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Immunohistochemical Localisation of Keratin in Human Lung Tumours

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Summary. Antisera against total keratin extracts of human callus have been used to identify keratins in lung tumours of different histological type. Forty-three were classified by the WHO scheme. Keratin immunoreactive cells were identified in all 8 epidermoid carcinomas; 6 out of 12 large cell carcinomas; 2 out of 6 adenocarcinomas; 2 out of 15 small cell carcinomas and in the only muco-epidermoid carcinoma. These cases demonstrate the heterogeneity of phenotypic expression in lung tumours not recognisable without the use of immunohistochemical techniques.

Key words: Keratin polypeptides – Bronchogenic carcinoma – Phenotypic modulation

Introduction

Keratin immunoreactive materials have been described in a number of normal and diseased human tissues (Schlegel et al. 1980; Krepler et al. 1980; Loning et al. 1981; Caselitz et al. 1981; Sieinski et al. 1981). Keratin is present within the basal cells and intermediate cells of the normal trachea and bronchus and in the ducts of the bronchial glands (Schlegel et al. 1980). More recently keratin has been described in a number of human tumours (Schlegel et al. 1980; Sieinski et al. 1981), including two squamous cell carcinomas of the bronchus and negative to weak staining was noted in three adenocarcinomas (Schlegel et al. 1980). These results, when viewed in the light of the detailed ultrastructural histogenetic classification of lung tumours by McDowell et al. 1978 and 1980) suggest that the use of antikeratin antisera may provide a reagent for the demonstration of epidermoid differentiation not normally detectable at the light microscope level. In this report 43 tumours of varying histological pattern were stained using antikeratin antibodies and the results are discussed in the context of the modulation of phenotypic patterns in lung tumours and the potential use

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of these sera to detect sub-populations of cells within human neoplasms. The potential of detecting clones of cells that may show differential responses to treatment is briefly considered.

Materials and Methods

Antibody Preparation and Immunohistochemical Techniques

Keratin was purified from human callus as described by Sun and Green (1978) and analysis on polyacrylamide gels revealed a pattern of bands identical to that obtained by these authors. Antisera was raised by dissolving 1 mg of keratin in 500 µl of 1 M Urea and 5 µl of mercaptoethanol in PBS: this was emulsified with 1 ml of Freund's complete adjuvant, and injected subcutaneously into the nuchal region of rabbits at monthly intervals. The resultant antisera gave a single immunoprecipitin band on Ouchterlony diffusion against purified keratin. Second antibodies were obtained by affinity purification of a sheep anti-rabbit gamma globulin that was conjugated to alkaline phosphatase using gluteraldehyde (Avrameas and Ternynck 1971).

The specimens were obtained from bronchial biopsies or lobectomies. Material was selected purely on the grounds of availability, which may reflect the interest of the oncologists in this centre rather than the spectrum of tumour types in the region as a whole. Four cases were also included from small cell carcinomas in which post mortem material was available. Tumours were classified according to the WHO nomenclature (1967). All tissues were fixed in neutral formol saline, processed in isopropyl alcohol and inhibisol and embedded in paraffin wax. Sections were cut at approximately 4 µm and dewaxed in xylene. The sections were treated with 15% acetic acid to block endogenous alkaline phosphatase activity and incubated with the antikeratin antiserum at a dilution of 1/100 for 90 min, washed, and incubated with the enzyme-conjugated second antibody for a further one and a half hours. The stain was developed using napthol AS:B1 phosphoric acid plus Fast Red T.R. The slides were counterstained with Mayer's haemalum and mounted in buffered glycerin jelly.

Specificity of the staining was confirmed by absorption of the antiserum with 1 mg/ml of keratin.

Results

Of the forty-three tumours examined, keratin-containing cells were identified in 19 (Table 1).

Table 1. Bronchial carcinoma and immunoreactive keratin-containing cells

Classification (WHO, 1967)	No. of tumours	No. containing positive cells
Epidermoid	8	8
Large cell – without mucin	12	6
Large cell – giant cell	1	0
Adenocarcinomas – bronchogenic		
a) Acinar	5	1
b) Papillary	1	1
Small cell		
a) Polygonal	5	0
b) Fusiform	10	2
Muco-epidermoid	1	1

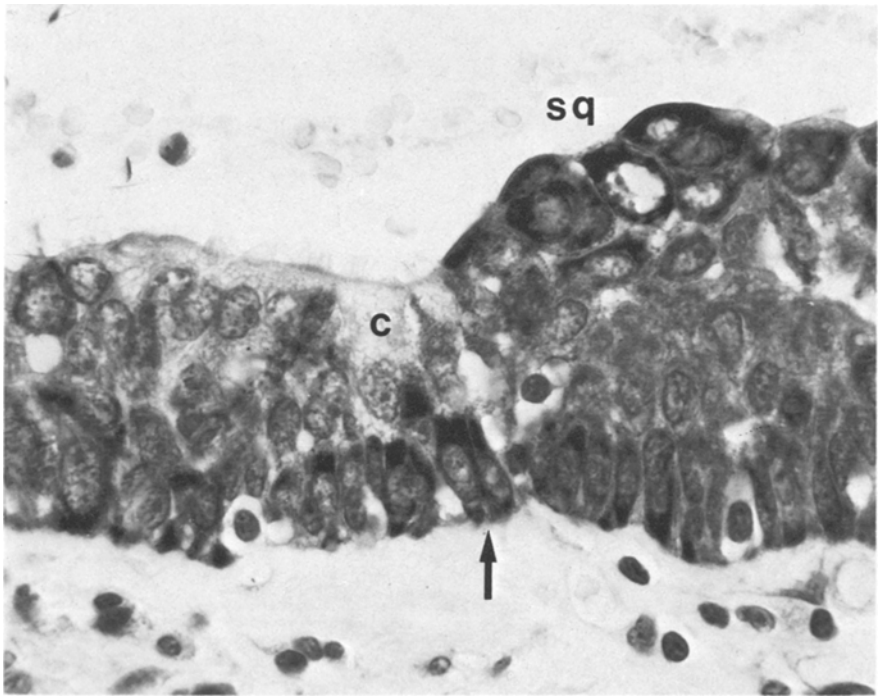

Fig. 1. Photomicrograph of human bronchial mucosa stained to demonstrate the presence of keratin. Basal cells (*arrow*) are strongly positive as are the cells in the area of stratified squamous epithelium (*sq*). Ciliated cells (*c*) do not contain keratin-like immunoreactive material. Magnification ×600

During these studies the presence of keratin in basal cells and intermediate cells of the normal bronchial mucosa was confirmed and as expected areas of squamous metaplasia were also strongly positive (Fig. 1). The material available for this study has not permitted a detailed investigation of dysplastic lesions, but the presence of squamous carcinoma in situ in one tumour was associated with a similar distribution of staining in the infiltrating tumour. It was also noted that in the bronchial glands both the ducts and serous elements were positive as were occasional mucin depleted cells in the mucinous acini (Fig. 2). In patients with hyperplastic bronchial epithelium it was possible, using a double staining technique developed by one of the authors (D. Mitchell, in preparation), to demonstrate cells containing both acid mucin and keratin.

In the epidermoid carcinomas the majority of the cells contained stainable keratin (Fig. 3) but in the other tumours only a minor proportion of the cells were positive as demonstrated in the case of the fusiform small cell carcinoma (Fig. 4) and the large cell anaplastic carcinoma (Fig. 5). In some of the poorly differentiated and moderately differentiated epidermoid carcinomas with a trabecular or solid growth pattern, there was a gradation of staining with the basaloid cells adjacent to the connective tissue being negative and an increased staining towards areas of keratinizing partially necrotic debris. The growth pattern of these tumours may be described as epidermoid in that there is a stratification

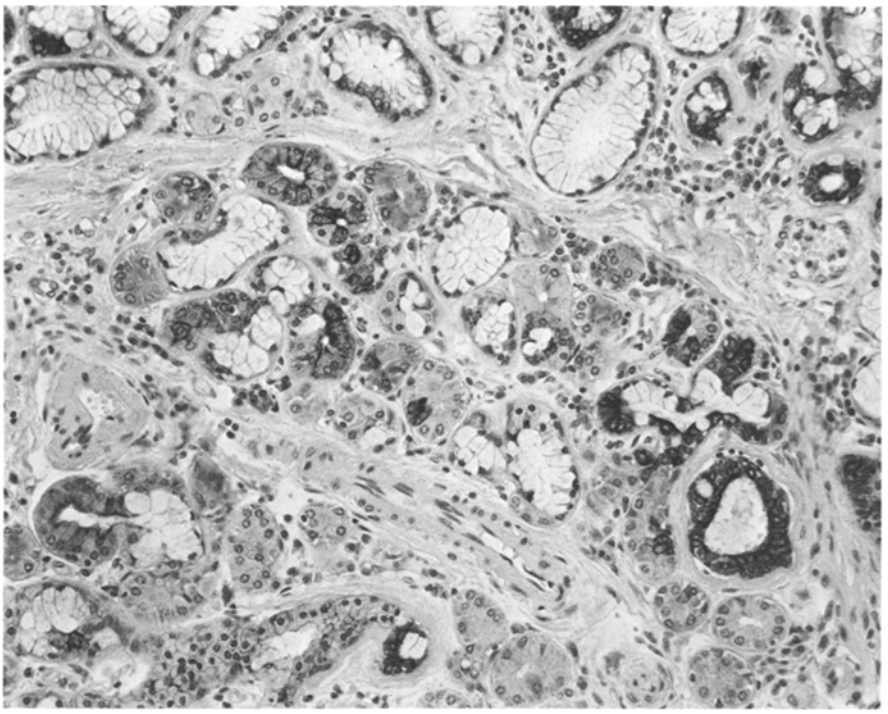

Fig. 2. Photomicrographs of human bronchial glands stained to demonstrate keratin in the cells lining the ducts of the bronchial gland and in cells within both the serous and mucous acini. Magnification $\times 160$

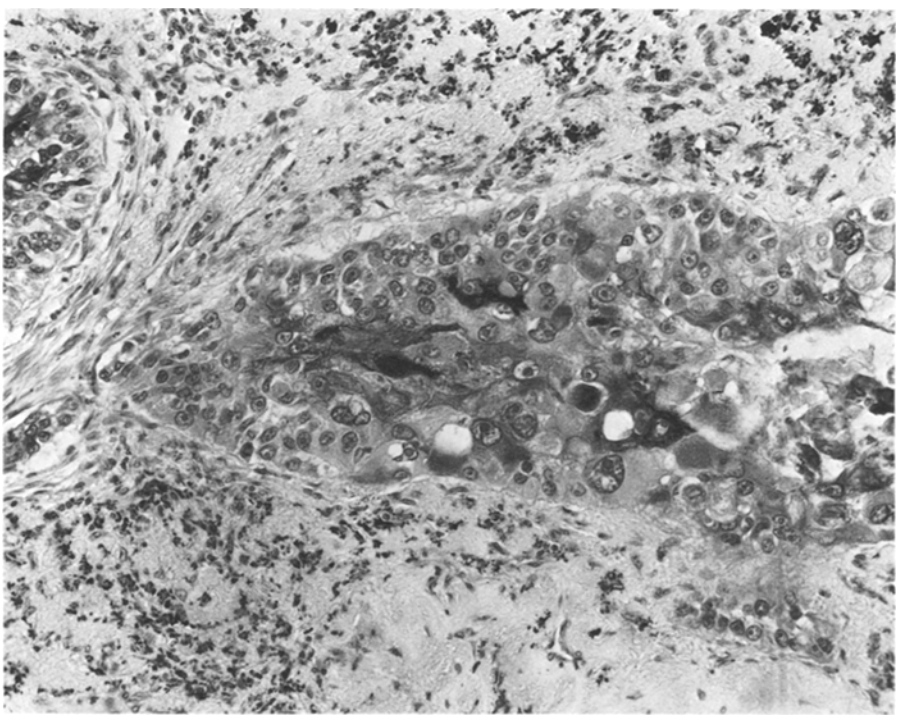

Fig. 3. Photomicrograph of an epidermoid carcinoma demonstrating very positive cells in the central regions of the infiltrating tumour and more weakly positive cells at the periphery. Magnification $\times 157$

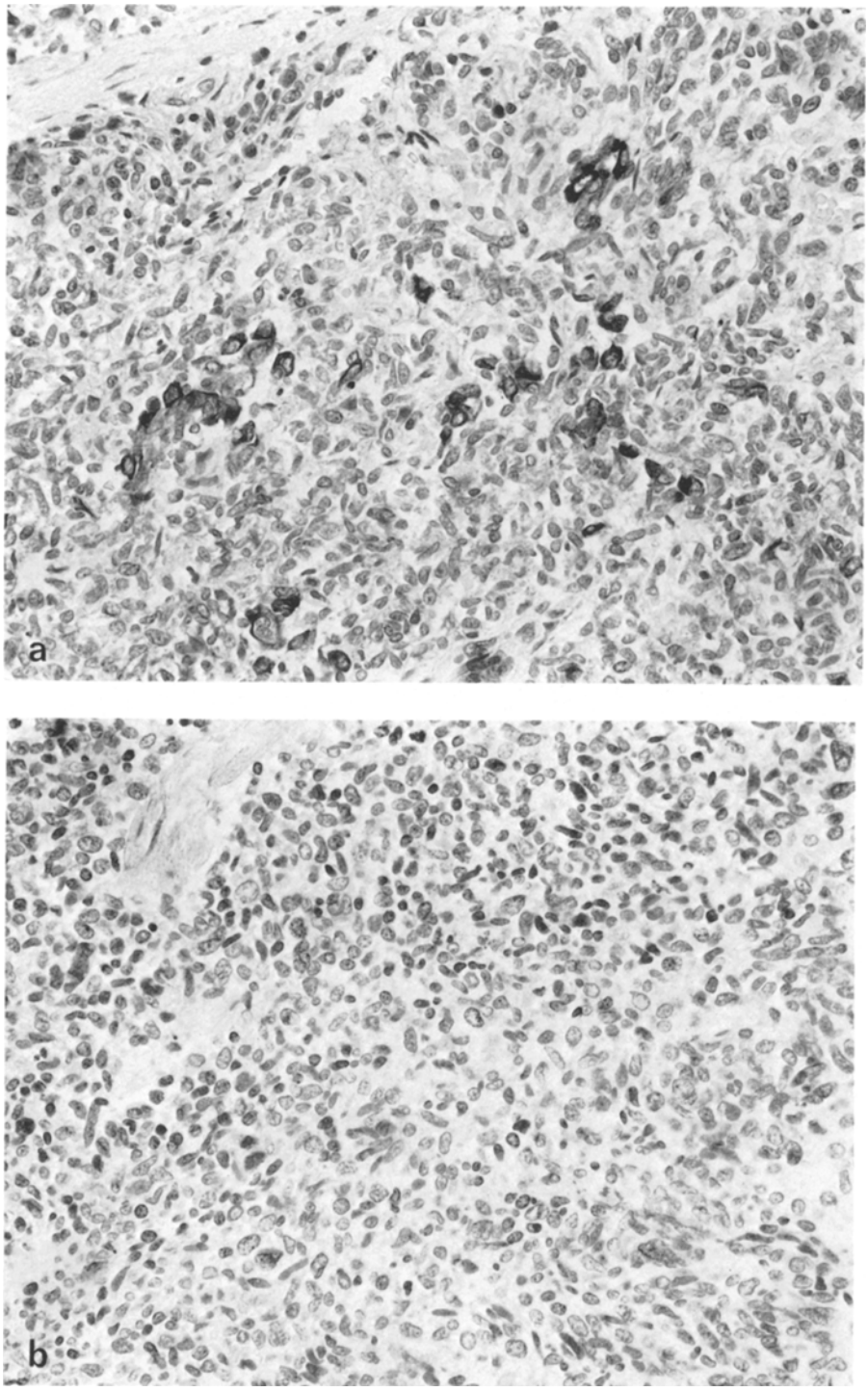

Fig. 4a, b. Photomicrographs of a small cell anaplastic carcinoma (fusiform type) demonstrating positively stained cells (**a**) and a control slide of the same area (**b**) in which the antibody has been previously absorbed with a purified keratin extract. Magnification $\times 250$

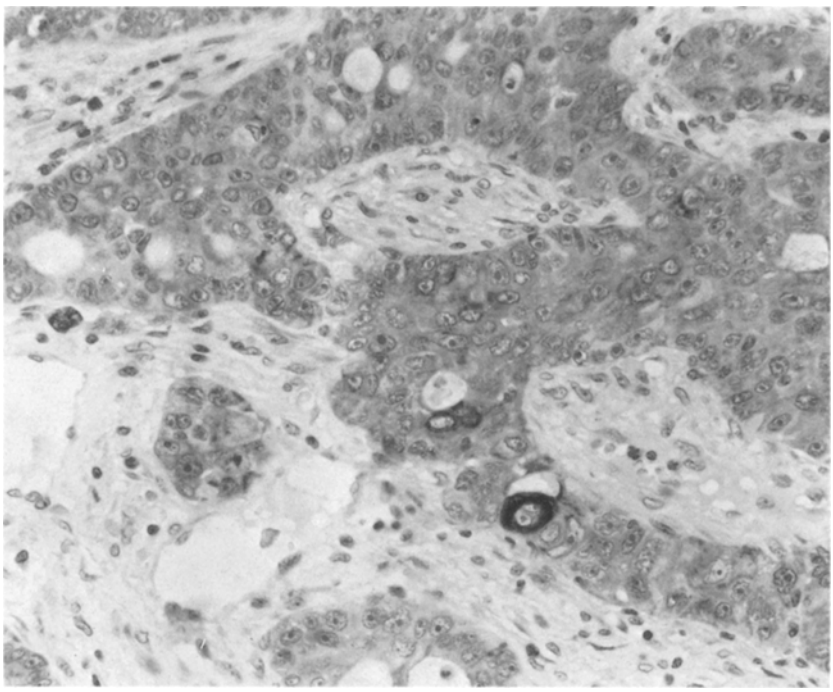

Fig. 5. Photomicrograph of a large cell analplastic carcinoma stained with anti-keratin antiserum. The majority of the cells are positively stained as seen by comparison with the negative stroma – occasional cells are very strongly positive. Magnification ×200

of the cells in a manner analogous to areas of squamous metaplasia in the bronchial mucosa.

Of the small cell carcinomas, only two contained keratin and these positive cells were present in metastases within the brain and the adrenal gland. The keratin containing cells were indistinguishable from the other components of the tumour using haematoxylin and eosin staining and were only identified using the immunohistochemical technique. Control sections of normal tissues showed that both of these sites are devoid of stainable keratin. In one case a bronchial tumour at the primary site appeared to contain small foci of keratin containing cells, but these could be identified as incorporated normal ductal structures.

In the two adenocarcinomas the staining was unevenly distributed throughout the tumour with no specific localisation. The single muco-epidermoid carcinoma showed positive staining of the squamous elements and negative glandular elements.

Discussion

The original concept that there is 'only one entity carcinoma of the lung' was proposed by Willis (1948). Since that time there have been numerous attempts to classify bronchial carcinoma using morphological criteria of epider-

moid or glandular differentiation. Such classifications, including the WHO nomenclature (1967) used here have failed to demonstrate a correlation between histological types and clinical behaviour (with the exception of the small cell group). This is not surprising as it is well documented that many tumours show a predominance of one morphological pattern with foci of alternative phenotypes in other areas of the tumour (Willis 1976; McDowell et al. 1978). In order to detect evidence of glandular or epidermoid differentiation not recognisable using standard histological techniques it is necessary to resort to electron microscopy (McDowell et al. 1980). The data presented here suggest that the use of specific antisera may provide a method of detecting early epidermoid change, which is more convenient and subject to fewer sampling problems.

Many of the cells lining the bronchial tree, including the Kultschitsky cells have been shown to contain tonofilament-like structures at the ultrastructural level (Hage 1974; Cutz et al. 1974; McDowell et al. 1980). Similar filaments have also been reported in carcinoid tumours in vitro (Bensch et al. 1976) and in small cell carcinomas (Ewing et al. 1980; Churg et al. 1980). The ultrastructural studies of McDowell have suggested that there is evidence of epidermoid differentiation in both large and small cell carcinomas with a large proportion of tumours exhibiting elements of both glandular and epidermoid differentiation (McDowell et al. 1978). The demonstration of tonofilaments in small cell carcinomas and the presence of dense core neurosecretory granules in well differentiated squamous and adenocarcinomas (McDowell et al. 1981) supports Willis' original concept and indicates that standard classification systems are based on quantitative phenotypic expression rather than the recognition of distinct clinico-pathological entities. In the data presented here it can be seen that tumours of different morphological types contain evidence of epidermoid differentiation not detectable in haematoxylin and eosin sections. This is consistent with the ultrastructural data of other workers recently reviewed (McDowell et al. 1980).

It has been suggested that the heterogeneity of phenotypic expression seen in bronchial carcinomas is due to responses of the tumour to local microenvironmental factors (McDowell et al. 1978) in a manner analagous to the phenotypic modulation seen as goblet cell hyperplasia and squamous metaplasia in the bronchial mucosa. It would be of interest to consider whether some of these potential modulations may be responsible for tumour recurrence after treatments and the future challenge is to develop markers of biological behaviour rather than purely of potential histogenetic interest. On the basis of past experience it is very unlikely that single markers will provide the necessary tools for the reclassification of tumours, but it is to be hoped that future classification will be functionally as well as morphologically based. Ellison (1977) has proposed that the association of peptide hormone production with a high proportion of lung tumours may be associated with local selection of cells more suited to survive in the local environment. Thus, certain peptides may confer a selective advantage on the tumour cells. Such concepts may lead to a more rational appraisal of lung cancer and to the reclassification based on a number of immunological reagents, one of which may be the use of antikeratin antisera to demonstrate epidermoid differentiation.

In this study an obvious feature was the inter-tumour and intra-tumour heterogeneity with respect to the degree of positivity of the staining. The figures clearly demonstrate that in all the major tumour categories some cells were very strongly positive while other cells were completely negative with gradation between the two extremes. At the present time it would be highly speculative to consider this as representing more than quantitative differences in the amount of detectable keratin-like materials, but it is possible that the use of band specific anti prekeratin antibodies such as these produced by Loning et al. (1980) would enable the demonstration of sequential changes in the degree of epidermoid differentiation. The true biological heterogeneity of tumours is still to be explored, but it could be speculated that these techniques in combination with other functional markers may provide the necessary tools to identify therapeutically responsive tumours.

At the present time the presence of keratin and tonofilaments is accepted as evidence of epidermoid differentiation (McDowell et al. 1980) and in this paper these criteria are used. Recently, however, keratins have been identified as part of the cytoskeleton of cells not usually associated with the ability to undergo squamous metaplasia (i.e. myoepithelial cells – Schlegel et al. 1980). It may be necessary in the future to reconsider the present usage of the term epidermoid and to refer instead to the presence or absence of keratin so avoiding the use of potentially confusing nomenclature.

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