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Neuroendocrine and Epithelial Markers in Diagnostic Bronchial Lung Cancer Biopsy Specimens

Heather E. Burnett, Hani D. Zakhour and Carol Walker

The incidence of neuroendocrine and epithelial markers was investigated by immunocytochemistry in archival, lung cancer, bronchial biopsy specimens (n = 48). No correlation of antigenicity with histological type was observed. 79% non-small cell lung carcinoma (NSCLC) and 61% small cell lung carcinoma (SCLC) were positive for epithelial markers. HuTu-m3 did not discriminate adenocarcinomas and squamous cell carcinomas from SCLC. 83% SCLC and 93% NSCLC were positive for one or more neuroendocrine marker. Multiple neuroendocrine markers were found in 61% SCLC, 83% NSCLC and 83% squamous cell carcinomas, this incidence being greater in the NSCLC group, and in the squamous carcinomas in particular, than previously reported.
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INTRODUCTION

LUNG CANCER may be clinically and histologically sub-divided into small cell (SCLC) (chemosensitive) and non-small cell lung cancers (NSCLC) (surgically excised where possible) [1]. A subgroup of NSCLC, morphologically indistinguishable from other NSCLC by conventional microscopy, exhibits some of the neuroendocrine features of SCLC [1, 2], and may be responsive to chemotherapy [2–5]. Furthermore, some SCLC have been shown to express epithelial antigens [6], and those that are cytokeratin-positive may have a better prognosis [7]. These observations suggest that classification of lung cancers according to their biological properties as well as their morphological appearance may lead to improvements in the treatment of this disease [1]. However, histological diagnosis of the majority of lung cancers is restricted to examination of formalin-fixed, paraffin-embedded bronchial biopsies. This study has investigated the incidence of immunocytochemical markers in a series of archival bronchial biopsies taken for initial diagnosis.

MATERIALS AND METHODS

Biopsy material

48 bronchial biopsy specimens, reported as primary lung cancer at Arrowe Park Hospital were selected, representing the ratio of the histological types diagnosed in the district.

Immunocytochemical staining

Antibodies (Table 1) were applied to each case using the avidin-biotin-peroxidase technique [8]. For each biopsy the entire section was examined; sections in which 30% or more tumour cells stained were considered positive.

Pre-adsorption of anti-NSE

Nine NSE-positive tumours, both SCLC and NSCLC, were stained with anti-neuron-specific enolase (anti-NSE) antibody pre-adsorbed with pure γγ-enolase (NSE) [Cambridge Research Biochemicals] at concentrations from 0.01 nmol/l to 1 μmol/l. Optimally diluted antibody was mixed with the enzyme at 20°C overnight. The pre-adsorbed antibodies were then applied to sections at the primary antibody stage of the standard procedure.

Table 1. Antibodies used in the study

Antigen	Antibody	Dilution	Control	Supplier
Neuron-specific enolase (NSE) γ-enolase	Rabbit polyclonal	1:200	Carcinoid	Dako
Synaptophysin (38 kD synaptic vesicle protein)	Mouse monoclonal Clone: SY38	1:20	Carcinoid	Dako
PGP 9.5 (ubiquitin C-terminal hydrolase)	Rabbit polyclonal	1:1000	Carcinoid	Ultraclone
Epithelial membrane antigen (EMA)	Mouse monoclonal Clone: E29	1:50	Appendix	Dako
Cytokeratins 8, 18 and 19	Mouse monoclonal Clone: Cam5.2	1:50	Appendix	Becton-Dickinson
Squamous and adeno lung carcinoma	Mouse monoclonal Clone: HuTu-m3 (IgM)	1:20	Squamous lung carcinoma	Serotec

RESULTS

The immunocytochemical staining characteristics of the tumours studied are shown in Table 2.

If the tumour stained for more than one neuroendocrine antibody, the pattern of staining was the same with each; PGP 9.5 nearly always gave stronger staining than synaptophysin or NSE. Positivity for neuroendocrine markers in squamous cell carcinomas was usually diffuse and often seen in the nucleus as well as the cytoplasm. In SCLC, staining was either localised

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Table 2. Immunocytochemical profiles and histological type

	NSE	SYN	PGP	EMA	CAM	HUM
SCLC						
	+	+	+	+	+	+
	+	+	+	+	+	−
	+	+	+	+	−	−
	+	+	+	+	−	−
	+	+	+	−	−	+
	+	+	+	−	−	−
	+	+	+	−	−	−
	+	+	+	−	−	−
	+	+	+	−	−	−
	+	ND	+	+	+	−
	+	−	+	−	−	+
	−	−	+	+	+	−
	−	−	+	+	+	−
	−	−	+	+	−	−
	−	+	−	−	+	+
	−	−	−	+	−	−
	−	−	−	−	+	−
	−	−	−	−	−	−
Squamous cell carcinoma						
	+	+	+	+	+	−
	+	+	+	+	−	+
	+	+	+	+	−	+
	+	+	+	−	−	+
	+	+	+	+	−	−
	+	+	+	+	−	−
	+	+	+	+	−	−
	+	+	+	−	−	−
	+	+	+	−	−	−
	−	+	+	+	−	+
	−	+	+	+	−	+
	−	+	+	+	−	−
	+	−	+	+	−	−
	+	−	+	+	−	−
	+	−	+	+	+	+
	+	−	+	−	+	+
	+	−	+	−	+	−
	+	+	−	−	−	−
	+	−	+	ND	−	−
	−	ND	+	+	+	+
	−	−	+	−	+	−
	−	−	ND	−	−	−
	−	−	−	+	+	+
	−	−	−	+	+	−
Large cell carcinoma						
	−	+	+	+	−	−
	+	−	+	ND	−	−
	+	−	−	−	−	−
Adenocarcinoma						
	+	+	+	+	+	+
	+	+	+	+	+	+
Undifferentiated carcinoma						
	+	+	+	+	−	−

ND = not done (insufficient material in block), SYN = synaptophysin, PGP = PGP 9.5, EMA = epithelial membrane antigen, CAM = Cam 5.2, HUM = HuTu-m3.

to the cytoplasm or spread diffusely through the cells and extracellular matrix.

89% (42/47) of tumours in this series (SCLC 15/18, NSCLC 27/29) showed positive staining with at least one neuroendocrine marker. 11/18 SCLC and 24/29 NSCLC expressed multiple neuroendocrine antigens. The majority of the tumours examined in the NSCLC group were squamous cell carcinomas, of which 91% (21/23) were positive for one or more neuroendocrine marker, multiple markers being present in 19/23 tumours.

Sections of NSE-positive tumours treated with anti-NSE, pre-absorbed with γγ-enolase, showed a partial loss of staining with 0.1 μmol/l NSE and total extinction with 1 μmol/l NSE, indicating genuine recognition of γ-enolase.

72% (33/46) of tumours studied were positive for either Cam 5.2 or EMA or both. In the squamous carcinoma group, 78% (18/23) showed positivity for one or both of the markers with 10 of the 18 positive for EMA alone. 61% (11/18) of SCLC were positive for one or both epithelial markers.

31% of the tumours were positive with HuTu-m3 (15/48). Staining patterns varied from focal membranous positivity in what appeared to be keratinising regions in squamous carcinoma to diffuse cytoplasmic staining of adenocarcinomas. Different samples of SCLC showed diffuse (possibly extracellular) or focal staining. Normal bronchial epithelium also stained with HuTu-m3.

DISCUSSION

No correlation between antigenicity and histological type could be observed. A considerable overlap occurred in the expression of neuroendocrine and epithelial markers in all histological groups, consistent with the hypothesis that all bronchial carcinomas arise from pluripotent epithelial cells [1, 2].

HuTu-m3, contrary to the manufacturer's claims, was an unreliable discriminator of squamous and adenocarcinomas from SCLC.

The incidence of staining for one or both epithelial markers (79% [22/28] NSCLC and 61% [11/18] SCLC) agreed favourably with other studies [6, 9].

The most striking aspect of this study was the high incidence (93%) of NSCLC which stained for neuroendocrine markers; this exceeded the percentage of SCLC (83%) expressing neuroendocrine features and was greater than has been reported previously [5, 10, 11]. The significance of neuroendocrine markers is thought to be greater when two or more markers are detected [12]. Multiple neuroendocrine markers in the NSCLC group were found in 86% of the tumours examined here, a much higher percentage than the 10–30% usually quoted for NSCLC [6, 9, 13]. It is notable that 21/23 of the squamous tumours examined stained for one or more neuroendocrine marker and 19/23 stained for two or more. These results contrast with those of other workers [13, 14], who found, using a similarly sensitive technique but antibodies to different neuroendocrine antigens, that neuroendocrine markers were more commonly expressed in adenocarcinomas and large cell carcinomas and only rarely in squamous cell carcinomas. Neuroendocrine differentiation in squamous-cell carcinomas, has been reported previously, but to a lesser extent than found here [11, 15]. Our results for the incidence of one or more neuroendocrine markers in SCLC compared reasonably with that of other workers [11, 12], although expression of multiple markers was less commonly observed here [13]. Other studies, using similarly small numbers of tumours reported lower incidences of these markers [9, 12, 15].

In view of the possible responsiveness of neuroendocrine positive NSCLC to chemotherapy [2–5], further studies are important to determine whether the incidence of neuroendocrine differentiation found in this study is typical of the lung tumours

treated in this geographical region and to further characterise the pattern of neuroendocrine differentiation in these tumours, in order that the group which may be eligible for chemotherapy be correctly identified.

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Phase II Study of Mitozolomide in Advanced Soft Tissue Sarcoma of Adults: the EORTC Soft Tissue and Bone Sarcoma Group

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INTRODUCTION

TREATMENT OF advanced inoperable and metastatic soft tissue sarcoma remains disappointing with the chemotherapeutic drugs available. Doxorubicin, ifosfamide and dacarbazine are the only active drugs available. Phase II studies therefore remain mandatory.

In the EORTC Soft Tissue and Bone Sarcoma Group we investigated mitozolomide, an imidazoltetrazine. Mitozolomide, with a general formula of $C_7H_7C_1N_6O_2$, showed activity by intraperitoneal route, in experimental tumour in mice such as the L_{1210}, P_{388} leukemia. Noteworthy is that there is also activity to solid tumours such as Lewis lung carcinoma and B_{16} melanoma [1, 2]. The mechanism of action has not been fully elucidated, but seems related to mechanism of action of nitrosureas, via DNA interstrand cross-link formation.

Clinical studies were done with a single dose regimen and a 5 times daily [3]: dose limiting toxicity was thrombopenia. The duration of the leukopenia and thrombopenia followed a pattern of nitrosurea derivatives, the nadirs occured with a median of 26 and 22 days, with a range of recovery up to 6 weeks. As a result of the phase I studies a dose of 100 mg/m^2 was advocated for previously untreated patients and a dose of 90 mg/m^2 for previously treated patients.

PATIENTS AND METHODS

Patients in the age range of 15–75 years were eligible, with a histologically proven, measurable locally advanced and/or

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