1—Neuroendocrine differentiation in lung tumours

Mary N Sheppard

Great advances in knowledge of neuroendocrine lung tumours have resulted in the past 20 years from the application of new techniques, including electron microscopy, cell culture, immunocytochemistry, radioimmunoassay, and more recently molecular biology. These techniques have added greatly to our understanding of these complicated and often fatal tumours but they also raise many further questions, to which we do not yet have answers. For pathologists this is reflected in the multiple and at times complicated classifications applied to these tumours, depending on the techniques used and the tissues studied. For clinicians this explosion in knowledge has provided many new avenues for investigation and the possibility of developing new therapeutic tools. The subject of neuroendocrine differentiation in lung tumours is only now being unravelled. These new findings may soon help to unlock some of the secrets of lung carcinogenesis and help patients by providing diagnostic, prognostic, and possibly therapeutic information.

Neuroendocrine cells in the lung

It was Feyrter¹ in 1938 who first described clear cells in the respiratory epithelium of the human airway and included them in a "diffuse endocrine system" with other clear cells in the gastrointestinal tract and pancreas. Fröhlich² found that these cells were argyrophilic (taking up silver salts) and with foresight (but no evidence) he considered them to be chemoreceptors capable of monitoring gas tensions in the airways and releasing hormones. Hamperl³ saw the similarity of these cells to the Kulschitsky or K cell of the gastrointestinal tract and suggested this name for them. Electron microscopy showed that the cells contained cytoplasmic dense core granules similar to granules found in endocrine cells elsewhere and in neurones.⁴ The cells were therefore called neuroendocrine cells to emphasise the association between the endocrine and the nervous system. Immunocytochemical and sensitive radioimmunoassay techniques confirmed the presence of several amines and peptides within these neuroendocrine cells, including serotonin, gastrin releasing peptide (GRP), the C flanking peptide of preproGRP, calcitonin, leu-enkephalin, calcitonin gene related peptide (CGRP), and endothelin.^{5,6} Many of these peptides are also found in neurones and nerves of the central and peripheral nervous system, again showing the close link between the endocrine and nervous systems.

This widespread collection of peptide containing cells and nerves is known as the diffuse neuroendocrine system.^{6,7} All these peptides have potent actions on airway smooth muscle, vasomotor tone, and airway secretion and have been proposed as important modulators of physiological changes in the lung.⁸ The peptides may carry out their effects in a classical endocrine manner via the bloodstream, via nerve transmission (neuroendocrine), or in a local ("paracrine") fashion. Not surprisingly, given the extensive nature of this peptide containing system in the human respiratory tract, lung tumours can produce many of these neuroendocrine features.

Neuroendocrine lung tumour classification

The secretion of what were originally considered to be "ectopic" hormones by lung tumours has been known for some time.⁹ Adrenocortical overactivity leading to Cushing's syndrome was described in a patient with lung carcinoma in 1928. Sensitive assay and cell culture techniques have permitted the detection of peptide hormone secretion by tumours, particularly lung tumours,^{10,11} in the absence of clinically overt syndromes. Overt endocrinological disturbance and biologically active peptide hormone production is common in small cell carcinomas and carcinoid tumours; but almost all lung cancers, irrespective of histological type, are capable of producing small amounts of peptide.¹²

Both carcinoid tumours and small cell carcinomas can be recognised by light microscopy. Dense core neurosecretory granules in small cell carcinomas and carcinoid tumours added an ultrastructural feature to establish neuroendocrine differentiation^{4,13} and showed the close association between these tumours and normal pulmonary endocrine cells, which also contain dense core granules. Thus both carcinoid tumours and small cell carcinomas are considered neuroendocrine tumours of the lung and included in the World Health Organisation histological classification of lung tumours.¹⁴ Carcinoid tumours are considered to form the benign end of the spectrum and small cell carcinomas the highly malignant end. A distinct subgroup of carcinoid tumours, labelled atypical carcinoids, with histological and clinical evidence of malignancy and with a high rate of metastases,¹⁵ were described in 1972. These lung neuroendocrine tumours form a link between carcinoid tumours and small cell carcinomas. To reflect the overlap between these tumours it was suggested that

Department of
Lung Pathology,
National Heart and
Lung Institute,
Royal Brompton
National Heart and
Lung Hospital,
London SW3 6NP
M N Sheppard

Reprint requests to:
Dr Sheppard

Classifications of neuroendocrine carcinomas of the lung

WHO classification ¹⁴	Paladugu ¹⁶	Gould ²⁶	Travis ⁶¹
Carcinoid	KCC* 1	Carcinoid	Carcinoid
Atypical carcinoid	KCC 2	Well differentiated neuroendocrine carcinoma Neuroendocrine carcinoma intermediate cell carcinoma	Atypical carcinoid Neuroendocrine carcinoma large cell carcinoma
Small cell carcinoma	KCC 3	Neuroendocrine carcinoma Small cell carcinoma	Small cell carcinoma

*Kulschitsky cell carcinoma.

they should be reclassified as Kulschitsky cell carcinomas to indicate their spectrum of aggressiveness and emphasise their origin from the pulmonary endocrine or Kulschitsky cell¹⁶ (table).

Immunocytochemical techniques using antibodies to general neuroendocrine markers, including neurone specific enolase,¹⁷ PGP 9-5, synaptophysin, and chromogranin,^{18,19} were applied to neuroendocrine lung tumours by ourselves and others. Sensitive radioimmunoassay techniques were also used to detect these markers in serum and monitor tumour progression and recurrence.^{20,21}

These new techniques clarified the neuroendocrine nature of the tumours, suspected from light microscopy, and produced some unexpected findings, which at first were difficult to explain. The presence of dense core granules, peptide or amine hormone production, and neuroendocrine immunocytochemical markers in undifferentiated large cell carcinomas, squamous cell carcinomas and adenocarcinomas gave rise to the concept of atypical endocrine tumours,^{22,23} non-small cell carcinomas with neuroendocrine features,²⁴ and large cell neuroendocrine tumours of the lung.²⁵ These categories have caused much confusion among pathologists and clinicians concerning their behaviour, prognosis, and response to treatment.

The important question is whether these tumours with neuroendocrine features behave aggressively like small cell carcinomas and whether they therefore respond to chemotherapy. Alternatively, is neuroendocrine differentiation of no significance, merely reflecting the general heterogeneity of lung tumours? Another possibility is that neuroendocrine differentiation may indicate a more benign behaviour as typical carcinoid tumours, which display many neuroendocrine features, are usually indolent.

In an attempt to answer these questions Gould²⁶ suggested a spectrum comprising several related but distinct types of neuroendocrine neoplasms in the lung (table). He retained the typical carcinoid tumour group but labelled atypical carcinoids as well differentiated neuroendocrine carcinomas to emphasise their malignant behaviour. Small cell carcinomas are categorised as neuroendocrine carcinoma of small cell type. In addition, he introduced a new category, which he called

neuroendocrine carcinoma of intermediate cell type, and included many large cell undifferentiated carcinomas and tumours with squamous and glandular differentiation in this group (table). His clinical follow up of patients with these tumours shows a spectrum of behaviour, the intermediate cell type behaving in a fashion similar to that of the small cell type and being more aggressive than other large cell carcinomas.²⁷ He considers that neuroendocrine differentiation in non-small cell carcinomas indicates aggressive behaviour and suggests that chemotherapy should be assessed for such tumours.

This classification is useful in delineating a spectrum of endocrine lung tumours with predictable behaviour but falls short on several points. Firstly, the description of the neuroendocrine carcinoma of intermediate cell type is too vague and non-specific to allow this to be separated from other undifferentiated tumours by light microscopy in my experience. Secondly, it is unfortunate that this category was named intermediate cell type as it is often confused with the "intermediate" small cell carcinoma subgroup of the WHO classification.¹⁴ Thirdly, the classification presupposes a histogenetic relationship that has not yet been established. There is now sufficient evidence to regard carcinoid tumours as separate from small cell carcinomas and other lung tumours. Carcinoid tumours occur in a younger population and are not related to smoking habits or other risk factors for the more common forms of lung cancer. Furthermore, small cell carcinomas are rarely seen in association with carcinoid tumours. The initial response of a small cell carcinoma to combination chemotherapy and the poor long term prognosis contrast with the chemoresistance and good prognosis of most carcinoid tumours. These observations call into question the value of attempts to devise a terminology for a spectrum of neuroendocrine tumours and the classification of these tumours remains in a state of flux.

Difficulties in the diagnosis of neuroendocrine lung tumours

The more recent classifications of neuroendocrine lung tumours are difficult for pathologists to apply, particularly for the non-small cell types. Classification requires basic light microscopy plus electron microscopy and the use of immunocytochemical markers, which are expensive and time consuming. A patient with an unusual lung tumour suspected of being a neuroendocrine tumour should probably be referred to a specialist centre for appropriate investigations; formal follow up is essential to establish their behaviour and prognosis.

A second problem is that lung tumours show heterogeneity with both light microscopy and electron microscopy, which can lead to problems in interpreting neuroendocrine features. Up to 7% of lung tumours have a mixed morphology according to light microscopic appearances. Multiple sampling of a tumour by electron microscopy may reveal glandular, squamous, and neuroendocrine

Figure 1 Typical carcinoid tumour of lung with islands and ribbons (trabeculae) of cells showing uniformity of nuclei and cytoplasm. There is hyperchromatism of the nuclei at the periphery of the islands.

Figure 2 Dense granules in the cytoplasm of a carcinoid tumour cell. (Electron micrograph by A Dewar, National Heart and Lung Institute.)

areas even within the same cell; the significance of large or small areas of neuroendocrine differentiation in a mixed tumour remains to be assessed. For definitive diagnosis large quantities of tissue may be required for special investigations. This may be a problem with bronchial biopsy, the only diagnostic technique used for many lung tumours as the patients are inoperable. Most neuroendocrine classifications rely heavily on the architecture of the cells within the tumour and this may not be seen easily in small biopsy specimens. Crush artefact, cellular distortion, and degeneration may make it difficult at times to separate carcinoid tumours from small cell carcinomas, a fact of great importance for treatment and prognosis.

At electron microscopy the definition of a dense core neurosecretory granule, the hallmark of neuroendocrine differentiation, is not clear and several studies show a wide divergence in the size, shape, and distribution of these granules. Differentiating them from small lysosomes may be difficult.

Finally, the specificity of the general neuroendocrine markers has been questioned as these markers have been found in non-neuroendocrine tumours, such as breast or kidney tumours. The use of only one marker for immunocytochemical diagnosis of neuroendocrine differentiation is open to criticism and a panel of markers is now recommended (see below).

Carcinoid tumours of the lung

About 12% of all carcinoids arise in the lung but less than 1% of lung tumours are carcinoid.²⁸ Hamperl²⁹ was the first to separate carcinoids from other bronchial adenomas and point out their similarity to gastrointestinal carcinoids. The highest incidence occurs in the 31–40 year age group, with a female preponderance (62%). The tumour usually arises in a main bronchus (85%) but may be peripheral (15%).³⁰ Multicentric growths scattered throughout the lung have been described³¹ and carcinoids can be associated with endocrine tumours elsewhere as part of a pluriglandular syndrome.³²

Carcinoid tumours contain uniform cells with small round to oval nuclei, few nucleoli, and granular eosinophilic cytoplasm. The cells are most commonly in islands interweaving with one another in a mosaic pattern (fig 1), as interconnecting ribbons of cells in a trabecular pattern, or in an adenopapillary pattern; mixed forms are also seen.³³ In a typical carcinoid nuclear pleomorphism and mitoses are rare.

At the ultrastructural level the main feature of all carcinoids is the large numbers of dense core neurosecretory granules in the cytoplasm of virtually all tumour cells⁴ (fig 2). Most carcinoid tumours are strongly immunoreac-

tive for general neuroendocrine markers^{17 18 34} (fig 3), and immunocytochemical methods and radioimmunoassay show that bronchial carcinoids produce a wide range of amines and peptide hormones.^{12 26} Gould found that 5-hydroxytryptamine was the most frequently occurring immunoreactive substance in pulmonary carcinoids, followed in descending order of frequency by gastrin releasing peptide, vasoactive intestinal polypeptide, leu-enkephalin, somatostatin, calcitonin, and adrenocorticotrophic hormone. A single tumour may produce several peptides as well as 5-hydroxytryptamine. The latter has been put forward as an immunocytochemical marker of carcinoids but is found in only half of foregut carcinoids, which limits its diagnostic usefulness.³⁵ S-100 positive sustentacular cells have been reported in pulmonary carcinoids³⁶ and labelled as "paraganglioid" carcinoids. The feature that distinguishes these from the rare primary pulmonary paraganglioma is that paragangliomas rarely express keratin and are strongly immunoreactive for neurofilaments.³⁷

Carcinoid tumours are generally easy to diagnose by light microscopy and do not require further investigations. They have an excellent prognosis generally. Five per cent of typical cases with no features of malignancy metastasise¹⁵ so the behaviour of individual tumours cannot be predicted. Deeply invasive tumours,³⁸ tumours greater than 3.0 cm in diameter, and lymph node metastases predispose to recurrence.³⁹

Atypical carcinoid tumours

Arrigoni¹⁵ first identified a group of tumours with a carcinoid architecture but with evidence of malignancy, characterised by nuclear pleomorphism, mitoses, and necrosis. The tumours were larger and more likely to be peripheral, and their behaviour was more aggressive than that of typical carcinoids, with regional or distant metastases in 70% of patients and a mortality of 30%.

In two studies that examined stage 1 resectable tumours diagnosed initially as small cell or large cell undifferentiated carcinomas 25%⁴⁰ and 80%⁴¹ turned out to be atypical carcinoids or well differentiated neuroendocrine carcinomas. These tumours were associated with a very much better survival rate at two years (60–75%) than small cell carcinomas even when these were resectable (9%).⁴⁰ The improved survival described for stage 1 resectable small cell carcinoma^{42 43} and the subsequent resurgence of interest in resecting small cell carcinomas must be looked at closely in view of these findings. Strict criteria must be used in labelling any peripheral lung lesion as a small cell carcinoma.

Dense core neurosecretory granules are far less numerous than in true carcinoids and they tend to be smaller (80–140 nm). Intertwined cytoplasmic processes are prominent and the granules are usually concentrated there. In a typical carcinoid more than half of the cells contain granules with at least five per cell whereas atypical carcinoids possessed granules

Figure 3 Positive immunostaining for chromogranin A in the cell cytoplasm of a carcinoid tumour.

Figure 4 Small cell carcinoma of the lung showing the small hyperchromatic nuclei, nuclear moulding, little cytoplasm, and high cell death rate.

Figure 5 Small cell carcinoma of the lung giving a focal intense signal for chromogranin A messenger RNA. (From Hamid *et al*²⁸; reproduced by courtesy of the Journal of Pathology.)

in half of the cells or less with less than five per cell.²⁵

These tumours are immunoreactive for many of the neuroendocrine, peptide hormone and epithelial antigens already described for carcinoids.^{18 26 34} No immunocytochemical marker, however, distinguishing this tumour from typical carcinoid or small cell carcinoma has so far been discovered.

Small cell carcinoma

The WHO classification¹⁴ divides small cell carcinomas into three main types: (1) small or oat cell carcinoma, consisting of small round to elongated nuclei with finely granular chromatin, no nucleoli, and little cytoplasm (fig 4); (2) intermediate or polygonal cell carcinoma, composed of slightly larger cells with more abundant cytoplasm and with nuclear characteristics similar to those of oat cell carcinoma; (3) small cell combined with squamous cell carcinoma or adenocarcinoma (only about 1% of tumours fall into this category).

These tumours often produce hormonal syndromes and contain dense core neurosecretory granules at the ultrastructural level¹³ confirming

their neuroendocrine nature. The granules are usually scanty and small (80–140 nm) and multiple sampling of a tumour is necessary to detect them. The use of electron microscopy has introduced new problems in the diagnosis of small cell carcinoma. Several authors report the absence of dense core granules in what appears at light microscopy to be classic small cell carcinoma. The presence of tonofilaments and desmosomes led Churg *et al*⁴⁴ to label them small cell squamous tumours whereas Nomori *et al*⁴⁵ labelled them undifferentiated carcinoma, small cell type. Patients with these tumours have been reported to survive longer than those with tumours with classical granules, but numbers of cases are small.⁴⁶

There has also been controversy about the prognostic significance of the subdivisions of small cell carcinomas. Some studies have led to claims that the small cell type has a better prognosis and is more responsive to chemotherapy,^{47 48} whereas others suggest that the intermediate cell type does best.⁴⁹ Two further studies suggest no difference.⁵¹ This controversy, as well as the difficulty of separating the small cell from the intermediate cell type histologically, has led many pathologists and clinicians to abandon this subclassification.

The mixed small cell/large cell carcinoma was first proposed as a separate entity in 1982. These mixed tumours, originally thought to be rare, are being recognised more frequently especially in biopsy and necropsy specimens from patients treated intensively with radiation or chemotherapy. They represent 5–12% of all small cell carcinomas and have shown a poor response to chemotherapy with a shorter survival.^{52 53}

The International Association for the Study of Lung Cancer (IASLC) has recommended that small cell carcinoma should be divided into three groups: (1) small cell carcinoma, combining the classic small cell and intermediate cell types (90% of untreated small cell carcinomas are of this type); (2) small cell/large cell carcinoma, in which at least 24% of the cells have large granular or more open nuclei with prominent eosinophilic nucleoli (4–6% of untreated small cell carcinomas fall into this category); (3) carcinoma of mixed type similar to WHO group 3.

This classification is supported by experimental studies on small cell carcinoma. Many continuous cell lines and xenographs have been established.⁵⁰ Most contain dense core granules, express neuroendocrine and other markers associated with small cell carcinoma, and are radiosensitive. The cytological features of these “classic” cell lines are of the small cell subtype of small cell lung carcinoma. Some small cell lines have a “variant” morphological appearance and resemble small/large cell carcinomas or large cell carcinomas. They lack dense core granules and other neuroendocrine features. They are sometimes associated with amplification of the c-myc oncogene and they grow more rapidly, are cloned more efficiently, and are radioresistant. These variant cell lines may develop from classic small cell carcinomas, especially if these have been treated with

chemotherapy, or from mixed small cell/large cell carcinomas. The development of large cell features in a small cell carcinoma after chemotherapy may be due to the treatment or to the survival of large cells already present in the tumour before treatment. There is wide support for the above classification, though some studies suggest no difference in prognosis.⁵¹

Small cell carcinomas are immunoreactive for general neuronal markers.^{17 18 34} Serum concentration of neuroendocrine markers, such as neurone specific enolase, have been used as tumour markers for small cell carcinoma and may be used to monitor treatment and predict relapse.^{20 21} Unfortunately from the diagnostic and prognostic point of view, in general the more malignant the neuroendocrine tumour is the less likely it appears to be to give positive immunocytochemical results for neuroendocrine antigens or peptide hormones.²⁶

A surprising immunocytochemical finding is that gastrin releasing peptide, an established growth factor, is rarely expressed in small cell carcinomas. This may be due to the production of abnormal forms of the precursor preproGRP molecule, and the C terminal peptide in particular. Antibodies to C terminal peptide show strong immunostaining in 12% of typical carcinoids, in 56% of atypical carcinoids, and in 70% of small cell carcinomas,¹⁸ and these appear to be associated with a shorter survival than those without the immunoreactive peptide. The more malignant a tumour is the more likely it is to produce an abnormal peptide precursor. The mechanism underlying any of the paraneoplastic syndromes, such as neuropathies, encephalomyelitis, and myopathies, is unknown, though production of antigens by the tumour similar to neuronal antigens and the subsequent induction of antibodies by the body in response to the antigens are thought to explain many of the syndromes.⁵⁵ This suggests that many more tumour products remain to be discovered.

Immunocytochemistry and radioimmunoassay provide useful data on stored and secreted peptide; information on gene expression and regulation requires molecular biology techniques, including Northern and Southern blotting, in situ hybridisation, and the polymerase chain reaction. When these techniques are applied the gene for gastrin releasing peptide has been found in all carcinoids and small cell carcinomas⁵⁶ and in most large cell carcinomas.⁵⁷ We have localised the neuroendocrine antigen chromogranin A gene in small cell carcinomas⁵⁸ (fig 5).

These techniques have also helped to detect oncogenes and anti-oncogenes and abnormalities in their expression in neuroendocrine tumours. The major oncogenes implicated in neuroendocrine tumours are the *myc* and *ras* families. *Myc* amplification appears to be associated with tumour progression rather than pathogenesis. *C-jun* is also amplified in small cell lung cancer.⁵⁹

Restriction fragment length polymorphism (RFLP) analysis indicates that deletions of chromosome arms 3p, 13q, and 17p are frequent in small cell lung cancer. Chromosome

13q is the site of the retinoblastoma gene and 17p of the p53 gene, and 3p is the site of an as yet unidentified anti-oncogene.⁶⁰

Non-small cell neuroendocrine carcinomas

The first description of non-small cell neuroendocrine carcinomas, which by light microscopy had been diagnosed as large cell or squamous cell carcinomas or adenocarcinomas, came in 1981, when McDowell *et al*²² described seven cases in the periphery of the lung. Electron microscopy subsequently showed dense core neurosecretory granules, thus establishing them as neuroendocrine tumours (in 4% of 150 lung tumours). The tumours were argyrophilic and contained 5-hydroxytryptamine, confirming endocrine differentiation at the level of light microscopy. McDowell labelled them atypical endocrine tumours of the lung. Another report²³ suggested that as many as 9% of non-small cell carcinomas in the lung contained neurosecretory granules, and that median survival and rates of response to treatment were in the ranges seen with non-small cell carcinoma. Others,^{24 25} however, claimed that they behaved more aggressively, like small cell carcinoma, and labelled them large cell neuroendocrine tumours or non-small cell carcinomas with neuroendocrine features. Gould²⁶ included them as intermediate cell type in his spectrum of neuroendocrine carcinomas and suggested that cell nesting with peripheral palisading of nuclei would help to identify this tumour by light microscopy, a point emphasised by Mooi *et al*.²⁴ Travis *et al*⁶¹ emphasised that the cells are large with prominent nucleoli in the nuclei, a high mitotic rate, and a low nuclear:cytoplasmic ratio and have an organoid, trabecular, and palisading pattern. In a later study and follow up by Gould's group the tumours behaved in a fashion similar to that of small cell carcinoma.²⁷ The clinical relevance of a separate group of non-small cell neuroendocrine carcinomas remains to be determined; most studies are small and lack follow up.⁶¹

Specificity of neuroendocrine markers

Several workers applying neuroendocrine markers to lung tumours have found that a proportion of all tumour types give positive results.^{34 62} This may reflect the heterogeneity of lung tumours; others consider that the markers lack specificity for neuroendocrine tumours.⁶³ We found that, although neurone specific enolase and PGP9.5 are positive in most neuroendocrine tumours,³⁴ they are also present in 10–60% of non-neuroendocrine lung tumours and are not now considered as reliable markers of neuroendocrine differentiation. Chromogranin, Leu-7, and synaptophysin appear to be more useful markers but they stain fewer neuroendocrine tumours than neurone specific enolase and PGP9.5.^{34 61} Use of a panel of markers rather than a single marker is advised, with a combination of neurone specific enolase, PGP9.5, chromogranin, and synapto-

physin. A positive result with two of the four markers is recommended before a tumour can be labelled as neuroendocrine.

Conclusion

Neuroendocrine lung tumours are proving a fruitful area of research for understanding the basic mechanisms of carcinogenesis. Rapid advances in the molecular and cell biology of these tumours should help to determine their spectrum of behaviour. New studies are essential, using light microscopy, electron microscopy, immunocytochemistry, and molecular biology techniques, for clarifying different subtypes and designing future treatments, particularly now that many growth factors are known to be produced by these tumours. A classification based on morphological and functional criteria may prove to be of therapeutic importance in the future.

- Feyrter F. *Ueber diffuse endokrine epitheliale Organe*. Leipzig: Bath, 1938.
- Fröhlich F. Die "Helle Zelle" der Bronchialschleim haut und ihre Beziehungen zum problem der Chemoreceptoren. *Frankfurt Z Pathol* 1949;60:517-59.
- Hamperl H. Über argyrophile Zellen. *Arch Pathol Anat Physiol* 1952;321:482-507.
- Bensch KG, Gordon GB, Miller LR. Electron microscopic and biochemical studies on the bronchial carcinoid tumour. *Cancer* 1965;18:592-602.
- Giaid A, Hamid QA, Springall DR, Yanagisawa M, Shinmi O, Sawamura T, et al. Detection of endothelin immunoreactivity and mRNA in pulmonary tumours. *J Pathol* 1990;162:15-22.
- Springall DR, Polak JM. Localization of peptides in the lung. In: Piper PJ, ed. *Advances in the understanding and treatment of asthma*. New York: Academy of Sciences, 1991:1-20.
- Polak JM, Bloom SR. Occurrence and distribution of regulatory peptides in the respiratory tract. In: Havemann K, Sorenson G, Gropp C, eds. *Recent results in cancer research*. Berlin: Springer, 1985:1-16.
- Becker KL. Lung Cancer. Peptide hormones and their possible functions in the normal and abnormal lung. In: Havemann K, Sorenson G, Gropp C, eds. *Recent results in cancer research*. Berlin: Springer, 1985:17-28.
- Brown WH. A case of pluriglandular syndrom. *Lancet* 1928;ii:1022-3.
- Ratcliffe JG, Podmore J. Ectopic hormones. In: Boelsma E, Rumke Ph, eds. *Tumour markers: impact and prospects*. Amsterdam: Elsevier/North Holland Biomedical Press, 1979:3-23.
- Baylin SB, Mendelsohn G. Ectopic (inappropriate) hormone production by tumours: mechanisms involved and the biological and clinical implications. *Endocr Rev* 1980; 1(No 1):45-77.
- Kameya T, Kodama T, Shimosato Y. Morphology of lung cancer in relation to its function. In: Shimosato Y, Melamed MR, Nettesheim P, eds. *Morphogenesis of lung cancer*. Vol 2. Florida: CRC Press, 1982:107-29.
- Bensch KG, Corrin B, Pariente R, Spencer H. Oat cell carcinoma of the lung: its origin and relationship to bronchial carcinoid. *Cancer* 1968;22:1163-72.
- World Health Organisation. *Histological typing of lung tumours*. 2nd ed. Geneva: WHO, 1981.
- Arrigoni MG, Wooner LB, Bernatz PE. Atypical carcinoid tumours of the lung. *Journal of Thoracic and Cardiovasc Surg* 1972;64:413-21.
- Paladugu RR, Benfield JR, Pak HY, Ross RK, Teplitz RL. Bronchopulmonary Kulchitzky cell carcinomas. A new classification scheme for typical and atypical carcinoids. *Cancer* 1985;55:1303-11.
- Sheppard MN, Corrin B, Bennett MH, Marangos PJ, Bloom SR, Polak JM. Immunocytochemical localisation of neuron-specific enolase in small cell carcinoma and carcinoid tumours of the lung. *Histopathology* 1984;8: 171-81.
- Addis BJ, Hamid Q, Ibrahim NBN, Fahey M, Bloom SR, Polak JM. Immunohistochemical markers of small cell carcinoma and related neuroendocrine tumours of the lung. *J Pathol* 1987;153:137-50.
- Wiedenmann B, Huttner WB. Synaptophysin and chromogranins/secretogranins—widespread constituents of distinct types of neuroendocrine vesicles and new tools in tumor diagnosis. *Virchows Arch B Cell Pathol* 1989;58: 95-121.
- Carney DN, Idhe DC, Cohen MH, Marangos PJ, Bunn PA, Minna JD. Serum neuron-specific enolase a marker for disease extent and response to therapy of small-cell lung cancer. *Lancet* 1982;ii:583-5.
- Burghuber OC, Worofka B, Schernthaner G, et al. Serum neuron-specific enolase is a useful tumour marker for small cell lung cancer. *Cancer* 1990;65:1386-90.
- McDowell EM, Wilson TS, Trump BS. Atypical endocrine tumours of the lung. *Arch Pathol Lab Med* 1981;105:20-8.
- Neal MH, Kosinski R, Cohen P, Ohrenstein JM. Atypical endocrine tumours of the lung. A clinicopathologic study of 19 cases. *Hum Pathol* 1986;17:1264-77.
- Mooi WJ, Dewar A, Springall DR, Polak JM, Addis BJ. Non-small cell lung carcinomas with neuroendocrine features. A light microscopic, immunohistochemical and ultrastructural study of 11 cases. *Histopathology* 1988; 13:329-37.
- Hammond ME, Sause WT. Large cell neuroendocrine tumors of the lung. *Cancer* 1985;56:1624-9.
- Gould VE, Linnoila I, Memoli VA, Warren WH. Neuroendocrine cells and neuroendocrine neoplasms of the lung. In: Sommers SC, Rosen PF, eds. *Pathology annual*. Appleton, Connecticut: Century-Crofts, 1983:287-330.
- Warren WH, Penfield Faber L, Gould VE. Neuroendocrine neoplasms of the lung. A clinicopathologic update. *J Thorac Cardiovasc Surg* 1989;98:321-32.
- Godwin JD. Carcinoid tumours. *Cancer* 1975;36:560-9.
- Hamperl H. Über Gutartige Bronchial Tumoren (cylindrome und carcinoide). *Virchows Arch A Pathol Anat Histol* 1937;300:46-8.
- Oike N, Bernatz PE, Woolner LB. Carcinoid tumors of the lung. *Ann Thorac Surg* 1976;22:270-7.
- Skinner C, Ewen SWB. Carcinoid lung: diffuse pulmonary infiltration by a multifocal bronchial carcinoid. *Thorax* 1976;31:212-9.
- Williams ED, Celestin LR. The association of bronchial carcinoid and pluriglandular adenomatosis. *Thorax* 1962; 17:120-7.
- Jones RA, Dawson IMP. Morphology and staining patterns of endocrine tumours in the gut, pancreas and bronchus and their possible significance. *Histopathology* 1977;1: 137-50.
- Sheppard MN, Morittu L, Moss F, et al. A study of epithelial, neuroendocrine and natural killer cell antibodies in adult lung and lung tumours. *Lung Cancer* 1988;4:70-2.
- Shaw PAV. Comparison of immunologic detection of 5-hydroxytryptamine by monoclonal antibodies with standard silver stains as an aid to diagnosing carcinoid tumours. *J Clin Pathol* 1988;41:265-72.
- Barbareschi M, Frigo B, Mosca L, Carboni N, Arrigoni GP, Leonardi E, et al. Bronchial carcinoids with S-100 positive sustentacular cells. A comparative study with gastrointestinal carcinoids, pheochromocytomas and paragangliomas. *Pathol Respir Pract* 1990;186:212-22.
- Hoefler H, Denk H, Lackinger E, Helleis G, Polak JM, Heitz PU. Immunocytochemical demonstration of intermediate filament cytoskeletal proteins in human endocrine tissues and (neuro-) endocrine tumours. *Virchows Arch A Pathol Anat* 1986;409:609-26.
- Hajdu SI, Winawer SJ, Myers WPL. Carcinoid tumours: a study of 204 cases. *Am J Clin Pathol* 1974;61:521-8.
- McCaughan BC, Martini N, Bains MS. Bronchial carcinoids. Review of 124 cases. *J Thorac Cardiovasc Surg* 1985;89:8-17.
- Warren WH, Memoli VA, Jordan AG, Gould VE. Re-evaluation of pulmonary neoplasms resected as small cell carcinomas. Significance of distinguishing between well-differentiated and small cell neuroendocrine carcinomas. *Cancer* 1990;65:1003-10.
- Kron IL, Harman PK, Mills SE, et al. A reappraisal of limited stage undifferentiated carcinoma of the lung. *J Thorac Cardiovasc Surg* 1982;84:734-7.
- Karrer K, Shields TW, Denck H, Hrabar B, Vogt-Moykopf I, Salzer GM. The importance of surgical and multimodality treatment for small cell bronchial carcinoma. *J Thorac Cardiovasc Surg* 1989;97:168-76.
- Prasad US, Naylor AR, Walker WS, Lamb D, Cameron EWJ, Walbaum PR. Long term survival after pulmonary resection for small cell carcinoma of the lung. *Thorax* 1989;44:784-7.
- Churg A, Johnston WH, Stulbarg M. Small cell squamous and mixed small cell squamous-small cell anaplastic carcinomas of the lung. *Am J Surg Pathol* 1980;4:255-63.
- Nomori H, Shimosato Y, Kodama T, Morinaga S, Nakajima T, Watanabe S. Subtypes of small cell carcinomas of the lung: morphometric ultrastructural and immunohistochemical analysis. *Hum Pathol* 1986;17:604-13.
- Wei-Li MD, Hammar SP, Jolly PC, Hill LD, Anderson RP. Unpredictable course of small cell undifferentiated lung carcinoma. *J Thorac Cardiovasc Surg* 1981;81:34-43.
- Nixon DW, Murphy GF, Sewell CW, Kutner M, Lynn MJ. Relationship between survival and histologic type in small cell anaplastic carcinoma of the lung. *Cancer* 1979; 44:1045-9.
- Davis S, Stanley KE, Yesner R, Kuang DT, Morris JF. Small-cell carcinoma of the lung—survival according to histologic sub-type. *Cancer* 1981;47:1863-6.

- 49 Katlic M, Carter D. Prognostic implications of histology, size and location of primary tumours. In: Muggia F, Rozenzweig M, eds. *Lung cancer: progress in therapeutic research*. New York: Raven Press, 1979:143-50.
- 50 Carney DN, Gazdar AF, Bepler G, *et al*. Establishment and identification of small cell lung cancer cell lines having classic and variant features. *Cancer Res* 1985;45:2913-23.
- 51 Bepler G, Neumann K, Holle R, Havemann K, Kalbfleisch H. Clinical relevance of histologic subtyping in small cell lung cancer. *Cancer* 1989;64:74-9.
- 52 Radic PA, Matthews MJ, Ihde DC, Gazdar AF, Carney DN, Bunn PA, *et al*. The clinical behavior of "mixed" small cell/large cell bronchogenic carcinoma compared to "pure" small cell subtypes. *Cancer* 1982;50:2894-902.
- 53 Hirsch FR, Osterlind K, Hansen HH. The prognostic significance of histopathologic subtyping of small cell carcinoma of the lung according to the World Health Organization classification. *Cancer* 1983;52:2144-60.
- 54 Hirsch FR, Matthews MJ, Aisner S, *et al*. Histopathologic classification of small cell lung cancer: changing concepts and terminology. *Cancer* 1988;62:973-7.
- 55 Graus F, Elkon KB, Cordon-Cardo C, Posner JB. Sensory neuropathy and small cell lung cancer. *Am J Med* 1983;80:45-52.
- 56 Hamid QA, Bishop AE, Springall DR, *et al*. Detection of human pro-bombesin mRNA in small cell carcinoma of the lung: in situ hybridisation with a cRNA probe. *Cancer* 1989;63:266-71.
- 57 Hamid QA, Corrin B, Dewar A, Hoefler H, Sheppard MN. Expression of gastrin-releasing peptide (human bombesin) gene in large cell undifferentiated carcinoma of the lung. *J Pathol* 1990;161:145-51.
- 58 Hamid Q, Corrin B, Sheppard MN, Polak JM. Localisation of chromogranin mRNA in small cell carcinoma of the lung. *J Pathol* 1991;163:293-7.
- 59 Minna JD. Autocrine growth factor production, chromosomal deletions and oncogene activation in the pathogenesis of lung cancer. *Lung Cancer* 1988;4:6-10.
- 60 Gazdar AF. 1990 Oncogenes, antioncogenes and growth factors in the pathogenesis of small cell lung cancer (SCLC). In: Fiorentini G, Marangolo M, Rosti G, eds. *Proceedings of the Second International Conference on Small Cell Lung Cancer* Ravenna: Augustea, 1990:23-5.
- 61 Travis WD, Linnoila RL, Tsokos ME, *et al*. Neuroendocrine tumours of the lung with proposed criteria for large-cell neuroendocrine carcinoma: *Am J Surg Pathol* 1991; 15:529-53.
- 62 Reeve JG, Stewart J, Watson JV, Wulfrank D, Twentyman PR, Bleehen NM. Neuron specific enolase expression in carcinoma of the lung. *Br J Cancer* 1986;53:519-28.
- 63 Leader M, Collins M, Patel J, Henry K. Antineuron specific enolase staining reactions in sarcomas and carcinomas: its lack of neuroendocrine specificity. *J Clin Pathol* 1986; 39:1186-92.