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Immunohistochemical Localisation of Keratin in Small Cell Carcinoma of the Lung: Correlation with Response to Combination Chemotherapy

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Abstract—*Immunohistochemical localisation of keratin was assessed on 45 diagnostic specimens of small cell carcinoma of the lung in patients who subsequently received combination chemotherapy. Nine out of 45 (20%) contained keratin immunoreactive cells. Six of these achieved a complete response to treatment compared to 12 of the tumours which did not show positive staining for keratin. For 2 patients the tumours were shown to contain nests of keratin immunoreactive cells. Both of these are alive and free of disease more than 5 yr after the initial diagnosis. The results indicate that the presence of keratin immunoreactive cells may not directly equate with squamous differentiation and therefore not constitute an adverse prognostic factor in terms of response to chemotherapy.*

INTRODUCTION

SMALL cell anaplastic carcinoma of the lung (SCCL) is recognised clinically as a distinct entity from other types of lung carcinoma. Compared to epidermoid carcinomas, it is characterized by its chemosensitivity, although the long-term benefits can be disappointing [1–3]. The extent of disease at presentation and response to chemotherapy are the main factors influencing survival [4, 5], underlining the need to define other prognostic factors. Evidence of histological heterogeneity with SCCL at the microscopic level [6, 7] has been confirmed by ultrastructural studies [8, 9], which glandular or epidermoid differentiation have been identified both before and after treatment [9–11], but attempts to correlate histological sub-types of SCCL with the clinical outcome have so far failed to demonstrate any significant relationship [12, 13].

The ultrastructural histogenic classification of lung tumours proposed by Hess *et al.* [14] and preliminary work using anti-keratin antisera [15] have suggested that such antisera may provide a reagent to demonstrate epidermoid differentiation, not normally detectable by light microscopy. In view of previous results reporting the presence of keratin immunoreactive cells in a minority of SCCL biopsy specimens [15], and knowing the relative chemoresistance of epidermoid lung carcinoma, we were interested in assessing the incidence of keratin immunoreactive cells in a larger series of SCCL and in identifying any possible correlation with response to chemotherapy and survival.

We present here the results of immunocytochemical staining for keratin in 45 diagnostic specimens of SCCL in patients who subsequently received combination chemotherapy.

MATERIALS AND METHODS

Antibody preparation and immunohistochemical techniques

Antisera was raised in rabbits using purified human keratin as the immunogen as previously described [15]. The specimens were obtained from bronchial biopsies in 41 patients, from node biopsy in 2 and from lobectomy in 2. Material was selected both on the grounds of availability and according to a retrospective analysis of response to chemotherapy.

The 45 tumours were classified using WHO criteria as small cell anaplastic in type [6]. Tissues were fixed in neutral formol saline, processed in isopropyl alcohol and inhibisol and embedded in paraffin wax. Immunocytochemical localisation

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of keratin was performed using an alkaline-phosphatase conjugate as previously described [15].

Patients characteristics

All the patients received combination chemotherapy. Two drug combinations were used and have been described elsewhere [16, 17]. Response to treatment was assessed after 2 courses of combination chemotherapy. The minimum follow-up for all the patients was 2 yr and survivals were calculated according to the Kaplan–Meier method. Comparisons of survival were done using the method described by Peto *et al.* [18].

RESULTS

Keratin immunoreactivity

Nine of the 45 (20%) tumours examined were shown to contain keratin immunoreactive cells. When normal bronchial mucosa adjacent to the tumour was present, keratin was demonstrated in basal and intermediate cells. A minor proportion of cells within the 9 tumours were stained positively. There were unevenly distributed and were not distinguishable using conventional haematoxylin and eosin staining from the other components of the tumour.

In 2 tumours, small rounded nests of positive cells were present (Fig. 1) scattered throughout the lesions. Their growth pattern suggested keratin pearl formation, although there was no evidence of keratinization in haematoxylin–eosin sections.

Correlation with response and survival

Seven out of the 9 keratin-positive tumours did show objective signs of response to combination chemotherapy, 6 of them achieving a complete response, whereas 19 of the 36 keratin negative tumours achieved a response to combination chemotherapy, with only 12 complete responses (Table 1). However, no significant differences in median survival could be demonstrated between the 2 groups.

Two patients with tumours containing nests of keratin positive cells achieved long-term survival, remaining free of disease for 5 and 6 yr respectively after the initial diagnosis.

Keratin immunoreactivity did not correlate with the extent of the disease at presentation.

DISCUSSION

These results are somewhat in contradiction with our initial postulate; namely, that squamous differentiation is associated with chemo-insensitivity. Although the numbers are too small to reach conclusions with respect to survival, we found a higher rate of complete responders in the group of tumours with keratin immunoreactive cells. Our findings raise 3 main questions:

1. The histological diagnosis of bronchogenic carcinomas is usually made by bronchoscopy; material is sampled in limited amounts and is sometimes even insufficient to ascertain the diagnosis. It is therefore possible that specimens analysed are not representative of the whole tumour. There is also evidence that some biochemical markers detected by immunocytochemistry at the primary site may not be present in distant metastases [19]. Although in our series only 2 tumours were diagnosed by lymph-node biopsy, 1 was keratin-negative and the other was keratin-positive: the keratin content of the primary was not assessed, and therefore we cannot exclude differences between the primary tumours and metastases as a possible source of error.

2. Although keratin and tonofilaments are generally considered to be features of epidermoid differentiation, keratin has also been recently detected in cells not normally known to undergo squamous metaplasia [20]. This may well apply to small cell carcinoma of the lung, which is thought to be of neuroendocrine origin. As previously suggested [15], the use of antibodies to the different subunits of pre-keratin might provide a means to study sequential changes in the degree of epidermoid differentiation, and consequently clarify the meaning of keratin immunoreactive cells in small cell carcinoma of the lung.

3. Finally, we concentrated on diagnostic material and did not assess, by serial investigations for the same patient, the keratin content at diagnosis, during remission and at the time of relapse. Brereton *et al.* reported a 24% incidence of foci of squamous cell carcinoma detected at the

Table 1. Correlation between keratin immunoreactivity and response to chemotherapy

No. of tumours	Keratin immunoreactivity	Partial response (%)	Complete response (%)	Overall response (%)
9	(+)	1 (11)	6 (67)	7 (78)
36	(-)	7 (20)	12 (33)	19 (53)

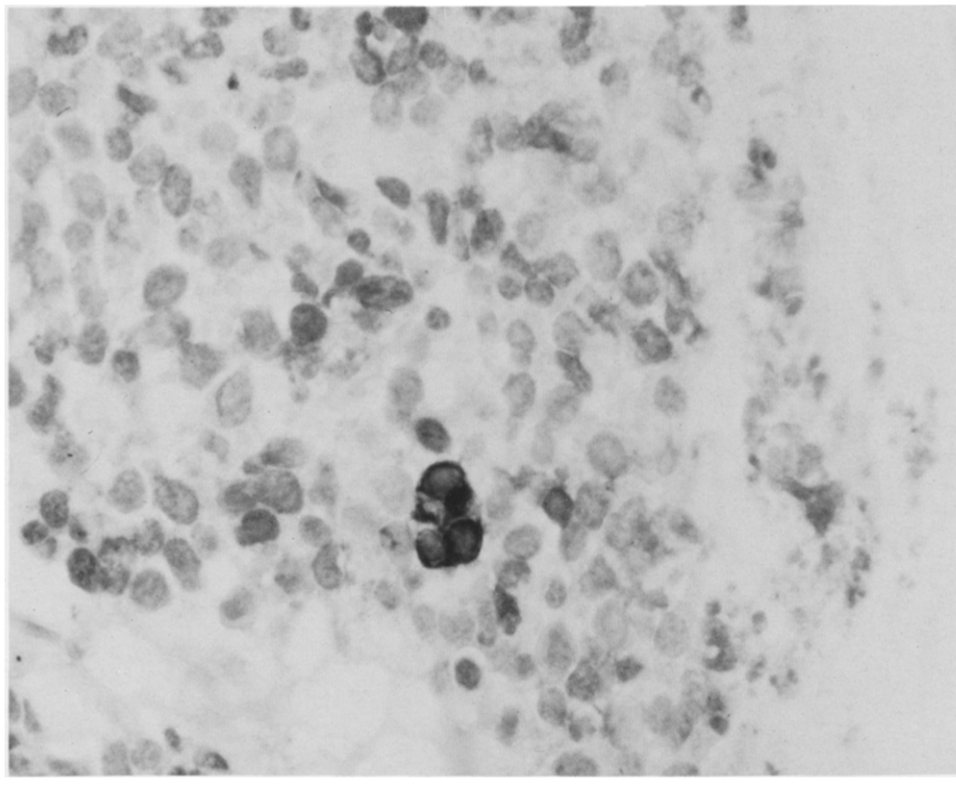

Fig. 1. *Photomicrographs of small cell anaplastic carcinoma of the lung showing focal keratin immunoreactive cells.* ×580.

light microscopic level in an autopsy series of patients who had previously been treated with combination chemotherapy for histologically proven small cell carcinoma of the lung [10]. A similar prevalence assessed morphologically and biochemically has been described in a group of patients who received radiotherapy and chemotherapy [11]. Such results suggest that treatment either favours differentiation of anaplastic cells into squamous cells or eliminates the more sensitive small cell populations, although in 2 patients who did not receive anti-tumour therapy, foci of glandular differentiation within the small cell primary tumour were seen at autopsy. Serial assessment of keratin immunoreactivity is expected to clarify the role exerted by treatment on histological heterogeneity at presentation and also determine more precisely its clinical relevance. By analogy with adult germ-cell tumours, the persistence or appearance, if confirmed, of cells expressing such a phenotype could characterise a slow-growing type of cell, resistant to chemotherapy but potentially amenable by surgery [21].

In conclusion, our data confirm the pathologic heterogeneity of newly diagnosed small cell carcinoma of the lung: 20% of 45 biopsy specimens contained keratin immunoreactive cells and, surprisingly, complete responders to combination chemotherapy appear perhaps to be commoner in this sub-group of patients. We are currently investigating the use of combined markers, such as ACTH, calcitonin and neurone-specific enolase, with an emphasis on serial biopsies within the same patients to confirm the possible favourable prognostic implications of keratin immunoreactive cells in small cell carcinoma of the lung.

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