glick JH (1981) Adenocarcinoid tumor of the colon arising in preexisting ulcerative colitis. Cancer 48:833–839

Matsuyama M, Suzuki H (1970) Differentiation of immature mucous cells into parietal, argyrophil, and chief cells in stomach grafts. Science 169:385–387

Matthews MJ (1976) Problems in morphology and behavior of bronchopulmonary disease. In: Israel L, Chahinian AP (ed). Lung cancer, natural history, progress and therapy. Academic Press, New York, pp 23–62

McDonald TJ, Jornvall H, Nilsson G, Vagne M, Ghatei M, Bloom SR, Mutt V (1979) Characterization of a gastrin releasing peptide from porcine non-antral gastric tissue. Biochem Biophys Res Commun 90:227–233

McDowell EM, Trump BF (1981a) Pulmonary small cell carcinoma showing tripartite differentiation in individual cells. Hum Pathol 12:286–294

McDowell EM, Wilson TS, Trump BF (1981b) Atypical endocrine tumors of the lung. Arch Pathol Lab Med 105:20–28

Moody TW, Pert CB, Gazdar AF (1981) High levels of intracellular bombesin characterize human small-cell lung carcinoma. Science 214:1246–1248

Nakanishi S, Inoue A, Kita T, Nakamura M, Chang ACY, Cohen SN, Numa S (1979) Nucleotide sequence of cloned cDNA for bovine corticotropin β-lipotropin precursor. Nature 278:423–427

Noorden SV, Polak JM, Pearse AGE (1977) Single cellular origin of somatostatin and calcitonin in the rat thyroid gland. Histochemistry 53:243–247

Pearse AGE, Polak JM, Bloom SR (1977) The newer gut hormones, cellular sources, physiology, pathology and clinical aspects. Gastroenterology 72:746–761

Phifer RF, Orth D, Spicer SS (1974) Specific demonstration of the human hypophyseal adrenocortico-melanotropic (ACTH/MSH) cell. J Clin Endocrinol Metab 39:684–692

Ratzenhofer M (1977) Über enterale Hyperplasien und Geschwulste der disseminierten endokrinen (parakrinen) Helle Zellen Feyrters unter Berücksichtigung amphikriner Zellwucherung. Verh Dtsch Ges Pathol 61:7–24

Rossen RD, Morgan C, Hsu KC, Butler WT, Rose HM (1968) Localization of 11S external secreting IgA by immunofluorescence in tissues lining the oral and respiratory passages in man. J Immunol 100:706–717

Saint-Guillain M, Dessy C, Masson B, Herlant M (1974) Identification en microscopie électro-

nique des cellules corticotropes chez le Porcelet. C R Séances Acad Sc Paris 278 (Sér D): 2185–2188
Sano T, Kagawa N, Hizawa K, Saito H, Saito S (1980) Demonstration of somatostatin production in medullary carcinoma of the thyroid. Jpn J Clin Oncol 10:221–228
Sidhu GS (1979) The endodermal origin of digestive and respiratory tract APUD cells; histologic evidence and review of the literature. Am J Pathol 96:5–20
Shimosato Y, Kameya T, Nagai K, Hirohashi S, Koide T, Hayashi H, Nomura T (1976) Transplantation of human tumors in nude mice. J Natl Cancer Inst 56:1251–1260
Shimosato Y, Kameya T, Kodama T (1979a) Morphology, histogenesis and prognosis of small cell carcinoma of the lung. In: Wilkins PM (ed). Advances in medical oncology 11. Clinical cancer principle, sites 2. Pergamon Press, Oxford New York, pp 29–38
Shimosato Y, Kameya T, Hirohashi S (1979b) Growth, morphology, and function of xenotransplanted human tumors. In: Sommers SC, Rosen PP (eds). Pathology annual 1979 (Pt. 2), vol 14. Appleton-Century-Crofts, New York, pp 215–257
Sternberger LA (1979) Immunohistochemistry (2nd edn), John Wiley & Sons, New York
Tahara E, Haizuka S, Kodama T, Yamada A (1975) The relationship of gastrointestinal endocrine cells to gastric epithelial changes with special reference to gastric cancer. Acta Pathol Jpn 25:161–177
Tanaka I, Nakai Y, Jingami H, Fukata J, Nakao K, Oki S, Nakanishi S, Numa S, Imura H (1980) Existence of γ-melanotropin (γ-MSH)-like immunoreactivity in bovine and human pituitary glands. Biochem Biophys Res Commun 94:211–217
Tateishi R (1973) Distribution of argyrophil cells in adult human lungs. Arch Pathol 96:198–202
Wharton A, Polak JM, Bloom SR, Ghatei MA, Solcia E, Brown MR, Pearse AGE (1978) Bombesin-like immunoreactivity in the lung. Nature 273:269–270
WHO International Reference Centre for the Histological Definition and Classification of Lung Tumours (1981) Histological typing of lung tumours. (2nd edn) World Health Organization, Geneva
Willis RA (1961) The incidence and histological types of pulmonary carcinoma, with comments on some fallacies and uncertainties. Med J Aust 48:433–440
Wood SM, Wood JR, Ghatei MA, Lee YC, O'Shaughnessy D, Bloom SR (1981) Bombesin, somatostatin and neurotensin-like immunoreactivity in bronchial carcinoma. J Clin Endocrinol Metab 53:1310–1312
Yamaguchi K, Abe K, Miyakawa S, Ohnami S, Sakagami M, Yanaihara N (1980a) The presence of macromolecular vasoactive intestinal polypeptide (VIP) in VIP-producing tumors. Gastroenterology 79:687–694
Yamaguchi K, Abe K, Miyakawa S, Ohnami S, Adachi I, Oka Y, Ueda M, Kameya T, Yanaihara N (1980b) Multiple hormone production in endocrine tumors of the pancreas. In: Miyoshi A (ed). Gut peptides; secretion, function and clinical aspects. Kodansha, Tokyo, pp 343–350
Yamaguchi K, Abe K, Kameya T, Adachi I, Taguchi S, Otsubo K (1983) Yanaihara N Production and molecular size heterogeneity of immunoreactive gastrin releasing peptide in fetal and adult lungs and primary lung tumors. Cancer Res in press
Yanaihara N, Sakagami M, Sato H, Yamamoto K, Hashimoto T, Yanaihara C, Ito Z, Yamaguchi K, Abe K (1977) Immunological aspects of secretin, substance P, and VIP. Gastroenterology 72:803–810
Yanaihara N, Yanaihara C, Mochizuki T, Iwahara K, Fujita T, Iwanaga T (1981) Immunoreactive GRP. Peptides (Fayetteville) 2 (Suppl 2):185–191
Yesner R (1979) Pathologic diagnosis of lung cancer. In: Muggia FM, Rozencweig M (ed). Lung cancer, progress in therapeutic research in progress in cancer research and therapy, vol 11. Raven Press, New York, pp 79–82

Accepted March 17, 1983