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Biological markers in human lung carcinoma: an immunopathological study of six antigens

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ABSTRACT Immunohistological methods were used to investigate the presence of calcitonin, thyrotrophin, carcinoembryonic antigen, beta pregnancy specific glycoprotein, human placental lactogen, and the beta subunit of human chorionic gonadotrophin in formalin fixed lung tumour tissue sections. Carcinoembryonic antigen was observed in 71% of 101 tumours studied (70%), $beta_1$ pregnancy specific glycoprotein in 66 of 97 tumours (68%), beta subunit of human chorionic gonadotrophin in 35 of 97 (36%), human placental lactogen in 19 of 97 (20%), calcitonin in 10 of 71 (14%), and thyrotrophin in one of 27 lung tumours studied. There appeared to be no direct association between the presence of any given marker and the presence of any other. Similarly, the association between the presence of a tumour marker and histological type was poor. This study shows that the presence of tumour markers is relatively common in human lung tumours.

Several distinct clinical syndromes have been recognised in association with the production of certain polypeptides by lung tumours.[1,2] Indeed, Broder[3] suggested that lung carcinoma may be the most frequent malignancy associated with ectopic hormone production. Cell lines derived from human lung carcinomas have been shown to produce hormones and tumour markers in vitro, including human growth hormone,[4] human chorionic gonadotrophin,[5] adrenocorticotrophin and carcinoembryonic antigen.[6] Most investigations have focused on biochemical measurements of the relevant products in the serum or plasma of patients with lung tumours and correlations have been found between the level of a specific substance and the clinical syndrome produced in that patient. Recently, the advent of specific immunocytochemical techniques has enabled the demonstration of some of the relevant antigens within histological sections of conventionally processed material. Accordingly, we have applied these specific and sensitive techniques to a retrospective study of human lung tumours. We investigated six immunohistological markers—calcitonin, thyrotrophin, carcinoembryonic antigen, $beta_1$ pregnancy specific glycoprotein, human placental lactogen, and the beta subunit of human chorionic gonadotrophin to determine the incidence of such substances in the various histological subtypes of human lung carcinoma.

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Methods

Surgical specimens of 123 primary lung tumours were fixed in 10% buffered formalin, embedded in paraffin, and sectioned at intervals of 5 μm. The sections were stained routinely with haematoxylin and eosin and periodic acid Shiff with and without diastase and alcian blue. Each of the tumours was classified after study of sections stained with these techniques according to the revised World Health Organisation histological classification of lung tumours.[7] Six immunohistochemical markers were studied: calcitonin (by courtesy of Dr J Polak), thyrotrophin, carcinoembryonic antigen, $beta_1$ pregnancy specific glycoprotein, human placental lactogen, and the beta subunit of human chorionic gonadotrophin (the last four from Dakopatts) by a modification of the DNP hapten sandwich staining technique described by Jasani *et al*[8] and fully detailed elsewhere.[9] Table 1 shows the dilutions of primary antiserum used, incubation time, and the respective positive controls. Preliminary studies showed that trypsinisation was useful for the demonstration of some markers. Trypsinisation for one hour was optimal for calcitonin. Trypsinisation for four hours was optimal for $beta_1$ pregnancy

Table 1 *Dilutions and incubation times of specific antisera*

Specific antiserum	*Dilution*	*Incubation time (h) with primary antiserum*	*Controls*
Calcitonin	1/1600	1	Rat thyroid and background human lung
Thyrotrophin	1/10 000	1	Human hypophysis
Carcinoembryonic antigen	1/1600	1	Colon adencarcinoma
Beta$_1$ pregnancy specific glycoprotein	1/1600	15	Human placenta
Beta subunit of human chorionic gonadotrophin	1/800	15	Human placenta
Human placental lactogen	1/1600	15	Human placenta

specific glycoprotein, human placental lactogen, and the beta subunit of human chorionic gonadotrophin. Trypsinisation was not performed for thyrotrophin or carcinoembryonic antigen. Appropriate positive and negative controls were included with each batch of staining. The negative controls consisted of replacing the specific primary antiserum by non-immune serum.

The degree of positivity was graded semi-quantitatively as follows: 0—negative; + (weak)—up to 5% of tumour cells were intensely or moderately stained; ++ (moderate)—5–33% of tumour cells were intensely or moderately stained; +++ (strong)—more than 33% of the tumour cells evinced intense or moderate staining.

Results

Tables 2–4 show the breakdown of the histological subgroups of lung tumour and the results of staining for the six immunohistological markers. In descending order of frequency, carcinoembryonic antigen was observed within the tumour cells of 70% of lung tumours studied, beta$_1$ pregnancy specific glycoprotein in 68%, the beta subunit of human chorionic gonadotrophin in 36%, human placental lactogen in 20%, calcitonin in 14%, and thyrotrophin in none.

Calcitonin was most frequently observed in small cell carcinomas regardless of subtype (table 4). It was also observed in a bronchial carcinoid tumour and two adenocarcinomas. The number of positive cells within a given tumour was small (1–5%) and they had to be searched for diligently. Occasional calcitonin positive cells were also seen in the normal bronchiolar epithelium in some cases but this did not relate to the positivity of the tumour cells.

In general, the tumours positive for carcinoembryonic antigen showed staining of a considerable proportion of cells but the two carcinoid tumours, which were positive, showed only sparse groups of positive cells (table 2). Most tumours showed diffuse cytoplasmic staining by carcinoembryonic antigen but in well and moderately differentiated adenocarcinomas the glycocalyx was also stained. This marker was occasionally found sparsely and focally within metaplastic bronchial epithelium of the background lung.

An appreciable proportion of tumours within each major histological subgroup was positive for one or more of the placental proteins—beta$_1$ pregnancy specific glycoprotein, human placental lactogen, and the beta subunit of human chorionic gonadotrophin (tables 3 and 4). For the most part the tumours showed diffuse intracytoplasmic staining for these markers. The antibody to beta$_1$ pregnancy specific glycoprotein, however, also stained the keratin rich areas in well and moderately differentiated squamous carcinomas and the glycocalyx

Table 2 *Number of tumours showing each grade of immunostaining for carcinoembryomic antigen*

Histological type	*Grade of immunostaining**				*Total*
	0	+	++	+++	
Epidermoid carcinoma	2	6	13	5	26
Small cell carcinoma	7	10	7	1	25
Adenocarcinoma	3	4	1	6	14
Large cell carcinoma	7	8	0	0	15
Adenosquamous carcinoma	2	3	3	0	8
Carcinoid tumour	7	2	0	0	9
Adenoid cystic carcinoma	1	0	0	0	1
Mucoepidermoid carcinoma	0	0	1	0	1
Carcinosarcoma	0	1	0	0	1
Malignant lymphoma	1	0	0	0	1
Total	30	34	25	12	101

*See under "Methods."

Table 3 *Number of tumours showing each grade of immunostaining for beta$_1$ pregnancy specific glycoprotein*

Histological type	*Grade of immunostaining**				*Total*
	0	+	++	+++	
Epidermoid carcinoma	4	14	16	7	41
Small cell carcinoma	9	2	1	1	13
Adenocarcinoma	1	4	6	2	13
Large cell carcinoma	13	2	0	0	15
Adenosquamous carcinoma	3	3	4	3	13
Mucoepidermoid carcinoma	0	0	1	0	1
Carcinosarcoma	1	0	0	0	1
Total	31	25	28	13	97

*See under "Methods."

Table 4 *Results of immunostaining for the beta subunit of human chorionic gonadotrophin (βHCG), human placental lactogen (HPL), calcitonin (CT), and thyrotrophin (TSH)*

Histological type	*Result of immunostaining (No of tumours)*							
	βHCG		*HPL*		*CT*		*TSH*	
	+	−	+	−	+	−	+	−
Epidermoid carcinoma	9	32	8	33	0	9	0	8
Small cell carcinoma	7	6	3	10	7	17	0	1
Adenocarcinoma	5	8	1	12	2	8	0	8
Large cell carcinoma	8	7	3	12	0	8	0	0
Adenosquamous carcinoma	6	7	4	9	0	8	0	1
Carcinoid tumour	ND		ND		1	8	0	9
Adenoid cystic carcinoma	ND		ND		0	1	ND	
Mucoepidermoid carcinoma	0	1	0	1	ND		ND	
Carcinosarcoma	0	1	0	1	0	1	ND	
Malignant lymphoma	ND		ND		0	1	ND	
Total	35	62	19	78	10	61	0	27

ND—not done.

in adenocarcinomas. The glycoprotein was often present in a considerable proportion of tumour cells. The beta subunit of human chorionic gonadotrophin was present in more than 5% of tumour cells in only one tumour (a poorly differentiated squamous carcinoma). Human placental lactogen was not present in more than 5% of cells in any tumour.

There did not appear to be any direct association between the presence of a given marker and the presence of any other. Some tumours were negative for all markers studied while some were positive for as many as six.

Discussion

This study shows that the tumour antigens studied are relatively common in lung tumours. Eighty six per cent of the tumours contained at least one antigen and 35% contained two or more antigens.

Raised concentrations of calcitonin have been reported in various lung tumours but the highest frequency has been associated with small cell carcinoma and bronchial carcinoid tumours. In small cell carcinoma raised calcitonin concentrations have been reported in 64–82% of cases.[2 10 11] Milhaud *et al*[12] reported raised concentrations in half of the bronchial carcinoids and all the oat cell carcinomas studied. Few studies using immunohistological techniques for the demonstration of calcitonin have been reported for pulmonary tumours. Cooney *et al*[13] reported calcitonin positive cells in six out of 10 bronchial carcinoid tumours. Delellis and Wolfe[14] reported calcitonin positive cells in a very rare type of tumour which arises from the lung and which possesses morphological characteristics similar to those of medullary carcinoma of the thyroid, the so called medullary carcinoma of lung. Dayal *et al*[15] reported calcitonin positive cells in two out of four bronchial carcinoids. Our results are similar to others although at a lower level of detection, but we must emphasise that calcitonin positive cells are only a small proportion of the total tumour cells (1–5%) and had to be searched for diligently. Possibly examination of several blocks for each tumour would raise considerably the number of positive tumours detected. This is in agreement with the observations of Cooney *et al*,[13] who noted calcitonin positive staining in only a small number of the tumour cells of bronchial car-

cinoids. Occasional calcitonin positive cells were also located, either singly or in small clusters, within the bronchial or bronchiollar epithelium of the background lung; Becker *et al*[16] reported similar findings in the human lung using a peroxidase-antiperoxidase technique for calcitonin. Our findings of calcitonin positive cells in two adenocarcinomas is interesting and accords with the demonstration of endocrine cells by electron microscopy within tumours not suspected of being endocrine by light microscopy.[17] A prospective study of serum calcitonin concentrations in 61 patients with bronchogenic carcinoma showed raised levels in 52% regardless of histological type; they were not related to the presence of osseous metastasis.[10] In the same study, however, investigation of six cases by selective thyroid venous sampling showed that the raised calcitonin concentrations could be thyroidal or ectopic in origin. Our study cannot refute or confirm the ectopic production of calcitonin by the lung tumour since the number of calcitonin positive cells was no greater in the tumour than within the background lung.

Several studies have shown raised concentrations of carcinoembryonic antigen in patients with lung tumours and it has also been demonstrated in the sections of some lung tumours. Our findings of carcinoembryonic antigen positivity in 70% of the tumours studied is broadly in agreement with those of other studies.[18–20] Some investigators have reported carcinoembryonic antigen positivity to be more common in the better differentiated than in more poorly differentiated carcinomas but we did not find this to be the case. Nevertheless, serum levels of carcinoembryonic antigen have consistently been shown to be greater in patients with adenocarcinomas than with the other major histological groups of lung cancer.[21]

The so called trophoblastic specific pregnancy proteins (beta$_1$ pregnancy specific glycoprotein, human placental lactogen, and the beta subunit of human chorionic gonadotrophin), which are normally synthesised by the human placenta, have been shown to be increased in the serum of some patients with various non-trophoblastic tumours. They have also been demonstrated by immunoperoxidase techniques in tissue sections of various non-trophoblastic tumours.[22,23] Few studies of these markers in lung tumours have been reported and then only of a small number of cases. Gropp *et al*[24] investigated 113 patients and found raised concentrations of beta subunit of human chorionic gonadotrophin in 19% of squamous, 33% of oat cell, and 26% of large cell undifferentiated pulmonary carcinomas. Rosen *et al*[25] investigated 187 patients and found raised human placental lactogen levels in 5 cases. Grudzinskas *et al*[26] found raised concentrations of beta$_1$ pregnancy specific glycoprotein in one out of 32 patients investigated. Using a peroxidase-antiperoxidase immunocytochemical technique, Wilson *et al*[27] found the beta subunit of human chorionic gonadotrophin in 84% of 61 lung tumours examined. We have found 36% of lung tumours examined to be positive for this protein—less than Wilson *et al*,[27] who had a higher rate of detection for each histological subgroup. Our finding of beta$_1$ pregnancy specific glycoprotein in the sections of 75% of lung tumours studied is surprisingly high in view of the reported low incidence of raised serum levels. In many respects it behaves similarly to carcinoembryonic antigen both in the pattern of staining and in the frequency of positivity. Both carcinoembryonic antigens and beta$_1$ pregnancy specific glycoprotein were often but not invariably present in the same tumour. Surprisingly, the latter was detected less often in large cell undifferentiated carcinomas than in the other major groups.

This study shows that tumour markers can be relatively frequently demonstrated in lung tumours. Accordingly, further studies are required to elucidate the role of tumour markers in monitoring and in prognosis and the prediction of clinical manifestations of lung tumours. It would appear from this study that beta$_1$ pregnancy specific glycoprotein, the beta subunit of human chorionic gonadotrophin, and carcinoembryonic antigen are likely to prove the most useful markers in this respect.

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