Human lung tumours may coexpress different classes of intermediate filaments

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SUMMARY Ninety four pulmonary neoplasms were examined immunocytochemically with two or three different monoclonal antibodies against the intermediate filament proteins cytokeratin, neurofilament, vimentin, and desmin. In normal tissues these have a different and non-overlapping distribution, and it is generally believed that tumours maintain the same pattern of expression as the tissues from which they arise. In this report, however, the coexpression of at least two (and less commonly three or four) different intermediate filaments was seen in 40% (37 of 94) of the cases of lung cancer. These results, especially if confirmed in other common types of human malignancy, have considerable implications for the use of anti-intermediate filament antibodies in diagnostic pathology.

The cytoskeleton of mammalian cells contains a meshwork of intermediate filament proteins whose biochemistry and tissue distribution have been subjects of considerable interest in recent years. Intermediate filaments can be classified into five subtypes—namely, cytokeratin, vimentin, desmin, neurofilament, and glial fibrillary acid protein (GFAP). They show different and generally non-overlapping patterns of tissue distribution.

From a clinical point of view the differences in distribution between the individual intermediate filament classes are of particular interest: it is generally believed that tumours contain intermediate filaments of the same type of tissue from which the neoplasm has arisen (table 1). This offers a valuable means of identifying the origin of human malignancies by immunocytochemical labelling, using antibodies specific for the different classes of intermediate filament.¹⁻⁶

The reliability of intermediate filaments as markers of tumour cell origin would be seriously weakened if it were shown that neoplastic transformation was accompanied by a tendency to express "inappropriate" classes of intermediate filaments. A few examples of such aberrant coexpression have been reported, notably for cytokeratin and vimentin.⁷⁻¹² This is generally thought to be a rare phenomenon and the fidelity with which the intermediate filament

profiles of neoplastic cells reflect their tissue of origin has not been seriously questioned.

In this paper we report an immunocytochemical analysis of a large series of surgical resected lung tumours in which it was shown that cases expressing two and sometimes more classes of intermediate filament were found relatively often. This suggests that the acquisition of new classes of intermediate filaments by neoplastic cells may be a more common event than has previously been thought and prompts a reassessment of the diagnostic application of anti-intermediate filament antibodies.

Material and methods

LUNG SPECIMENS

Ninety four pulmonary tumours were received as lung resection specimens fresh from the operating theatre. Portions were taken for histology, electron microscopy, and immunocytochemistry and dealt

Table 1 *Expression of intermediate filament proteins in normal cell types*

Cytokeratin	Keratinising and non-keratinising epithelium
Vimentin	Most mesenchymal tissues and cells such as fibrous tissue, cartilage, haemopoietic, endothelium, melanocytes, etc
Neurofilaments	Most neurons in peripheral and central nervous tissue
Desmin	Skeletal, cardiac, and smooth muscle
GFAP	Astrocytes

Table 2 Summary of immunocytochemical labelling of lung tumours with monoclonal antibodies against different classes of intermediate filaments

Tumour type	No of cases	Cytokeratin	Neurofilament	Vimentin	Desmin
Squamous cell carcinoma	60	60	12	18	4
Adenocarcinoma	17	17	1	4	0
Small cell carcinoma	9	9	2	2	1
Carcinoid	8	8	6	3	1
Total	94	94	21	27	6

Table 3 Monoclonal antibodies used for study of intermediate filament expression by human lung tumours

Intermediate filament	Monoclonal antibody	Source or reference
Cytokeratin	LP34 KL1 CAM 5.2	Dako ¹⁵ Becton-Dickinson
Neurofilaments	NR4 MNf (2F11)	Dako Eurodiagnostics ²⁰
Vimentin	V9	Dako
Desmin	MV1 (Vg) DE-R-11 D33	Eurodiagnostics ²⁰ Dako van Muijen (unpublished observations)
	MDE	Eurodiagnostics

Antibodies recognising GFAP were not included in this study.

with as described previously.¹³ The tumours were categorised independently according to the WHO classification system¹⁴ by two of the authors (KCG and MSD) (table 2).

IMMUNOCYTOCHEMISTRY

Table 3 shows details of the monoclonal antibodies used in this study. Monoclonal antibodies recognising GFAP were not included in this study as those antibodies available to us in our preliminary studies showed a wide range of cross reactivity with normal epithelial tissues. For practical diagnostic purposes, antibodies against GFAP would also not normally be

part of a panel for the assessment of lung tumours in man.

Cryostat sections were stained either by a three stage immunoperoxidase procedure or by the APAAP immunoalkaline phosphatase technique, as detailed previously.^{16 17}

Results

Tables 2 and 4 summarise the results of immunostaining 94 lung tumours. All of the tumours were positively labelled, usually homogeneously, for cytokeratins. In addition, however, about 40% of the tumours (37 of 94) also reacted with monoclonal antibodies against at least one other class of intermediate filament. Neurofilaments were coexpressed by 21 lung tumours (22%), vimentin by 27 tumours (28%), and desmin by six tumours (6%). Fourteen tumours showed staining for three different intermediate filaments, and one small cell carcinoma was positive for all four (detected with two or three different antibodies for each intermediate filament) (figure).

In most cases the labelling for intermediate filaments other than cytokeratin was focal or patchy (in contrast to the homogeneous pattern of cytokeratin labelling) and was observed with all of the monoclonal antibodies specific for a particular class of intermediate filament. There were a few cases, however, in which focal positivity (especially for neuro-

Table 4 Details of staining patterns seen in human lung tumours coexpressing different classes of intermediate filaments

Tumour type	No of cases	Cytokeratin	Neurofilament	Vimentin	Desmin
Squamous cell carcinoma	10	+	—	+	—
	7	+	+	+	—
	4	+	+	—	—
	2	+	—	+	+
	1	+	+	—	+
Adenocarcinoma	3	+	—	+	—
	1	+	+	+	—
Small cell carcinoma	1	+	+	+	+
	1	+	—	+	—
	1	+	+	—	—
Carcinoid tumour	3	+	+	+	—
	3	+	+	—	—

Figure Small cell carcinoma of lung expressing four different intermediate filaments: (a) cytokeratin; (b) neurofilament; (c) vimentin; and (d) desmin. Note entrapped bronchial mucosal gland (asterisk) showing typical epithelial pattern of intermediate filament expression.

filaments) was seen with only one or two of the antibodies specific for a particular intermediate filament.

Discussion

In recent years intermediate filaments have become the subject of intense research by cell biologists and pathologists. Initial studies suggested that there were no exceptions to the rule that epithelium expressed only cytokeratins, nerves only neurofilaments, etc (table 1). More recently there have been reports that vimentin is coexpressed with cytokeratins during embryonic development, in tumour cells growing in vitro, or in serous effusions,¹⁸⁻²⁰ and in human myocardium and myometrium.²¹ It has also become clear that some tumours such as renal cell carcinomas,⁸ salivary tumours,⁹ mesotheliomas,¹² and thyroid tumours¹¹ also commonly express vimentin as well as cytokeratin. Desmin or glial filaments may also be coexpressed with vimentin in some sarcomas.^{7 22 23}

The existence of exceptions such as those cited

above has led to a modification of the original simple concept that any individual tumour will only express a single class of intermediate filament, characteristic of its tissue of origin. This revised view has not caused pathologists to abandon the use of intermediate filaments for tumour classification, as it is generally held that such exceptions are rare. Furthermore, it has been stated that when more than one intermediate filament is found in a human cell, one of the members of the pair will always be vimentin.⁴ This empirical rule was barely formulated before it too was found to be subject to exceptions, notably among neuroendocrine tumours, as Merkel cell tumours,^{24 25} islet cell tumours,²⁶ parathyroid adenomas,²⁷ and bronchial carcinoid tumours²⁸ may coexpress neurofilaments and cytokeratins. The rarity of such exceptions was generally considered to pose little practical obstacle to the use of intermediate filaments as markers of tumour histogenesis.

This report indicates that the coexpression of at least two (and less commonly three or four) different

classes of intermediate filaments, far from being a rare phenomenon, is often observed in one of the commonest types of malignancy in man—lung cancer. The fact that this observation has been overlooked in the past may be attributed to several factors. The material in the present series was subject to careful microscopic scrutiny, so that focal areas of aberrant intermediate filament expression, which could escape detection on more cursory examination, were detected. The use of immunoenzymatic staining techniques (rather than immunofluorescence labelling) made it possible to establish with certainty that aberrant intermediate filaments were present in the tumour cells themselves (and not within non-neoplastic cells).

There is a further element, albeit difficult to assess, accounting for the failure of previous studies²⁹⁻³⁴ to detect such a high incidence of aberrant intermediate filament expression in lung tumours. A natural reluctance to destroy a beautiful hypothesis may have biased pathologists against recording unexpected immunocytochemical reactions as specific. Our initial response in the early stages of this study was also to seek alternative technical explanations for such findings, such as the detection of shared epitopes between different classes of intermediate filaments by certain monoclonal antibodies. The fact that most of the reactions in the present study, however, were confirmed using two or three unrelated monoclonal antibodies for each intermediate filament class (a control not found in previous studies) led us to abandon such an explanation.

It may be added that epitope sharing between different classes of intermediate filament cannot be excluded as an explanation of our findings, although there are theoretical grounds for thinking this unlikely (as there is no evidence of multiple genes coding for each intermediate filament other than cytokeratin). Even were such an explanation valid, it would not affect the practical implications of this report, which are concerned with the application of monoclonal antibodies against intermediate filaments for tumour classification and diagnosis.

Perhaps lung tumours are exceptional among human neoplasms in their tendency to express multiple intermediate filament types, although we have already seen similar examples of intermediate filament coexpression in smaller groups of thyroid tumours, small cell tumours of childhood, meningiomas, and different types of sarcoma. We are systematically studying other human tumours received in this department to establish whether or not the present findings are representative of a wider range of neoplasms.

The purpose of this report is not to condemn the use of antibodies to intermediate filaments in pathol-

ogy. On the contrary, we and others have found them extremely valuable in several difficult areas of diagnosis—for example, anaplastic tumours,³⁵⁻³⁷ metastases in bone marrow,³⁸ or lymph nodes.³⁹ They should, however, be used in conjunction with other reagents, such as antileucocyte common antigen, S100 protein, or epithelial membrane antigen. The dream of a panel of anti-intermediate filament antibodies solving all the problem areas of histopathology is over. What remains is a very useful group of reagents for use in conjunction with other antibodies and with all the conventional skills of histopathology.

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