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CLARA CELL 10 kDa PROTEIN mRNA IN NORMAL AND ATYPICAL REGIONS OF HUMAN RESPIRATORY EPITHELIUM

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We used RNA-RNA *in situ* hybridization to study expression of the human CC10 gene in morphologically normal and atypical areas of 32 non-neoplastic lung specimens resected from 26 non-small cell lung cancer patients. We scored strong, moderate or weak levels of CC10 mRNA expression in 3 distinct lung compartments. In morphologically normal lungs, strong and moderate levels of CC10 mRNA were observed in bronchioli and bronchi, respectively, but the expression was rarely observed in the alveolar region. Distinct alterations in CC10 mRNA expression were noted in specific histologic abnormalities within bronchi and the alveolar region. CC10 hybridization signal decreased markedly in bronchi containing diffuse goblet cell hyperplasia or squamous metaplasia, while CC10 mRNA expression remained unchanged in bronchi with basal cell hyperplasia or focal goblet cell hyperplasia. Bronchiolar CC10 mRNA levels remained unchanged in sections containing abnormalities elsewhere. Interestingly, in alveoli with bronchiolization of the alveoli, high levels of CC10 mRNA were observed. These regions also contained strongly stained keratin 14-positive cells, which may indicate a concurrent metaplastic process. In lungs with morphologic atypias, no correlation was found between abnormalities detected in bronchi and alveoli from the same lung. A comparison of mRNA expression and clinicopathologic features demonstrated that the amount of histologic abnormalities increased with smoking history (pack years); however, no correlation between CC10 mRNA expression and sex, age or smoking history was found.
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We and others have noticed an increase in the proportion of pulmonary adenocarcinomas, with up to 50% containing features suggestive of peripheral airway cell differentiation (Linnoila, 1990). In addition, these tumors are the most common tumor type in women, in younger age groups and in patients with no smoking history, suggesting a distinct biology for cancers arising in the peripheral lung. Pulmonary adenocarcinomas are generally resistant to radiation and chemotherapy, and the majority of patients develop distant metastases long before the diagnosis of lung cancer is made, calling for more effective early detection. However, unlike centrally located squamous cell carcinomas of the lung, for which the sequence of preneoplastic changes has been well described (Nasiell *et al.,* 1987), events preceding the more peripherally located pulmonary adenocarcinomas are not well understood.

One of the progenitor cells for the peripheral airway epithelium and adenocarcinomas arising in this region is the Clara cell, a morphologically defined subtype of non-ciliated secretory cells in bronchiolar epithelium (Massaro *et al.,* 1994). Prior to the availability of sequence information, the molecular weight of the major Clara cell secretory protein was estimated from SDS/PAGE to be about 10 kDa (Singh *et al.,* 1985) and for this reason has been referred to as CC10. The expression of CC10 was originally thought to be restricted to Clara cells (Singh *et al.,* 1988), often serving as a marker for these cells. We have previously demonstrated that non-ciliated secretory cells containing CC10 can be detected throughout the human tracheobronchial tree (Broers *et al.,* 1992; Linnoila *et al.,* 1992). While CC10 expression was abundant in non-neoplastic human lung, it was detectable in tumors and corresponding cell lines at markedly lower levels (Broers *et al.,* 1992; Linnoila *et al.,* 1992). Interestingly, CC10 levels were significantly lower in serum and bronchoalveolar lavage specimens obtained from smokers and lung cancer patients compared with specimens from healthy non-smokers (Bernard *et al.,* 1992).

It has been well documented that the entire lung exposed to pulmonary carcinogens such as tobacco smoke may show histologic abnormalities reflecting field cancerization (Auerbach *et al.,* 1961). The previously reported findings of alterations in CC10 expression during carcinogenesis prompted us to examine the morphological basis of CC10 dysregulation. Difficulty in obtaining normal lung tissue has led us to examine surrounding non-neoplastic lung obtained from patients undergoing resection for cancer.

In this study, we used RNA-RNA *in situ* hybridization to examine patterns of CC10 mRNA expression in morphologically normal and atypical areas of 32 non-neoplastic lung specimens obtained from 26 patients with non-small cell lung carcinomas (NSCLC) or mesotheliomas. Our findings suggest that the expression of CC10 mRNA becomes altered in distinct lung compartments and may implicate a role for CC10 in the development of pulmonary carcinomas.

MATERIAL AND METHODS

Tissues

Thirty-two non-neoplastic lung tissue specimens from 26 patients were reviewed in this study. All specimens were resected from patients who had NSCLC or mesotheliomas and were prepared as previously described (Broers *et al.,* 1992). Tumor histologies of the 26 cases are summarized in Table VII.

In situ hybridization

Preparation of the human CC10 kDa mRNA probe and tissue *in situ* hybridization were performed as previously described (Broers *et al.,* 1992) with the following modification. The 366 bp insert was re-oriented to avoid any incorporation of vector-derived material in the generated RNA probes. Results of the *in situ* hybridization were scored independently by 2 authors (R.I.L. and S.M.J.) for both the number of positive cells (distribution score: 0 = no positive cells; 1 for 1–10%; 2 for 11–50%; 3 for 51–100% of epithelial cells positive) and the labeling of grains per cell (0 = negative; 1 = weak; 2 = moderate; 3 = strong). Using the sum of these values, a hybridization signal index (HSI = distribution score + intensity score, possible values: 0, 2–6) was established for each lung compartment (Table I). All slides were hybridized in duplicate and exposed for 1 or 2 week intervals. Sections from one block were evaluated for 23 of the 26 patients studied, while 2, 3 and 4 separate blocks were reviewed for the remaining 3 patients.

Immunohistochemistry

The mouse monoclonal antibody (MAb) anti-keratin 14 (RCK 107) (Wetzels *et al.,* 1989) was used to identify areas of

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basal cell hyperplasia (a gift from Dr. J.L.V. Broers, University of Limburg, Maastricht, The Netherlands). Frozen sections were cut and thawed onto glass slides pre-coated with 0.1% poly-L-lysine and fixed by dipping once in −20°C methanol, and 3 times in 4°C acetone. Slides were air dried and stored at −20°C until use.

Immunohistochemical staining was performed using the Vectastain ABC staining kit (Vector, Burlingame, CA) following the vendor's instructions with modifications as described (Linnoila *et al.,* 1988).

Histopathology

Changes in 3 distinct lung compartments (bronchi, bronchioli, alveolar region) were recorded. Hematoxylin and eosin-stained sections of all tissues were screened for the following histologic abnormalities: basal cell hyperplasia, focal and diffuse goblet cell hyperplasia, squamous metaplasia, dysplasia, type II cell hyperplasia, fibrosis, and bronchiolization of the alveoli. The identification of histologic abnormalities was based on histologic characteristics described in previous reports (Nasiell, 1963; Nettesheim and Szakal, 1972; Adekunle *et al.,* 1991). Basal cell hyperplasia was scored when three or more basal cell layers were seen. Focal goblet cell hyperplasia was represented by normal appearing respiratory epithelium interrupted by clusters of 3 or more goblet cells with no more than 50% of bronchial lining cells composed of goblet cells, while diffuse goblet cell hyperplasia was scored in areas of more than 50% goblet cells. Squamous metaplasia was characterized by areas containing layers of flattened epithelial cells with cilia absent. Dysplasia was used to describe 2 types of lesions: 1) atypical squamous metaplasia (borderline carcinoma *in situ*) (Nasiell, 1966); and 2) atypia in epithelium that retained the general character of columnar epithelium with preserved cilia (Nasiell, 1963).

TABLE I – SCORING FOR CC10 mRNA EXPRESSION

Distribution score[1] (1–3)	Intensity score (1–3)	Hybridization signal index[2] (0, 2–6)
Negative = 0	Negative = −	Negative = 0
1 = 1–10	1 = +	Weak = 2
2 = 11–50	2 = ++	Moderate = 3–4[3]
3 = 51–100	3 = +++	Strong = 5–6

[1]Distribution score equals the percent of positive epithelial cells in high-power field.–[2]Hybridization signal index (HSI) equals the sum of distribution and intensity scores.–[3]For example, moderate score could potentially be distribution score 2 plus intensity score 1 = 3, as well as distribution score 2 plus intensity score 2 = 4.

Clinicopathologic analyses

Data were obtained from 26 NSCLC patients. Because multiple slides were obtained in 3 of the cases and one slide was available on each of the other 23 patients, data from each of the multiple slides were averaged to form a single value per patient. Multiple values obtained per case were averaged to form one value per compartment resulting in one HSI value for each of the 3 lung compartments per patient (Tables III–VII). Comparisons between 2 groups of patients' HSI values were made using the Wilcoxon rank sum test while the Kruskal-Wallis test was used to test for differences when multiple histologies were compared. Spearman rank correlation analysis was used to demonstrate the association between CC10 HSI values and pack years and age. All p values reported are two-sided.

RESULTS

CC10 mRNA in histologically normal human lung

Mean CC10 HSI was calculated for each of 3 lung compartments (bronchi, bronchioli, alveolar region) (Table II). A total

TABLE II – EXPRESSION OF CC10 mRNA IN LUNG COMPARTMENTS CONTAINING HISTOLOGIC ABNORMALITIES AND DIAGNOSIS OF CORRESPONDING TUMOR[1]

Conducting airways (number)	CC10 HSI[2] (mean ± SEM)	BCH	FGCH	DGCH	SQM	DYS	Diagnosis of tumor
Bronchi							
8—Control[3]	3.9 ± 0.31	−	−	−	−	−	ME (3), AD (2), SQ
1	5.0	+	−	−	−	−	SQ
4	4.0 ± 0.41	+	+	−	−	−	AD (2), CRC, ME
1	2.0	+	−	+	−	−	OT
1	3.0	−	+	+	−	−	CRC
2	5.5 ± 0.50	−	+	−	−	−	SQ (2)
1	3.0	−	+	−	+	−	CRC
1	3.0	+	−	+	−	+[4]	SQ
1	3.0	−	−	+	−	−	AD
1	5.0	−	−	−	−	+[5]	SQ
Bronchioles							
62—Control	5.0 ± 0.14	−	−	−	−	−	ME (6), AD (5), CRC (3) OT (3), ADSQ
1	5.0	−	−	−	−	+[5]	ADSQ

Alveolar region (number)	CC10 HSI[2] (mean ± SEM)	FIB	T2H	BOA	Diagnosis of tumor
11—Control[3]	0.5 ± 0.36	−	−	−	SQ (4), ME (3), AD (2), CRC, OT
8	0.0	+	−	−	AD (3), ME (2), CRC, SQ, ADSQ
7	0.4 ± 0.40	+	+	−	SQ (5), CRC, ME
1	3.0	+	−	+	OT
2	4.0 ± 1.00	+	+	+	ADSQ, AD

[1]BCH, basal cell hyperplasia; FGCH, focal goblet cell hyperplasia; DGCH, diffuse goblet cell hyperplasia; SQM, squamous metaplasia; DYS, dysplasia; FIB, fibrosis; T2H, type II cell hyperplasia; BOA, bronchiolization of the alveoli; AD, adenocarcinoma; ADSQ, adenosquamous; CRC, carcinoid; ME, mesothelioma; OT, other.–[2]HSI, hybridization signal index, the sum of distribution and intensity.–[3]Control, specimens containing no histologic abnormalities. Dysplasia was used to describe two types of lesions: [4]atypical squamous metaplasia (borderline carcinoma in situ), (Nasiell, 1966), and [5]atypia in epithelium that has retained the general character of columnar epithelium and preserved cilia (Nasiell, 1963).

TABLE III - COMPARISON OF SEX, PACK YEARS AND CC10 mRNA EXPRESSION

Sex (number)	Pack years[1]	Number of patients (%)	CC10 hybridization signal index [mean ± SEM (number of patients)]		
			Bronchi	Bronchioli	Alveolar region
Males (14)	0–49	10 (71)	4.0 ± 0.4 (4)	4.2 ± 0.4 (7)	1.0 ± 0.5 (9)
	50+	4 (29)	4.5 ± 0.8 (3)	5.4 ± 0.4 (2)	1.4 ± 0.8 (4)
	p_2=		0.59	0.30	0.86
Females (10)	0–49	6 (60)	3.5 ± 0.3 (5)	5.8 ± 0.2 (3)	0.5 ± 0.5 (5)
	50+	4 (40)	4.0 ± 1.0 (2)	4.6 ± 0.6 (3)	0.0 (4)
	p_2=		0.67	0.08	0.54

[1]Smoking history (pack years) was available for 24 of the 26 patients.

TABLE IV - COMPARISON OF SEX, AGE AND CC10 mRNA EXPRESSION

Sex (number)	Years (range)	Number of patients (%)	CC10 hybridization signal index [mean ± SEM (number of patients)]		
			Bronchi	Bronchioli	Alveolar region
Males (15)	0–55	2 (13)	4.0 (1)	4.5 ± 1.1 (2)	2.3 ± 0.8 (2)
	56+	13 (87)	4.3 ± 0.3 (8)	4.6 ± 0.4 (8)	1.0 ± 0.4 (12)
	p_2=		0.84	1.0	0.22
Females (11)	0–55	6 (55)	3.3 ± 0.4 (5)	5.2 ± 0.6 (4)	0.0 (5)
	56+	5 (45)	3.7 ± 0.7 (3)	5.4 ± 0.3 (3)	0.6 ± 0.6 (5)
	p_2=		0.75	0.85	0.42
Males	All ages	15	4.3 ± 0.3 (9)	4.6 ± 0.4 (10)	1.1 ± 0.4 (14)
Females	All ages	11	3.4 ± 0.3 (8)	5.4 ± 0.4 (7)	0.3 ± 0.3 (10)
	p_2=		0.10	0.15	0.11

TABLE V - CORRELATION OF CC10 mRNA HYBRIDIZATION SIGNAL INDEX WITH PACK YEARS AND AGE

	Spearman correlation coefficients/P_2/n		
	Bronchi CC10	Bronchioli CC10	Alveolar region CC10
Pack years	0.05/0.87/14	0.03/0.91/15	0.10/0.63/23
Age	0.35/0.17/17	−0.32/0.21/17	−0.12/0.58/24

TABLE VI - CORRELATION OF HISTOLOGIC ABNORMALITIES AND SMOKING HISTORY (PACK YEARS)

Atypia	Mean number of pack years ± SEM[2] (number of patients)		
	Bronchi	Broncioles	Alveoli
Normal	21 ± 7.3 (6)	37 ± 10.2 (14)	50 ± 12.1 (11)
Basal cell hyperplasia	55 ± 29.0 (4)		
Goblet cell hyperplasia[1]	58 ± 18.3 (9)		
Squamous metaplasia	100 (1)		
Dysplasia	45 ± 4.9 (2)	120 (1)	
Fibrosis			34 ± 11.9 (13)
Type II cell hyperplasia			58 ± 19.6 (6)
Bronchiolization of the alveoli			63 ± 28.9 (3)

[1]Includes focal and diffuse goblet cell hyperplasia.–[2]Mean number of pack years was calculated for all patients whose specimens contained one or more histologic abnormalities in each lung compartment.

TABLE VII - CORRELATION OF CC10 mRNA HYBRIDIZATION SIGNAL INDEX IN SURROUNDING NON-NEOPLASTIC LUNG WITH HISTOLOGIC TYPE OF THE CANCER

Histology	CC10 hybridization signal index (HSI) [mean ± SEM (number of patients)]		
	Bronchi	Broncioli	Alveoli region
Adenocarcinoma (5)	3.6 ± 0.4 (4)	4.9 ± 0.8 (3)	0.6 ± 0.6 (5)
Squamous (9)[1]	4.5 ± 0.4 (5)	5.0 ± 0.4 (6)	1.3 ± 0.5 (8)
Carcinoid (3)	3.7 ± 0.7 (3)	5.0 (1)	0.0 (3)
Mesothelioma (6)	4.0 ± 0.4 (4)	4.5 ± 0.6 (4)	0.5 ± 0.5 (6)
Other (3)[2]	2.0 (1)	5.1 ± 0.9 (3)	1.5 ± 1.5 (2)
p_2=	0.28	0.85	0.51
Total[3] (26)	(n = 17)	(n = 17)	(n = 24)

[1]One adenosquamous.–[2]Includes two sarcoma, one small cell tumor.–[3]Tissue specimens of the 26 patients we studied contained different combinations of bronchi, bronchioli and alveoli.

of 8 of 21 bronchi and 62 of 63 bronchioles examined were classified as controls, with an average HSI of 3.9 and 5.0, respectively. A total of 11 of 29 alveolar regions examined were classified as controls, with an average HSI of 0.5.

Large bronchi contained numerous scattered non-ciliated columnar cells demonstrating variable CC10 mRNA expression (Fig. 1*a,b*). We also detected focal hybridization signal in the epithelium of bronchial glands (Fig. 1*c,d*). In terminal bronchioli, both the distribution and intensity of CC10 mRNA expression was higher than that seen in large bronchi. It appeared that most cells did express CC10 mRNA (Fig. 1*e*). While grain density in epithelial cells was similar to that seen in terminal bronchioli, the total number of cells expressing CC10 mRNA in respiratory bronchioli was lower than that seen in bronchi and terminal bronchioli (Fig. 1*f*). CC10 mRNA expression was rarely observed in histologically normal alveoli (Fig. 1*g*).

Keratin 14 staining in histologically normal human lung

To aid in the detection of basal cell hyperplasia, we stained all tissue sections with the basal cell-specific keratin 14 MAb. As expected, keratin 14 immunoreactivity was strong in bronchial basal cells and glands (Fig. 2*a*), and only weakly expressed in bronchiolar basal cells (Fig. 2*b*). No immunoreactivity was observed in the alveolar region (Fig. 2*c*).

Distribution of histological abnormalities

When we examined the distribution of histologic abnormalities in a given lung we found no association between findings of atypia in either bronchial versus alveolar compartments. For example, 3 of the 6 (50%) control specimens that lacked histologic abnormalities in the bronchial compartment also

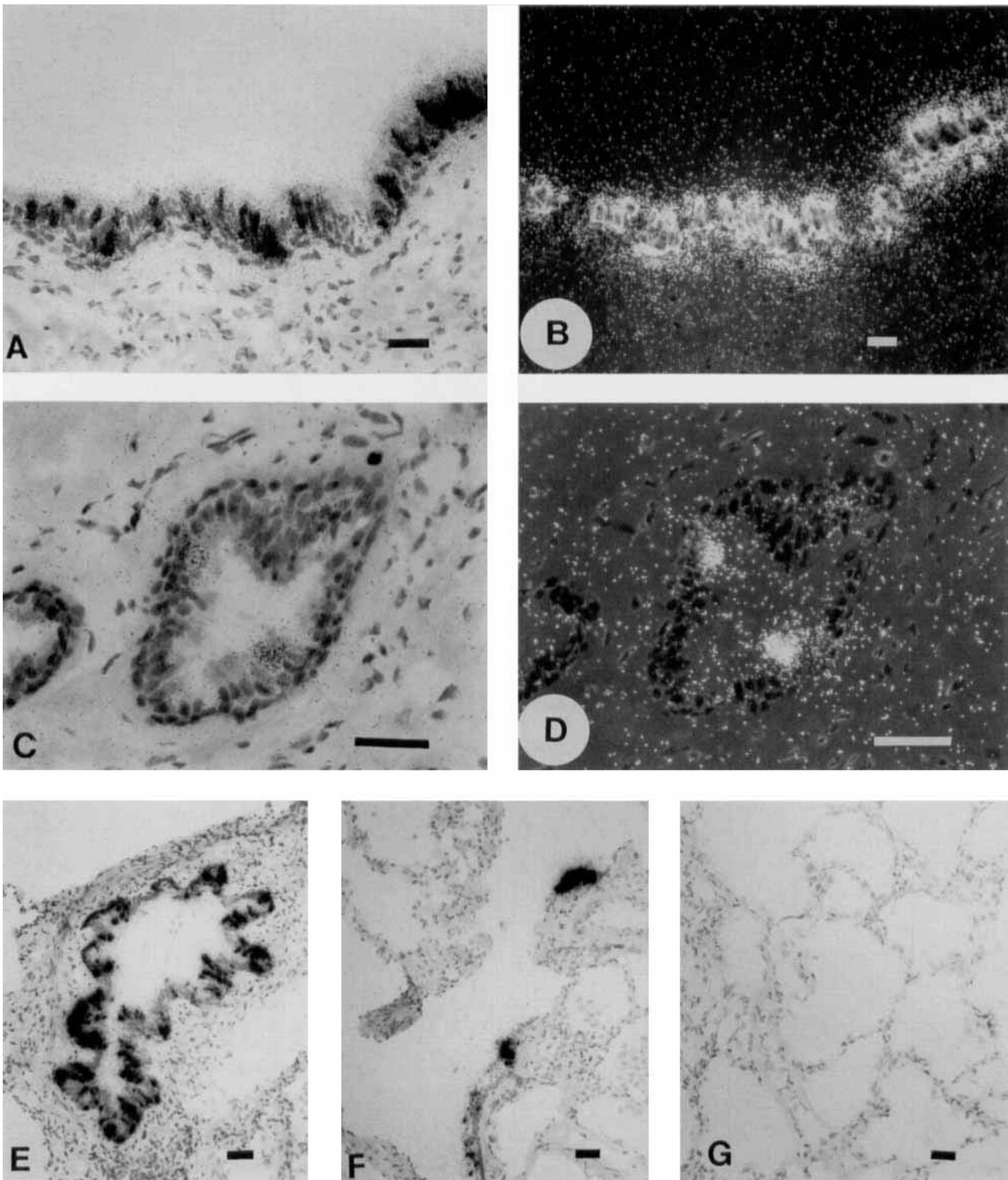

FIGURE 1 – Localization of CC10 mRNA in non-neoplastic lung without histologic abnormalities. *(a)* Bronchus. *(b)* Dark field view of the same bronchus. Note the intensity of grains for comparison with CC10 mRNA expression in abnormal lung compartments. *(c)* CC10 mRNA in bronchial glands. *(d)* Dark field of the same. *(e)* CC10 mRNA in terminal bronchiole. *(f)* A respiratory bronchiole. *(g)* Note the absence of CC10 mRNA in non-neoplastic alveoli. Bar = 50 μm.

lacked abnormalities in the alveolar compartment, while the other 50% contained alveolar abnormalities. Furthermore, of the 9 specimens containing bronchial abnormalities, 5/9 (55%) demonstrated normal alveolar regions and 4/9 (45%) showed atypia.

Despite numerous abnormalities in bronchi and alveoli, histologic abnormalities in bronchioli were rare. This disparity, and the fact that many changes occurred simultaneously in various compartments, prompted us to focus on defining CC10 mRNA expression patterns in individual compartments when-

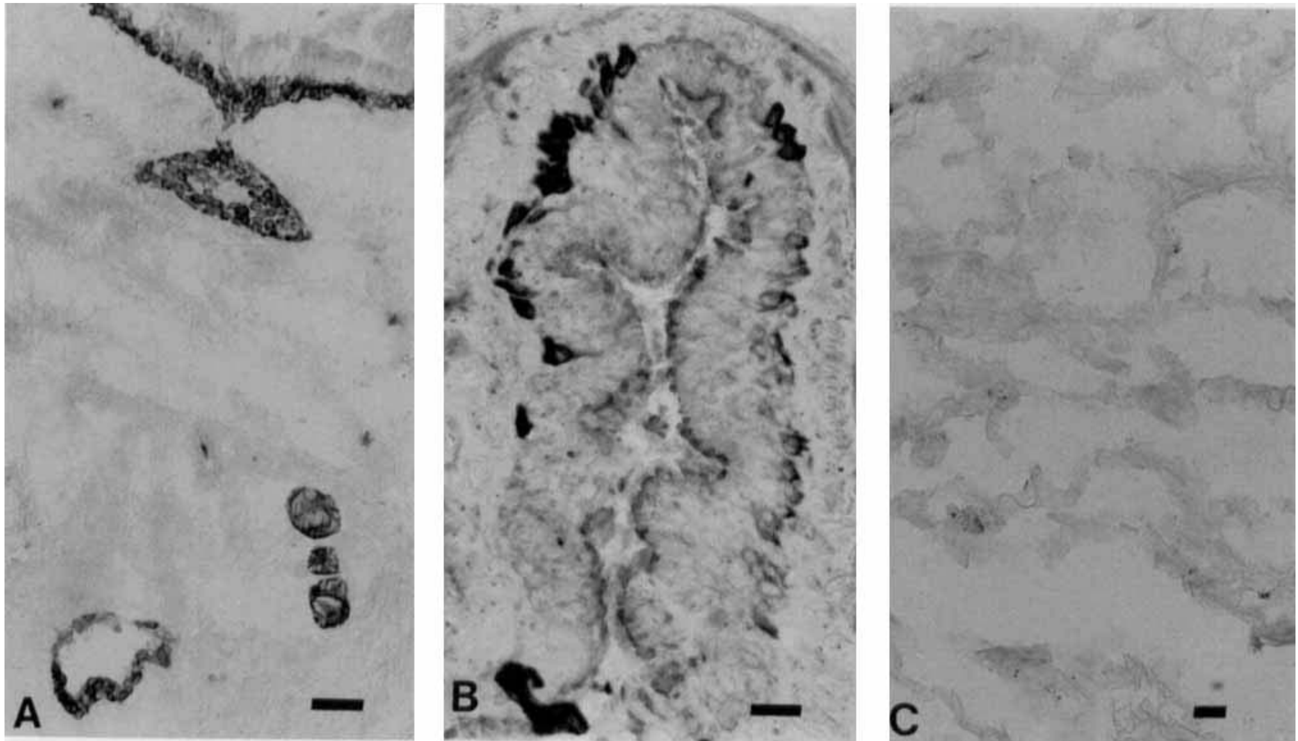

FIGURE 2 – Keratin 14 expression in human lung without histologic abnormalities. *(a)* Bronchial, ductal and glandular epithelium. *(b)* Bronchiolar epithelium. *(c)* Alveolar epithelium. Contrast the diffuse distribution pattern of keratin 14 immunoreactivity in bronchial basal cells, ducts and glands with the more focal staining pattern in bronchiolar basal cells. Note the absence of keratin 14 immunoreactivity in normal alveolar region. Bar = 50 μm.

ever they demonstrated histologic abnormalities and also to correlate the smoking history with each histologic abnormality (Table VI).

CC10 mRNA in histologically abnormal human lung

Histologic abnormalities in conducting airways. CC10 mRNA expression was examined in bronchi and bronchioli containing histologic abnormalities associated with the conducting airways (basal cell hyperplasia, focal and diffuse goblet cell hyperplasia, squamous metaplasia, dysplasia) (Table II). CC10 mRNA expression was detected in bronchial glands adjacent to abnormal bronchi, similar to that seen in normal controls (Fig. 2*c,d*). When compared with controls, CC10 mRNA expression increased in one bronchus containing basal cell hyperplasia only, while levels of expression remained unchanged in bronchi containing both basal cell hyperplasia and focal goblet cell hyperplasia. However, in bronchi containing diffuse goblet cell hyperplasia, the average CC10 HSI decreased from 3.9 to 2.8 (Fig. 3*a,b*). Squamous metaplasia was detected in one bronchus with an HSI of 3.0. We obtained discordant results for bronchi containing dysplasia with high CC10 mRNA levels in one (not shown) and decreased expression in the other (Fig. 3*c,d*). Keratin 14-positive basal cells did not express CC10 mRNA (Fig. 3*e,f*).

While histologic abnormalities were rarely detected in bronchioli, minor variation occurred in CC10 HSI. Only 1/63 demonstrated dysplasia in which CC10 mRNA expression remained unchanged (Table II).

Histologic abnormalities in the alveolar region. In sections containing histological abnormalities associated with the alveolar region (fibrosis, type II cell hyperplasia, bronchiolization of the alveoli), 8 alveolar regions contained fibrosis only and demonstrated no CC10 mRNA expression (Table II). In alveolar regions containing both fibrosis and focal type II cell hyperplasia (Fig. 4*a,b*), the average CC10 mRNA HSI remained unchanged from that of control alveoli. However, a marked increase in the average CC10 HSI (3.7) was observed in alveoli containing fibrosis, type II cell hyperplasia, and bronchiolization of the alveoli (Fig. 4*c,d*). These findings were most remarkable since little or no CC10 mRNA expression was detected in histologically normal alveoli (Fig. 1*g*).

Keratin 14 staining in histologically abnormal human lung

Histologic abnormalities in conducting airways. In histologically abnormal conducting airway epithelium, strong keratin 14 immunoreactivity was detected in bronchial basal cells containing areas of basal cell hyperplasia and squamous metaplasia, while immunoreactivity remained unchanged in bronchioli.

Histologic abnormalities in the alveolar region. While normal alveolar cells lacked immunoreactivity for the basal cell marker keratin 14, focal areas of strong keratin 14 immunoreactivity were observed in alveoli containing bronchiolization of the alveoli (Fig. 4*e,f*). The increase in CC10 mRNA expression, along with the appearance of keratin 14 positive cells, suggests that 2 distinct cell types may be associated with regions undergoing hyperplastic growth and squamous metaplasia in the alveolar region of human lung.

Clinicopathologic correlation

Histologic abnormalities and CC10 mRNA expression in various lung compartments were correlated with clinicopathologic features such as smoking history (pack years), sex, age and histologic diagnosis (Tables III–VII). Smoking history was available for 24 of the 26 patients.

To illustrate the correlation of CC10 and smoking history (pack years), we grouped these patients into those who smoked 0–49 pack years, or 50+ pack years. We found no statistically significant correlation between male or female smokers and mean CC10 mRNA levels in any of the 3 lung compartments (Table III).

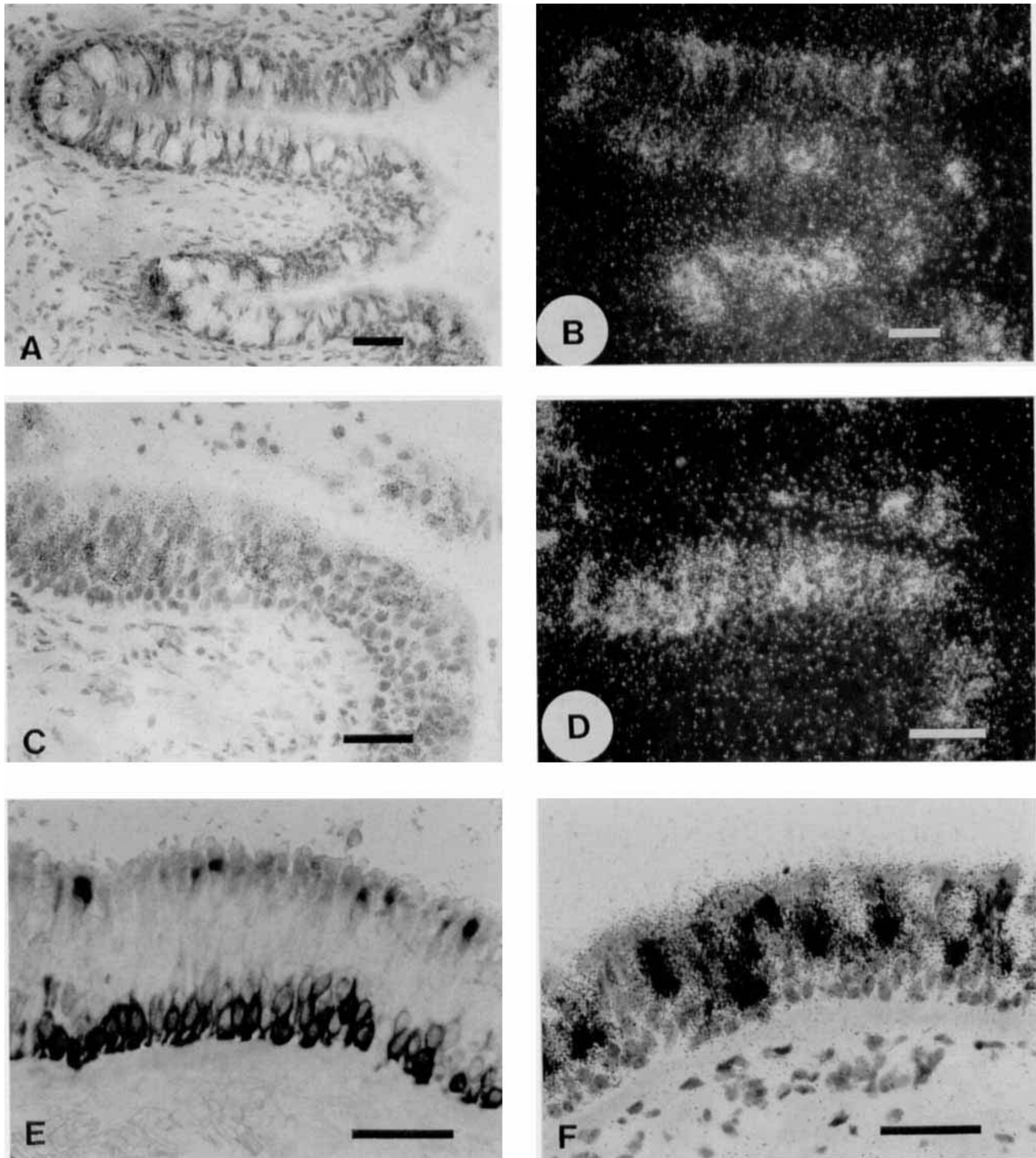

FIGURE 3 – CC10 mRNA expression in abnormal bronchi. *(a)* CC10 mRNA expression in bronchi containing diffuse goblet cell hyperplasia. *(b)* Dark field view of the same. *(c)* Bronchus with epithelial dysplasia. *(d)* Dark field view of the same bronchus hybridized with CC10 mRNA probe. Note decreased level of CC10 mRNA expression. *(e)* A bronchus containing basal cell hyperplasia strongly positive for keratin 14 (immunoperoxidase). *(f)* CC10 mRNA expression in the same bronchus. Note that basal cells are negative, while columnar cells are intensely positive. Bar = 50 μm.

We also compared sex and age of each patient with the expression of CC10 mRNA in each lung compartment. As expected, CC10 mRNA expression was highest in bronchioli. No significant correlation was found with age in either gender (Table IV). Spearman rank correlation similarly revealed no associations with CC10 expression and smoking history or age of patients (Table V).

In addition, we compared the expression of each histologic abnormality with smoking history. A greater than 50 pack year smoking history was associated with basal cell hyperplasia,

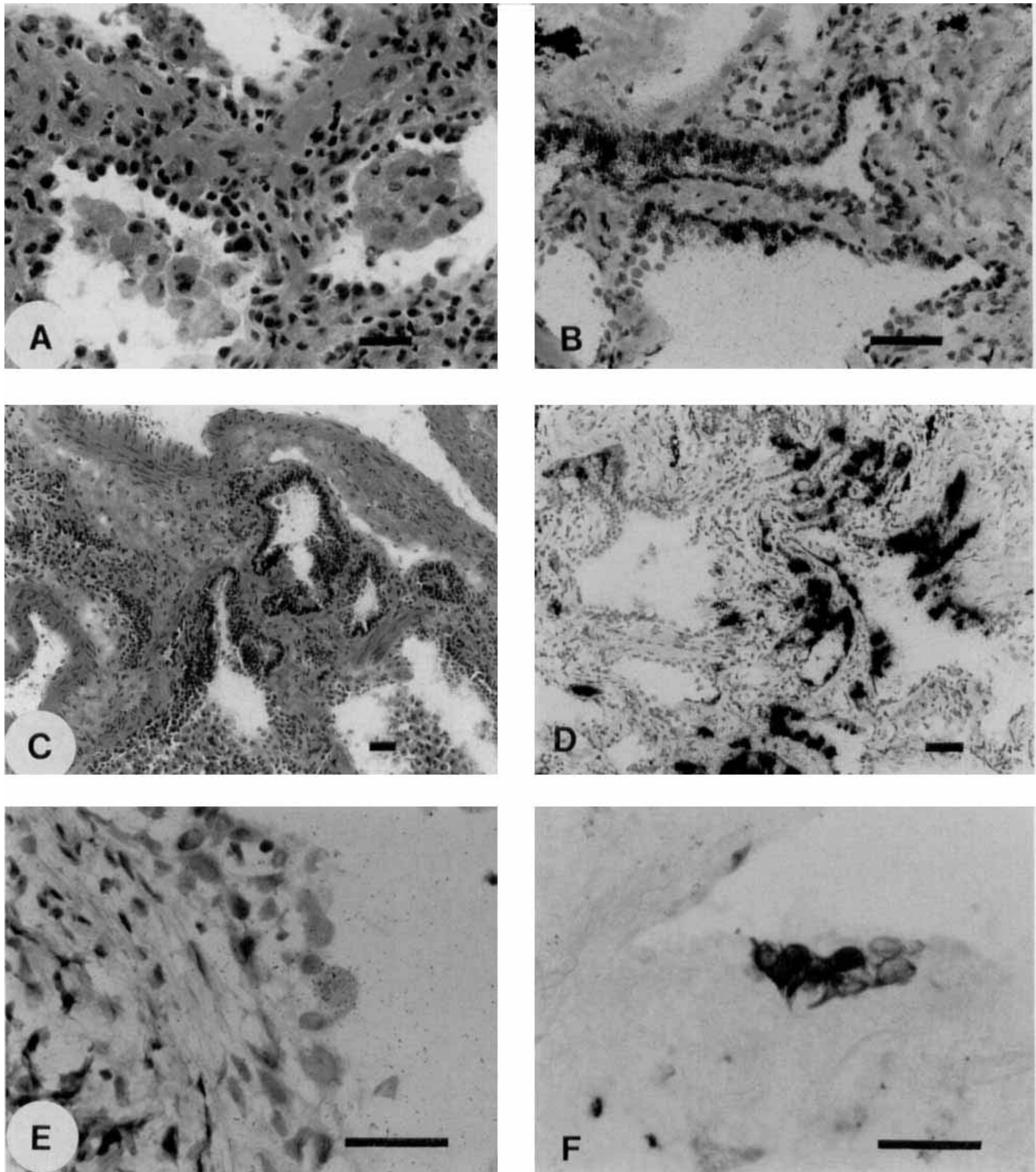

FIGURE 4 – CC10 mRNA expression in abnormal alveoli. *(a)* Type II cell hyperplasia and fibrosis of alveolar walls (H&E). *(b)* Corresponding region demonstrating moderate CC10 mRNA expression. *(c)* Bronchiolization of the alveoli (BOA) with fibrosis (H&E). *(d)* Corresponding region demonstrating strong CC10 mRNA. *(e)* High-power view of CC10 mRNA-positive alveolar cell. *(f)* coexpression of keratin 14-positive cells in the same area. Bar = 50 μm.

goblet cell hyperplasia, squamous metaplasia and dysplasia in the conducting airways, and type II cell hyperplasia and bronchiolization of the alveoli in the alveolar region (Table VI).

Lastly, we investigated CC10 mRNA expression and its possible correlation with histologic subtype of the resected tumor. To simplify the analysis, we combined squamous and adenosquamous histologies into one group (squamous, n = 9). Once again, CC10 mRNA levels were higher in bronchioli than bronchi in all groups; however, we observed no statistically significant correlation between CC10 mRNA expression and histologic subtype (Table VII).

DISCUSSION

Our study has demonstrated the wide range of changes associated with field cancerization in the human lung. We were able to show that smoking history correlated with an increase in histologic abnormalities that were unevenly distributed in the 3 main lung compartments. The least number of changes were observed in bronchioli. Histologic abnormalities were associated with marked changes in CC10 mRNA expression, which is a well-defined product of non-ciliated secretory epithelial cells. In bronchi, CC10 mRNA expression levels decreased in the presence of diffuse goblet cell hyperplasia and squamous metaplasia, while CC10 mRNA levels increased in the alveoli with bronchiolization of the alveoli.

The spectrum of histologic changes that we observed in the conducting airways was in accordance with previously published reports outlining events leading to squamous cell carcinoma (Nasiell, 1963). We also detected some of these changes in lungs from patients with adenocarcinoma. These findings are in agreement with those of Solomon *et al.* (1990), who showed focal areas of basal cell hyperplasia, squamous metaplasia and dysplasia in grossly unremarkable bronchi and bronchioles taken from lobectomy specimens containing primary adenocarcinoma. Histologic abnormalities that we observed in the alveolar region included bronchiolization of the alveoli and squamous metaplasia. These lesions are known to occur after a variety of insults to the lung (respiratory infection, exposure to chemical irritants and carcinogens) (Nettesheim and Szakal, 1972; Fukuda *et al.*, 1989); however, their significance in the development of lung cancer is unknown. The discordant manifestation of histological changes in various lung compartments may be due to the possibility that individual lung compartments respond to carcinogens at different rates. Many of the changes we observed were focal in distribution; therefore without evaluating an extensive number of tissue specimens, many of these lesions are likely to be missed (Slaughter *et al.*, 1953). Consequently, further studies are needed to establish the relationship between histologic abnormalities and the development of non-squamous cell carcinomas of the lung.

We have previously demonstrated that while CC10 mRNA was abundant throughout the conducting airways, expression was detected infrequently and at low levels in lung tumors (Broers *et al.*, 1992). Our current findings demonstrated that alterations in CC10 mRNA levels were associated with regions undergoing a change of cell type. While no change was detected in non-ciliated secretory epithelium with underlying basal cell hyperplasia, altered CC10 mRNA expression was observed in bronchi with diffuse goblet cell hyperplasia and squamous metaplasia and alveolar regions containing bronchiolization of the alveoli. Like the histologic abnormalities, the changes in CC10 may be focal and dependent on lung compartment. It is not clear whether CC10 mRNA levels are changing due to alterations in the pattern of gene expression in a given cell. Other approaches are necessary to elucidate this point further. Our findings of decreased CC10 levels in bronchi are in accordance with the studies that showed decreased CC10 protein expression in serum and bronchoalveolar lavage specimens of smokers and lung cancer patients (Bernard *et al.*, 1992). While a significant decrease in Clara cell number in distal airways of smokers has been described (Lumsden *et al.*, 1984), our results on CC10 expression in bronchioli were inconclusive. The decrease in Clara cell number may be of interest because Clara cells are active in the metabolism of xenobiotics (Boyd and Schuller, 1984).

CC10 protein is one of the major respiratory tract-derived proteins, amounting to 7% of the total protein content of lung lavages obtained from healthy non-smokers (Bernard *et al.*, 1992). While the function of CC10 protein has not been fully determined, it has been shown to inhibit phospholipase A2 (Singh *et al.*, 1990) and bind polychlorinated biphenyls, a major component of industrial pollution (Andersson *et al.*, 1991). Phospholipase A2 inhibition may modulate inflammation and chemotaxis in the human respiratory tract. Previous studies have demonstrated an imbalance of T-cell subsets (Ginns *et al.*, 1982) and antitumor activity of peripheral blood monocytes in sera of lung cancer patients (Mantovani *et al.*, 1979). Anti-inflammatory agents such as aspirin and piroxicam have been implicated in the suppression of neoplastic transformation in the gastrointestinal tract (Marnett, 1992). However, more studies are needed to determine the nature of pulmonary defenses against neoplastic disease. If CC10 does in fact provide a protective effect in the human lung, it is reasonable to assume that reduced CC10 levels may contribute to impaired respiratory tract function and subsequent neoplastic cell growth. The ability to bind polychlorinated biphenyls suggests that CC10 may be important in the clearance of harmful substances deposited within the respiratory tract.

We conclude that: 1) resected lung cancer specimens provide excellent material to study field effects; 2) the process is complex, and changes may be focal or proceed into different directions; 3) altered CC10 mRNA expression may indicate a change in cell type; and 4) more studies are needed to evaluate cancer-specific lesions.

In the future, it will be important to perform the following studies: 1) extensive tissue sampling to study alterations in bronchiolar CC10 expression; 2) determination of normal CC10 expression in various lung compartments from non-smoking subjects; and 3) assessment of CC10 levels as a potential marker for the early detection of lung cancer.

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