

# Atypical Goblet Cell Hyperplasia in Congenital Cystic Adenomatoid Malformation as a Possible Preneoplasia for Pulmonary Adenocarcinoma in Childhood: A Genetic Analysis

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Congenital cystic adenomatoid malformation (CCAM) of the lung is a congenital lesion that is sometimes complicated by bronchioloalveolar adenocarcinoma (BAC). In some cases foci of atypical goblet cell hyperplasia (AGCH) can be found within the cysts. It has been proposed that CCAM and AGCH predispose to the development of BAC. The present study used comparative genomic hybridization (CGH) to screen 22 cases of CCAM (epithelium, surrounding normal lung tissue, and both preneoplastic and neoplastic lesions) for chromosomal imbalances. Of these 22 cases, 10 were CCAM type 1, 10 were type 2, and 2 were type 3. Of the 10 cases of CCAM type 1, 2 were associated with AGCH, 1 was associated with atypical adenomatous hyperplasia (AAH) and associated tubular adenocarcinoma (AC), and 2 were associated with BAC (1 mucinous and 1 predominantly nonmucinous). The present study also involved immunohistochemistry for interleukin (IL)-13, IL-4 receptor- $\alpha$  (IL-4 $\alpha$ ), cytokines involved in the differentiation of goblet cells, and mucin 2 protein (Muc2). Chromosomal aberrations were not detected in the epithelium or the surrounding normal lung tissue, whereas varying

Congenital cystic adenomatoid malformations (CCAMs) are rare cystic lesions arising in the lung that were first grouped into 3 types based on their gross appearance: cystic, intermediate, and solid.<sup>1</sup> Stocker later recommended that they be renamed types 1, 2,

aberrations were found in the neoplastic lesions. The most frequent genomic imbalances observed in both AGCH and the carcinomas were gains in chromosomes 2 and 4. Interestingly, a predominance of gains was also reported in AC of nonsmokers. Chromosomal aberrations in AGCHs arising in CCAMs support their preneoplastic status. Nuclear expression of IL-13, IL-4 $\alpha$ , and Muc2 was detected in AGCH, whereas a cytoplasmic and nuclear reaction was seen in normal epithelium. This likely reflects an association with goblet cell differentiation, but it also drives proliferation in AGCH. *HUM PATHOL* 35:565-570. © 2004 Elsevier Inc. All rights reserved.

**Key words:** congenital cystic adenomatoid malformation, atypical goblet cell hyperplasia, preneoplasia, adenocarcinoma, comparative genomic hybridization.

**Abbreviations:** AAH, atypical adenomatous hyperplasia; AC, adenocarcinoma; AGCH, atypical goblet cell hyperplasia; BAC, bronchioloalveolar adenocarcinoma; CCAM, congenital cystic adenomatoid malformation; CGH, comparative genomic hybridization; DOP-PCR, degenerate oligonucleotide primer polymerase chain reaction.

and 3,<sup>2</sup> and later added types 0 and 4,<sup>3</sup> envisioning that the enlarged spectrum represented malformations of 5 successive groups of airways.

The pathogenesis of CCAMs is uncertain, but it has been suggested that they represent maturation defects at various points during organogenesis and differentiation. They are diagnosed mainly in still-born fetuses or newborn infants, but sometimes are not detected until adulthood.<sup>4</sup> Prenatal ultrasound scanning has also allowed their detection in utero.<sup>5</sup> Rare cases of CCAM show malignant transformation, usually bronchioloalveolar adenocarcinoma (BAC) within a type 1 lesion.<sup>6</sup>

In addition to BAC, type 1 CCAMs demonstrate focal atypical goblet cell hyperplasia (AGCH) in approximately 33% of cases,<sup>7</sup> although the incidence of carcinomatous transformation is less than 1%. However, the only evidence that these lesions represent true malignancies is the rare incidence of metastatic spread.<sup>8</sup> Furthermore, the relationship between AGCH and BAC in terms of carcinogenesis is uncertain.

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**TABLE 1.** Clinical Data for the 5 Patients With Preneoplastic Lesions or Adenocarcinoma

	Age	Sex	Clinical presentation	Follow-up
Patient 1	3 years	M	Cystic lung lesion	Followed-up for 3 years; fd
Patient 2	Newborn	F	Cystic lung lesion	fd
Patient 3	13 years	M	Cystic lung lesion, suspicious tumor	Follow-up for 11 years; fd
Patient 4	29 years	M	Tumor, cystic lung lesion	Follow-up for 2 years; alive, pd
Patient 5	3 years	F	Cystic lung lesion, nodular lesion	Follow-up for 16 years; fd

Abbreviations: fd, free of disease; pd, progressive disease.

Consequently, the primary aim of the present study was to investigate chromosomal aberrations in different types of CCAM, comparing changes in areas of AGCH and BAC with the surrounding cysts and lung parenchyma. In addition, because it is known that interleukin (IL)-13 is up-regulated in inflamed tissue and induces differentiation into goblet cells in animal models,<sup>9</sup> with up-regulation of mucin 2 gene (*Muc2*) via a pathway that involves the IL-4 receptor  $\alpha$  (IL-4R $\alpha$ ),<sup>10</sup> this study also analyzed the expression of IL-13, IL-4R $\alpha$ , and *Muc2* within areas of goblet cell proliferation.

## MATERIAL AND METHODS

### Study Samples

Participants in the European Project on Rare Pulmonary Diseases (funded by the Health & Consumer Protection Directorate General of the European Commission; project 2001/RD/10010) collected 43 cases of CCAM as formalin-fixed, paraffin-embedded tissue blocks. Fourteen of these specimens were CCAM type 1, 25 were CCAM type 2, and 4 were CCAM type 3. Two pathologists independently reviewed the typing of CCAM. No cases of types 0 and 4 were identified. Five cases of CCAM type 1 were associated with an AGCH, 1 case of CCAM type 1 was combined with a multifocal atypical adenomatous hyperplasia (AAH), and 5 cases of CCAM type 1 were associated with AC. Patient age ranged from 27 gestational weeks to 41 years.

Sixteen cases demonstrating either significant inflammation or massive hemorrhage were rejected, including cases with degraded DNA. In 2 cases with ACs and 3 cases with AGCH present in cysts, there was insufficient material for analysis by comparative genomic hybridization (CGH).<sup>11</sup> This left 22 cases for analysis, including 3 cases associated with foci of AC, 1 case containing foci of AAH, and 2 cases with cysts containing foci of AGCH (Table 1).

The tumor samples were examined and classified by an experienced pathologist. Sections for laser microdissection (Palm, Bernried, Germany) were stained with diluted Harris hematoxylin solution (Merck, Darmstadt, Germany).

In each case, epithelium lining the cysts and surrounding normal lung tissue, as well as areas of AGCH and BAC when present, were analyzed separately by CGH. AGCH was diagnosed if mucous cell proliferations were found embedded in the epithelium of the cysts or as small foci in adjacent alveoli. BAC was diagnosed when atypical cells formed single rows of cells on alveolar walls completely replacing the normal epithelium.

### Comparative Genomic Hybridization

Genomic DNA from the tumors was extracted using standard proteinase K digestion. DNA was amplified by degenerate oligonucleotide primer polymerase chain reaction (DOP-PCR) as described previously.<sup>12</sup> In each round of DOP-PCR, a normal control was coamplified and also analyzed using CGH. All hybridizations were performed in a sex-mismatch manner as an internal control. The Quips 3.1 software (Vysis, Downers Grove, IL) was used for CGH analysis. The well-known critical regions for CGH—1p36, 17p, 19p, 22, and the telomeric and centromeric regions—were not analyzed. Only cases where the sex mismatch was clearly identified by CGH were included. For each tumor sample, an average of 10 metaphase spreads was examined. Thresholds were set to 0.8 and 1.2.

### Immunohistochemistry

Anti-IL-13, anti-IL-4R $\alpha$ , and anti-*Muc2* (Santa Cruz Biotechnology, Santa Cruz, CA) were used as the primary antibodies as recommended. The incubation, washing steps, and detection procedures were performed in routine fashion. Microwave pretreatment was applied, with diaminobenzidine (ChemMed; Dako, Glostrup, Denmark) used as a chromogen, yielding a brown reaction product. Nuclei were counterstained slightly with hematoxylin.

## RESULTS

### Comparative Genomic Hybridization

Twenty-two cases of CCAM (10 type 1, 10 type 2, and 2 type 3) were analyzed by CGH. Two cases of

**FIGURE 1.** CCAM type 1 with AGCH inside the cyst (patient 1). (Hematoxylin and eosin; original magnification  $\times 100$ .)

**FIGURE 2.** AGCH within normal lung, adjacent to CCAM cysts (patient 2). (Hematoxylin and eosin; original magnification  $\times 50$ .)

**FIGURE 3.** AAH in normal lung adjacent to a CCAM cyst (patient 3). (A) Overview showing 4 of the AAH foci and also the tubular AC (small arrow), which is attached to the CCAM cyst wall. (B) One of the AAH foci enlarged (large arrow in A). (Hematoxylin and eosin; original magnification: A,  $\times 25$ , B,  $\times 100$ .)

**FIGURE 4.** Mucinous BAC arising outside a CCAM cyst (patient 4). (Hematoxylin and eosin; original magnification  $\times 200$ .)

**FIGURE 5.** Mixed mucinous and nonmucinous BAC adjacent to a CCAM cyst (patient 5). (Hematoxylin and eosin; original magnification  $\times 100$ .)



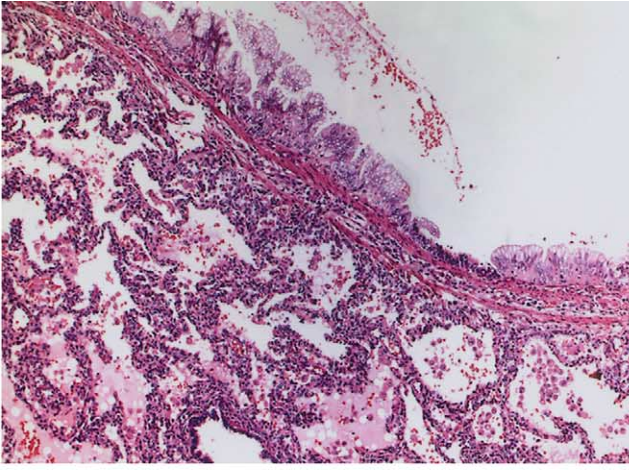


FIGURE 1

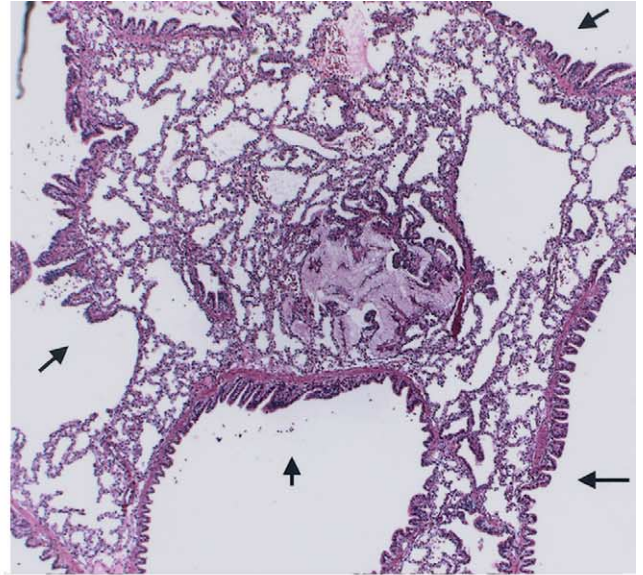


FIGURE 2

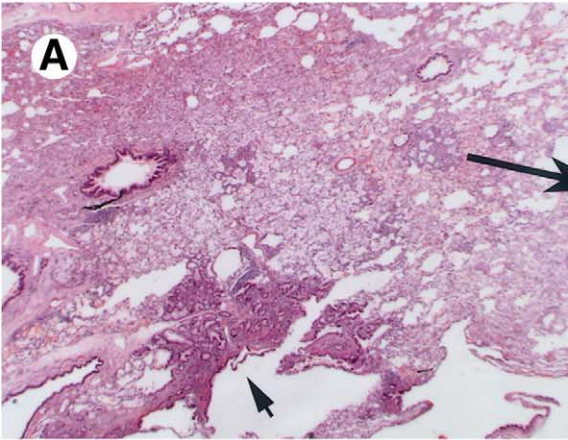


FIGURE 3

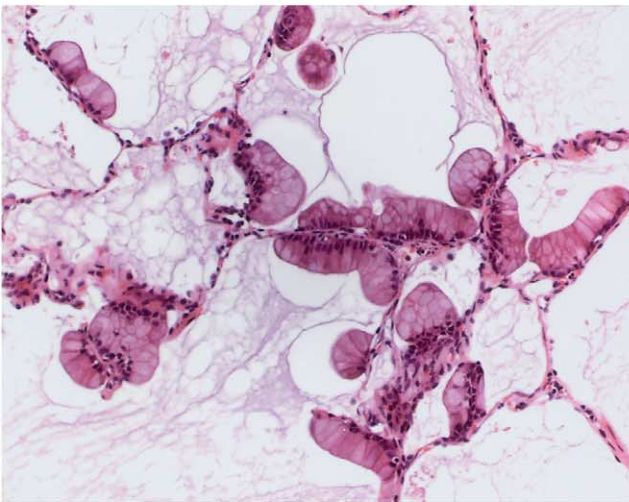
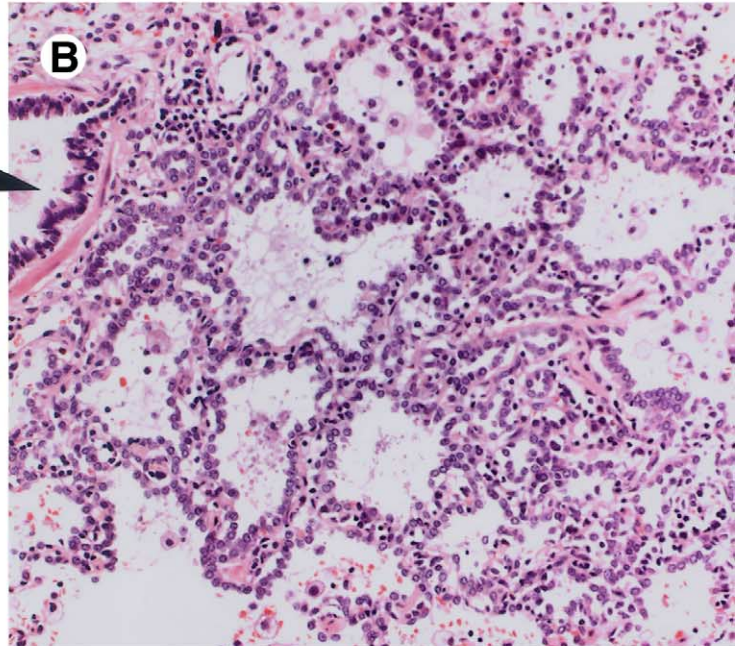


FIGURE 4

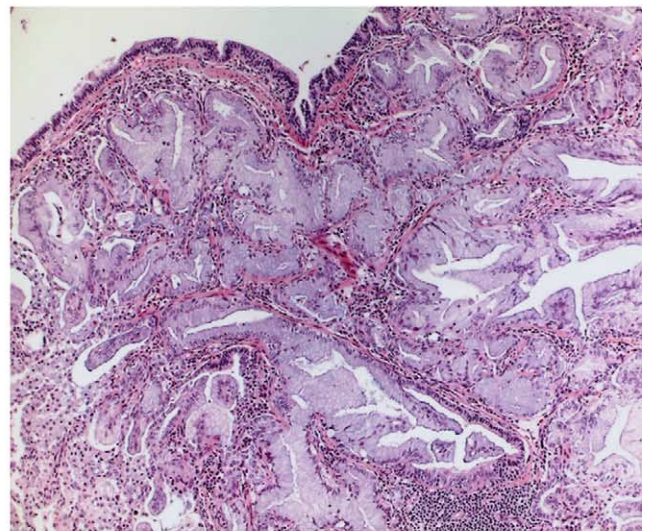
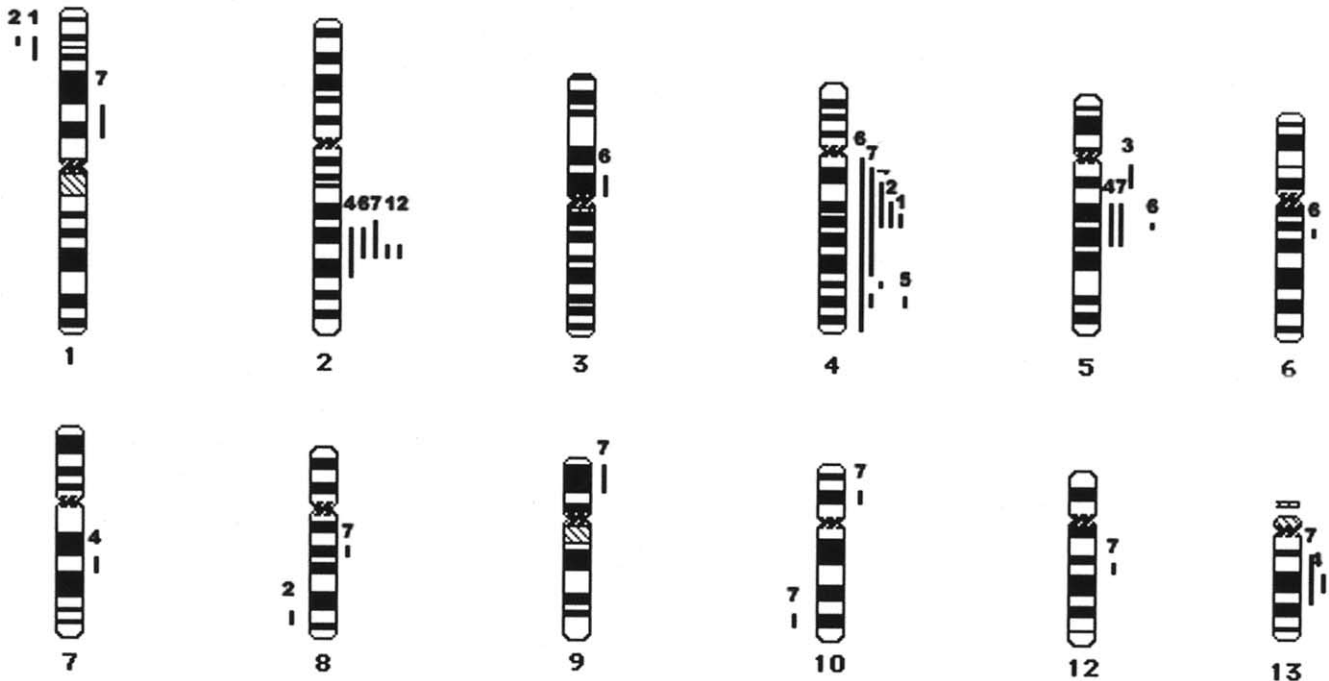


FIGURE 5





**FIGURE 6.** Summary of chromosomal aberrations in AGCH, AAH and AC, with gains on the right side and losses on the left side. 1, AGCH; 2, AGCH; 3, focus 1 of AAH; 4, pooled foci 2 to 5 of AAH; 5, nonmucinous BAC; 6, mucinous BAC; 7, tubular AC.

CCAM type 1 were associated with AGCH (Figs 1 and 2). These lesions were found within the cysts and also extending into the surrounding lung. One case was combined with a multifocal AAH (Fig 3), 1 case was combined with a tubular AC (Fig 4), and 2 cases were combined with a mucinous and nonmucinous BAC (Fig 5).

Chromosomal aberrations were not detected in the epithelium or in the surrounding normal lung tissue. Both cases of AGCH demonstrated aberrations detected by CGH (Fig 6). Four of the 5 AAH foci (all from patient 3) had to be pooled because of their small size. Predominantly gains, but sometimes losses, were found in AGCH, AAH, and AC (Fig 6).

#### Immunohistochemistry

IL-13 exhibited a positive nuclear reaction in the atypical goblet cells and a positive cytoplasmic and nuclear reaction in the adjacent epithelium as well as in infiltrating lymphocytes and plasma cells (Fig 7A and B). In addition, macrophages and pneumocytes demonstrated a positive nuclear and cytoplasmic reaction.

IL-4R $\alpha$  and Muc2 were exclusively positive in the nuclei of atypical goblet cells (Fig 8A), whereas in controls a positive cytoplasmic and nuclear reaction for IL-4R $\alpha$  and Muc2 was seen in pneumocytes and some leukocytes (Fig 8B). Bronchiolar (CCAM) epithelium was negative for Muc2, but demonstrated cytoplasmic and nuclear positivity using IL4R $\alpha$  antibodies.

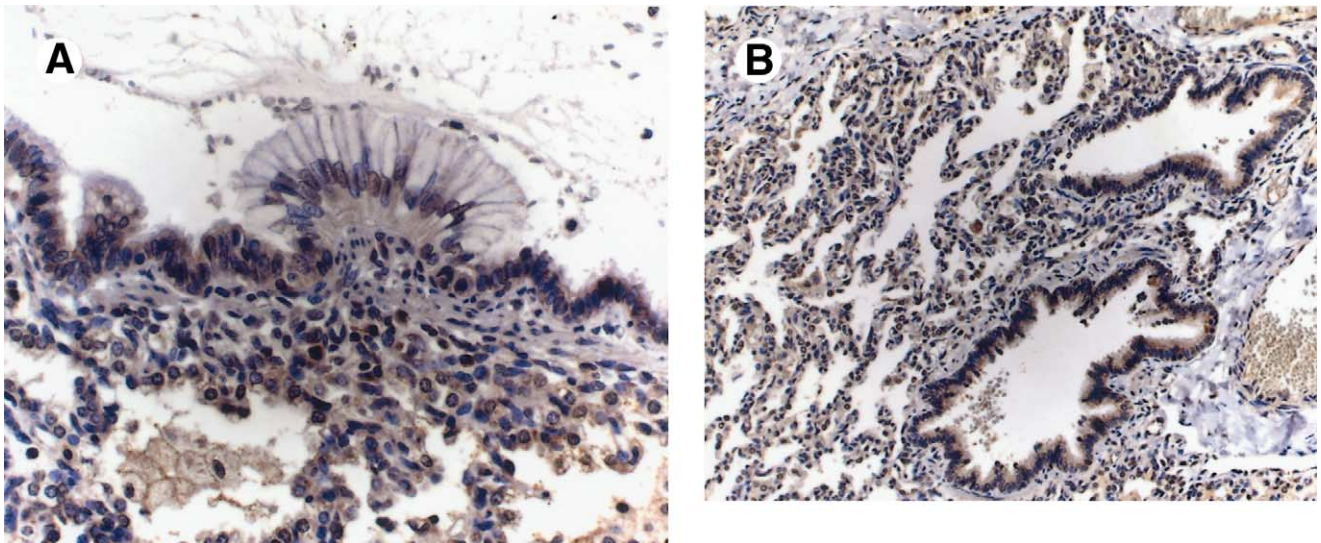
#### DISCUSSION

No chromosomal aberrations were detected in either epithelium lining cysts or surrounding normal tissues. One reason for this might be that CCAM results from regional deregulation of involved genes at a specific time point during organogenesis and differentiation, not by chromosomal imbalances. This process likely produces the different types of CCAM; for example, type 1 arises from cessation of development at the saccular stage. Point mutations and other alterations that cannot be detected by CGH may also account for this abnormal differentiation.

The AGCHs demonstrated loss in chromosome 1 and gains in chromosomes 2 and 4. In addition, 1 case of goblet cell proliferation showed gain in chromosome 5 and loss in chromosome 8.

However, the most frequent genomic imbalances were gains in chromosomes 2 and 4. These are apparently the initial chromosomal imbalances occurring during carcinogenesis. Therefore, despite the small number of cases, our study indicates that AGCH is a likely precursor to ACs arising from CCAMs, because the same aberrations were also detected in the sampled ACs.

It is also interesting that chromosomal gains were more common than losses. In a study on ACs from non-smokers,<sup>13</sup> gains also predominated among the chromosomal aberrations. Another study based on unselected AC cases, supposedly predominantly smokers, found that gains and losses were almost equally present.<sup>14</sup> Thus dif-



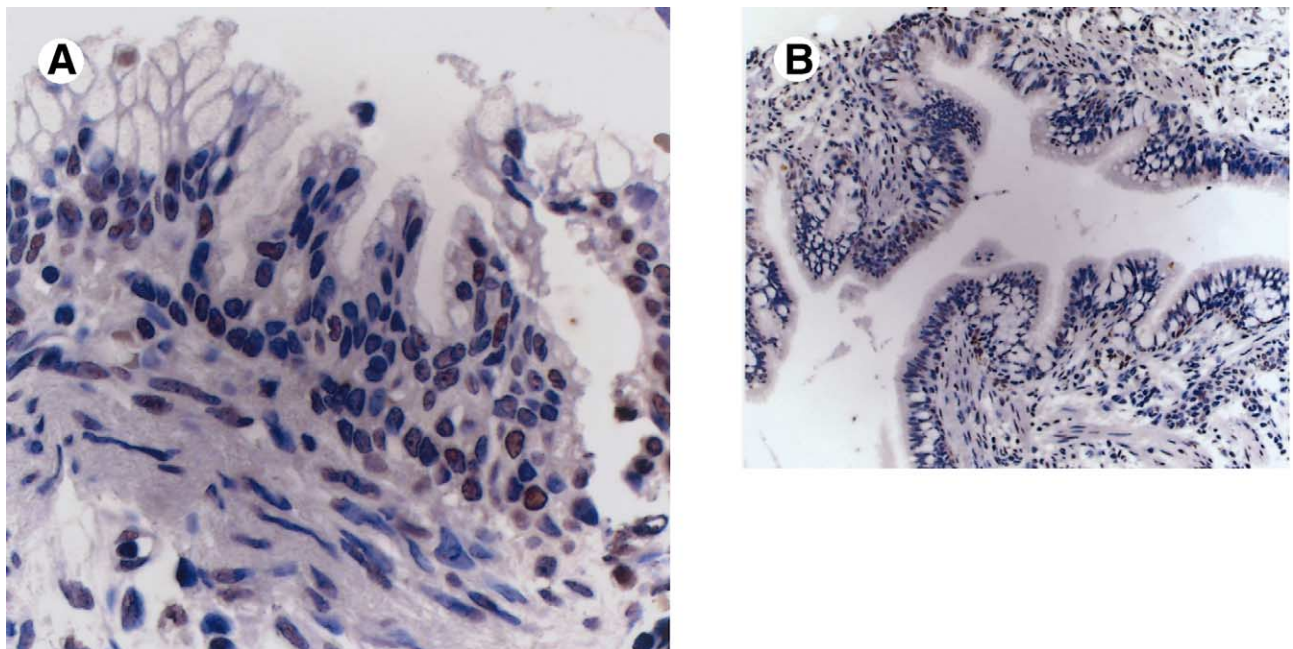
**FIGURE 7.** Immunohistochemistry for IL-13. (A) Exclusive nuclear staining in AGCH, with staining of nuclei and cytoplasm in adjacent normal epithelium as well as inflammatory cells. (B) Normal lung parenchyma outside CCAM, with an intense cytoplasmic as well as nuclear staining for IL-13 antibodies. (Original magnification: A,  $\times 400$ ; B,  $\times 100$ .)

ferent molecular pathways appear to be involved in the development of AC in smokers, nonsmokers, and childhood carcinomas arising in CCAM.

Another interesting finding was the exclusively nuclear staining for IL-4R $\alpha$  and Muc2 stimulated by IL-13. In contrast to internal controls outside CCAM, where IL-4R $\alpha$  and Muc2 could be demonstrated in the cytoplasm, cell membrane, and nucleus, the staining in AGCH was exclusively nuclear. Therefore, nuclear translocation of the IL-4R $\alpha$  and up-regulation of Muc2

not only may be responsible for goblet differentiation, but also may be involved in neoplastic transformation, similar to the role that has been described for epidermal growth factor receptor.<sup>15</sup>

In conclusion, whereas the respiratory-type surface epithelium in CCAMs does not demonstrate chromosomal aberrations detectable by CGH, these aberrations are present in putative precursor lesions (AGCH and AAH). Common genetic aberrations of putative precursor lesions and fully developed ACs are gains on chro-



**FIGURE 8.** Nuclear staining for Muc2 protein in AGCH (A), and focal cytoplasmic and nuclear staining in normal bronchial epithelium and stroma cells (B). (Original magnification: A,  $\times 400$ ; B,  $\times 200$ .)

mosomes 2 and 4. This again stresses the importance of complete resection of the cysts.

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