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Somatostatin and adrenocorticotrophic hormone like immunoreactivity in small cell carcinoma of the lung

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SUMMARY The immunocytological detection of adrenocorticotrophic hormone (ACTH) and somatotropin release inhibitor factor (SRIF) like immunoreactivity was carried out on tumour cells from bronchial brush smears in 39 cases of lung tumours. Results obtained were compared with the cytological and histological diagnosis and confirmed the high incidence of ACTH synthesis by malignant bronchial carcinoma cells: the same phenomenon also seems to occur for somatostatin. The concomitant detection of ACTH and SRIF like immunoreactivity seems to be highly suggestive of small cell carcinoma and indicates that the immunocytological detection of hormones carried out at the same time as cytological examination can improve the accuracy of the diagnosis.

Endocrine cells analogous to those found in the gastrointestinal tract and pancreas have been recognised in normal human bronchial mucosa by ultrastructural and histochemical techniques. Some types of anaplastic carcinoma may originate from such cells.[1-3] Bronchial carcinomas, mostly of oat cell type, are known to synthesise many different peptide hormones. Clinical syndromes are not always present, being reported in only 10% of all patients with lung cancer,[4] but in recent years the development of sensitive techniques has led to the detection of several peptides in 50% of cases. Ectopic production of somatostatin (somatotropin release inhibitory factor, or SRIF) has been reported to occur mainly in association with small cell carcinoma.[5 6]

We report here the immunocytological detection of both SRIF and ACTH hormones in cells obtained from bronchial brush smears and suggest that this detection used in conjunction with cytological examination may be useful in diagnosis.

Material and methods

PATIENTS

Thirty nine patients showing opacity on radiography and abnormal appearance on bronchoscopy were investigated. In all patients bronchial brush smears were obtained at the time of the fibroscopic examination.

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CYTOLOGICAL EXAMINATION

For each patient the bronchial brush smear sample was mixed with 5 ml of saline solution (0·9%) and centrifuged for 10 minutes in a Cytospin (Shandon). In this way cells were collected directly on the microscope slides. Depending on the size of the samples, we tried to obtain at least 12 slides, of which six were kept for the cytological study and six for the immunocytological assay. The cytological study was carried out using Papanicolaou's stain after fixation in a alcohol-ether mixture (50% vol/vol).

IMMUNOCYTOLOGICAL ASSAY

The presence of intracellular hormones was detected by the classical indirect immunofluorescence test after mild acetone fixation (3 minutes) with polyclonal but specific antihormone antisera (gift from MPD). Dilutions of antisera and the absence of cross reactivity between all antisera used were verified on frozen pancreas sections for the anti-SRIF (at 1/100 dilution) and human and guinea pig pituitary sections for the different anti-ACTH antisera (at 1/100 dilution). After incubation for one to two hours with the hormone antiserum and washing in phosphate buffer the cells were counterstained with a goat antirabbit IgG conjugated with fluorescein isothiocyanate (G/R FITC, Nordic Laboratories). The slides were then mounted in glycerol and visualised with a Zeiss orthoplan photomicroscope. As a result of the insufficient

number of slides obtained from some patients, only 28 patients were tested with the anti-1–24 ACTH and 31 with the anti-17–39 ACTH. For each patient one slide was kept as a control and the cells exposed to normal rabbit serum followed by the fluorescein isothiocyanate conjugated goat antirabbit IgG. The specificity of the staining reaction was tested by using the same antisera preabsorbed with the corresponding hormone.

For the absorption experiment 100 μl of the ACTH antiserum at 1/50 dilution were absorbed with 200 μl of ACTH 1–24 (Sigma) as follows: 0·5 mg of ACTH 1–24 diluted in 100 μl of sterile distilled water; 20 μl of the dilution was used for the absorption. The same method was used for the somatostatin.

Cells showing positive staining were identified by a double technique: after the immunofluorescence test the positive cells were photographed, the same slide was stained again by Papanicolaou's method, and each field photographed again. We were unable to test for the presence of both hormones in the same cells as both antihormone antisera (rabbit) used were obtained from the same animal.

Results

CYTOLOGICAL RESULTS

Malignant cells were diagnosed using the World Health Organisation classification criteria.[7] The study was focused mainly on the commonest types of lung tumours—the epidermoid and small cell types—because of the differential diagnostic problems encountered. Of the 39 specimens tested, 24 were identified as small cell carcinoma on cytological examination. This diagnosis was confirmed by histological study in 18 cases. Eight cases were identified as epidermoid carcinoma on cytological and histological examination. In four no malignant cells were detected on either the brush smear sample or biopsy specimen. They were taken as controls.

IMMUNOCYTOLOGICAL RESULTS

Positive staining with either the anti-SRIF or the anti-ACTH with both antisera was obtained in 26 cases (Table 1).

SRIF LIKE IMMUNOREACTIVITY

Positive cytoplasmic staining was detected with the

Table 1 *Histological and cytological diagnoses together with results of indirect immunofluorescence staining on bronchial brush smears for SRIF and ACTH like immunoreactivity in 35 men with lung tumours**

Case no	*Cytological diagnosis*	*SRIF*	*ACTH 1–24*	*ACTH 17–39*	*Histological diagnosis*
1	SCC	+	+	++	EC or SCC
2	SCC	+	+	−	SCC
3	SCC	−	+++	++	SCC
4	SCC	+++	++	−	No diagnosis
5	EC	−	+	−	EC
6	Suspect undetermined cells	−	−	−	EC
7	SCC	++	+	+++	SCC
8	SCC	+	++	+	SCC
9	SCC	−	+	−	SCC
10	EC	−	−	−	EC
11	SCC	−	+	++	No diagnosis
12	SCC	+	−	−	SCC
13	SCC	+	+	−	SCC
14	No diagnosis	−	−	−	SCC
15	SCC	++	+	−	No diagnosis
16	EC	+	−	−	EC
17	SCC	++	+	−	SCC
18	SCC	+	−	−	SCC
19	SCC	+	++	++	SCC
20	SCC	−	+++	++	SCC
21	SCC	++	++	−	SCC
22	EC	−	−	−	EC
23	SCC	+++	+	++	No diagnosis
24	EC	−		+	EC
25	EC or adenocarcinoma	−		−	Adenocarcinoma
26	EC	−		+	EC
27	SCC	+		+	SCC
28	EC	−		−	EC
29	SCC	+	−	+	SCC or EC
30	EC	−		−	EC
31	SCC	+	−		SCC
32	SCC	+	+		SCC
33	SCC	−	−		SCC
34	SCC	−	−		SCC
35	SCC	+		+	SCC
Total		19	17	13	

SCC = Small cell carcinoma; EC = Epidermoid carcinoma.
*In 26 men positive cells were found with either anti-SRIF or anti-ACTH antisera.

anti-SRIF antiserum in 19 cases; 18 of these were diagnosed as small cell carcinoma on cytological examination. Study of the biopsy specimen confirmed the diagnosis in 13 cases, did not allow a diagnosis in three, and was uncertain in two (Table 2).

ACTH LIKE IMMUNOREACTIVITY

In 22 cases, cells positive for either 1–24 or 17–39 ACTH immunoreactivity were detected. Thirteen of these cases were considered to be small cell carcinoma on both cytological and histological examination. Twenty four cases were tested with the two different 1–24 and 17–39 ACTH antisera. In eight cases both immunoreactivities were detected; all were found to be small cell carcinoma on cytological examination, but in five cases only was the diagnosis confirmed on histological examination (Table 3).

SRIF AND ACTH LIKE IMMUNOREACTIVITY

The existence of cells positive for both SRIF and ACTH immunoreactivity was shown only in cases of small cell carcinoma confirmed on both cytological and histological examination (11 of 18 cases). The number of cells stained by both antisera varied, and the intensity of staining was slightly different.

IMMUNOFLUORESCENCE (Fig. 1)

The immunofluorescent staining obtained was quite uniform in all the cases found to be small cell carcinoma; a thin ring of bright cytoplasmic staining in cells often forming clusters, the nucleus always remaining unstained. In the three cases of the epidermoid type, the cells appeared larger with more abundant cytoplasm that was positive with one or two of the tested antisera. The specificity of the staining was confirmed by the absorption experiment, as positivity was inhibited in all cases by preabsorption of the antiserum with the corresponding hormone.

No tumour cells were stained after exposure to normal rabbit serum, and only in some cases was a

Table 2 *The use of indirect immunofluorescence on cells obtained from bronchial brush smears, showing positive staining for SRIF and ACTH like immunoreactivity in 13 of 18 patients with SCC diagnosed by cytological and histological examination*

Type of tumour					
On cytological examination	*On histological examination*	*No of cases (n=35)*	*SRIF*	*ACTH*	*SRIF and ACTH*
SCC	SCC	18	13	13	10
SCC	No diagnosis	4	3	4	3
SCC	SCC or EC	2	2	2	2
No diagnosis	SCC	1			
EC	EC	8	1	2	0
Suspect undetermined cells	EC	1			
EC or adenocarcinoma	Adenocarcinoma	1			

SCC = Small cell carcinoma; EC = Epidermoid carcinoma.

Table 3 *Results of indirect immunofluorescence staining on cells from bronchial brush smears in eight patients with lung tumours with discrepancies in cytological and histological examination*

Case no	*Cytological diagnosis*	*Histological diagnosis*	*SRIF*	*ACTH 1–24*	*ACTH 17–39*	*Comments*
1	SCC	SCC or EC	+	+	++	Considered to be SCC, chemotherapy given
4	SCC	No diagnosis	+++	++	–	Considered to be SCC, chemotherapy given
6	Suspect undetermined cells	EC	–	–	–	Considered to be EC, pneumonectomy, performed
11	SCC	No diagnosis	–	+	++	Considered to be SCC, chemotherapy given
14	No diagnosis	SCC	–	–	–	Considered to be SCC, chemotherapy given
15	SCC	No diagnosis	++	+	–	Considered to be iliac metastasis, SCC, chemotherapy given
23	SCC	No diagnosis	+++	+	++	Considered to be cerebral metastasis. Patient refused chemotherapy
29	SCC	EC or SCC	+	–	+	Considered to be SCC, chemotherapy given

SCC = Small cell carcinoma; EC = Epidermoid carcinoma.

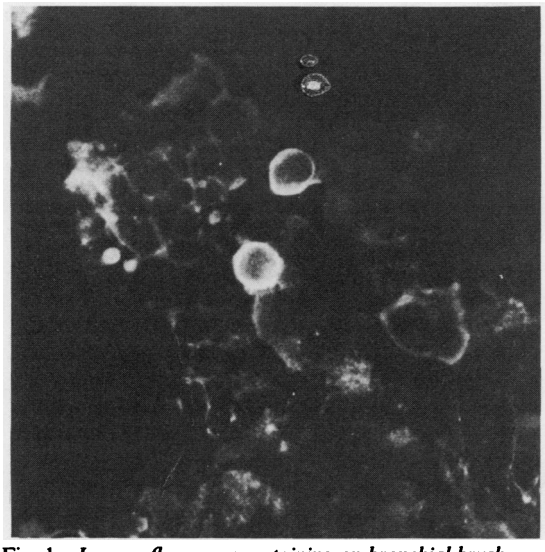

Fig. 1 *Immunofluorescence staining on bronchial brush smears in one case of small cell carcinoma with specific anti-SRIF antiserum.*

faint non-specific fluorescence of bronchial cells or macrophage detected. Similarly, no staining could be detected in the benign cases tested.

Discussion

Numerous peptides, including some neuropeptides, are known to be secreted by tumour cells; among pituitary hormones ACTH has been the most commonly reported.[4 8 9]

Bronchial carcinoma is one of the most common types of tumour associated with the production of ectopic hormone.[8] With the development of sensitive assays it has been recognised that all lung tumours may be associated with the production of peptides with hormonal activity, although this phenomenon seems to be more common in the small cell variety. Somatostatin in association with other neuropeptides[10 11] was recently detected in tumour extracts[5], tumour cell cultures,[12] and plasma in patients with oat cell carcinoma, but the immunocytological detection on tumour cells has not been reported.

Results obtained in our study support the known high prevalence of tumour cells producing ACTH and show that the same phenomenon can occur for somatostatin.

Many problems are still encountered in the histological classification of lung tumours, particularly anaplastic carcinoma as defined by the WHO classification.[7 13] Detection of hormone, in some cases at least, may help to establish the diagnosis at a fairly early stage of development and to identify the specific kind of tumour.[4] Our results confirm previous work and show the high prevalence of somatostatin in oat cell carcinoma. Clinical signs of excessive secretion of SRIF have not been described in association with lung carcinoma, but the immunoreactivity found in tumour extracts is sometimes accompanied by a detectable activity in plasma.[14] This has already been proved for several hormones and may reflect a low rate of synthesis and release, a rapid plasma clearance, or perhaps intermittent release.[15] Nevertheless, with the availability of sensitive radioimmunoassay raised SRIF plasma values may serve as a tumour marker. Pro-ACTH (a glycopeptide precursor) has also been proposed as an important tumour marker: whether it is or not needs to be investigated.[9]

The value of the cytological diagnosis on bronchial smears enriched by cytospinning has been proved. We have used this technique for over 10 years with good histological agreement. Immunochemical detection of hormones combined with cytological examination may further improve the value and rapidity of diagnosis and thus be useful in treatment. Although more patients and other types of tumour need to be tested, preliminary results obtained tend to show that the concomitant detection of the two hormones, ACTH and somatostatin, is highly suggestive of small cell carcinoma. Moreover, such findings may lead to a functional classification of lung tumours by studying the cytological characteristics of cells secreting specific peptides.

Many questions remain on the primitive or stem cell implicated in each type of lung tumour. The finding of transitional forms between small cell carcinoma, differentiated squamous cell carcinoma, and adenocarcinoma has led to a unified theory of lung cancer in man. The commonly associated production of ectopic hormone raises the question of different stages of cell differentiation. Results obtained in this study show that the detection of hormones with a panel of specific antisera or monoclonal antisera, or both, may well be useful in detecting tumour markers. Radioimmunoassay on the final histological specimen is needed to confirm the reliability of the cytological method.

Recent results obtained for the expression of neural filament type proteins in malignant cells of bronchial carcinoma[15] indicate that combining the two techniques will be even more useful.

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