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# Mucoepidermoid tumors of the bronchus. Ultrastructural and immunohistochemical study. Histiogenic correlations

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**Summary.** Bronchial mucoepidermoid tumors are uncommon neoplasms, morphologically similar to their salivary gland counterpart. The histogenesis is controversial. The aim of this study is to identify myoepithelial cells and speculate on their role in the origin of these tumors. Methods and Results: Sixteen bronchial mucoepidermoid tumor surgical specimens were formalin-fixed, paraffin-embedded and studied using a panel of nine antibodies in order to identify a myoepithelial differentiation. Additional antigens against several cytokeratins were performed in four cases and five of the biopies were studied using the electron microscopy. The different types of cells of the primary bronchial mucoepidermoid tumor (mucous luminal, intermediate and squamous) reacted strongly against AE1, CK7, 34bE12 and weakly with AE3, CK18 and CK8/18/19. S-100, α-smooth muscle actin, muscle actin HHF35 and α-actinin were consistently negative in all cell types. CD10 was positive in very few cells in just one case. Conclusion: The immunohistochemical and the ultrastructural study of bronchial mucoepidermiod tumors support a ductal unit origin, without a myoepithelial participation.

**Key words:** Mucoepidermoid, Immunohistochemical, Ultrastructural, Bronchial glands

## Introduction

Mucoepidermoid lung tumors of the trachea and bronchi are uncommon neoplasms, previously included under the term bronchial adenoma (Heitmiller et al., 1989). This tumor arises in the submucosal bronchial glands, which are identical to the mixed serous and mucous glands of the upper airway and similar to the major salivary glands (Klacsmann et al., 1979; Spencer, 1979). The histologic features are similar to those tumors initially described in 1945 by Stewart et al. in major salivary glands, and reported by Klacsmann et al. (1979) in bronchi. They are characterized by well-formed glandular spaces with a single layer of mucin-producing cells, papillary and tubular structures with mucin pools, and squamoid or intermediate cells.

Previous histochemical and ultrastructural studies on salivary tumors suggest that this tumor arises in the excretory duct (Eversole, 1971; Klacsmann et al., 1979; Stewart et al., 1945). There is just one study performed on bronchial mucoepidermoid tumors and it suggests the same origin for these tumors (Spencer, 1979), however, some authors argue a myoepithelial participation in the origin of the mucoepidermoid lung tumor (Dardick et al., 1984; Nikai et al., 1986).

The present report is based on the immuno-histochemical study of sixteen cases coming from a single institution. Ultrastructural study was performed in five of these surgical specimens. The results were compared to those previously reported by us in normal bronchial glands (Sanchez-Mora et al., 2005), in order to clarify the histogenesis of these tumors.

## Materials and methods

Sixteen surgically resected mucoepidermoid bronchial tumors were included in this study. They were obtained from the files of the Department of Pathology of the Gregorio Marañon Hospital between 1991-2003. The tumors were classified and graded according to the Klacsmann et al. criteria (Klacsmann et al., 1979). Demographic information was obtained from clinical records.

The results were compared to previously reported immunohistochemical profile by us of normal bronchial glands.

All specimens were fixed in 10% neutral tamponated formalin, paraffin-embedded and processed in a routine

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way. 4 µm sections were cut from the specimens and mounted on glass slides. The specimens were deparaffinized in xylene and rehydrated step by step with diminishing concentrations of ethanol. The sections were incubated at 37°C with 0.3% $H_2O_2$ in absolute methanol for 10 min, to block endogenous peroxidase. Then they were washed with phosphate-buffered saline (PBS) at pH 7.2 for 20 min. They were incubated with the primary antibodies for 45 min in a moist chamber at room temperature. Primary antibodies used were: AE1 (Biomeda, pre-diluted 1:2), AE3 (Biomeda, pre-diluted 1:2), 34bE12 (Dako, diluted 1:2), α-smooth muscle actin (Enzo, pre-diluted 1:2), smooth muscle actin HHF35 (Enzo, pre-diluted 1:2), α-actinin (Novocastra, diluted 1:60), CD10 (Novocastra, diluted 1:30), S-100 (Dako, diluted 1:1500), Glial Fibrillary Acidic Protein (GFAP) (Dako, dilution 1:1000), p53 (Novocastra, diluted 1:100) and Ki67 (Dako, diluted 1:200). In four cases the panel of cytokeratins was amplified including: CK7 (Novocastra, diluted 1:50), CK8 (Novocastra, diluted 1:100), CK10 (Biogenex, pre-diluted), CK18 (Novocastra, diluted 1:40), CK19 (Novocastra, diluted 1:150), CK20 (Novocastra, diluted 1:50), Cytokeratins 8/18/19 (Biogenex, pre-diluted 1:2.5). The sections were subsequently incubated with biotinylated anti-mouse and anti-rabbit Ig G and LBA (from DAKO) for 25 min at room temperature and then rinsed in PBS for 5 min, and then were immersed in avidin peroxidase complex for 25 min. Finally the peroxidase was localized by treatment of the sections with a fresh mixture of diaminobenzidine and sustrate in 10 min. After being washed with distilled water, the sections were lightly counterstained with haematoxylin, dehydrated in ethanol, cleared in xylene and cover slipped using Permount.

Tissue for electron microscopy was fixed in 2.5% glutaraldehyde, postfixed in 1.5% osmium tetroxide and dehydrated in graded ethyl alcohols. Then it was embedded in preinclusion resine. Thick sections cut at 1 micron were stained with toluidine blue. At least four blocks were examined from each case and the most representative block was used for electron microscopic examination. Silver-gold sections (800Å) were stained with uranyl acetate and lead citrate and examined with electron microscope (Jeol Jem- 100SX).

## Results

Eight patients (50%) were male and the other eight were female. Average age was 55.25 years (range: 20-77 years; DE=18.04 years). Lobectomy was performed in eleven cases (68.8%), bilobectomy in one case and tumorectomy in two cases. In two cases, surgical treatment was contraindication. Adjunctive radiation or chemotherapy was administered in three cases. Tumor size ranged from 1-9 cm in its greatest dimension (mean= 3.3 cm; median= 2.75 cm). Sections revealed a mixed solid and cystic tumor. Mucoepidermoid tumors were classified according to Klacsmann grading criteria (Klacsmann et al., 1979) as eleven low grade cases (68.8%) and five high grade cases (31.3%). Three patients (18.8%) showed lymph node metastases at the time of surgery.

Follow up data was available in all cases except one, follow-up ranged from one to one hundred and fifty six months. Eight patients were alive without evidence of disease at follow-up. Evidence of recurrent disease was found in four patients after a range of 6 to 45 months (mean=40.25 months). Three patients died at 1-57 months (mean=16.2 months after surgery). One patient died one month after surgery of causes unrelated with the tumor.

### *Histological features*

Microscopically, mucoepidermoid bronchial tumors were characterized by both solid and glandular components with cystic spaces filled with pools of mucous. The low grade tumors had a dominant glandular component, lined by tall columnar or goblet mucous cells (Fig. 1b). The extracellular mucin had a basophilic wispy appearance and stained positively with PAS. Areas of solid growth were composed of squamoid and transitional cells and they were the dominant component of the high grade tumors. The former cells were polygonal with a homogenous eosinophilic cytoplasm (Fig. 1a). Some intercellular bridges were observed. The mitotic activity ranged from none to 10 mitosis per ten high-power fields. Some tumors showed a variable amount of necrosis.

### *Immunohistochemical results*

All tumor cell types were strongly stained with monoclonal antibodies anticytokeratins AE1, CK7 and weakly with AE3, CK18, CK18, and CK8/18/19. The intermediate cells were strongly stained with monoclonal antibodies anticytokeratins CK34bE12 whereas the luminal cells were negative. None of these cells reacted against CK10, CK20, α-smooth muscle actin, HHF35 muscle actin, α-actinin, GFAP and S-100. CD10 was positive in just isolated cells in one case (Table 1 and Figs. 3, 4).

68.8% of cases (11/16) showed Ki67 antigen staining of less than 10%. All high grade tumors (5/16) showed a percentage stained cells of more than 25%. 31.35% of low grade mucoepidermoid tumors showed no nuclear p53 expression and 37.5% showed an expression of less than 25% of the tumoral cells. All high grade mucoepidermoid tumors stained for p53 and 18.75% of them showed an expression of more than 25% of the tumoral cells. These results showed a statistically significant association between high grade bronchial mucoepidermoid tumors and Ki67 and p53 nuclear expression greater than 25% in the tumoral cells ($p=0.0002$; $p=0.01$ respectively) (Table 2).

All internal controls were positive for all the antigens (normal bronchial gland epithelium for the cytokeratins, vascular smooth muscle for both actins,

erythrocyte membrane for actinin and nervous fibbers for S-100).

*Electron microscopy*

Five cases were examined with the electronic microscope. The relative number of each cell type in the low and high grade tumors varied, but the ultrastructure for this different cell types was similar. All the lesions revealed classic goblet cells with abundant mucus droplets (Fig. 2a). In addition, there were other cells with abundant mitochondria, glycogen and microvilli. There

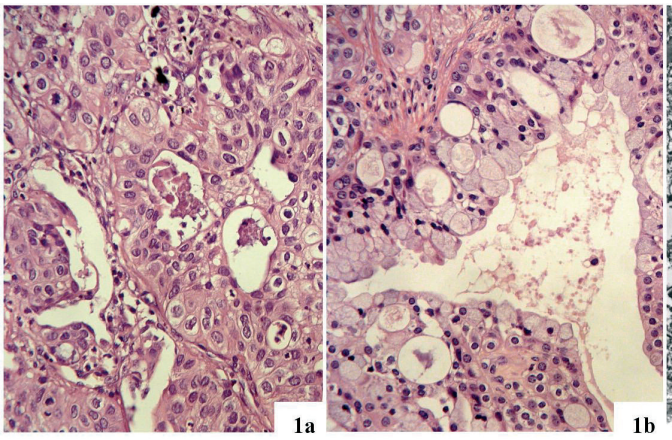



Figure 1

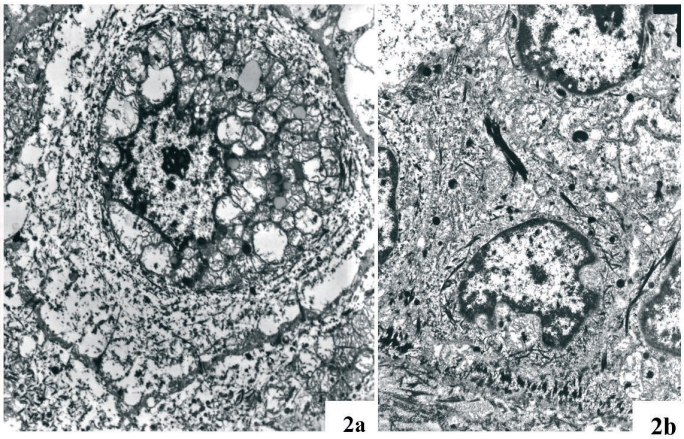
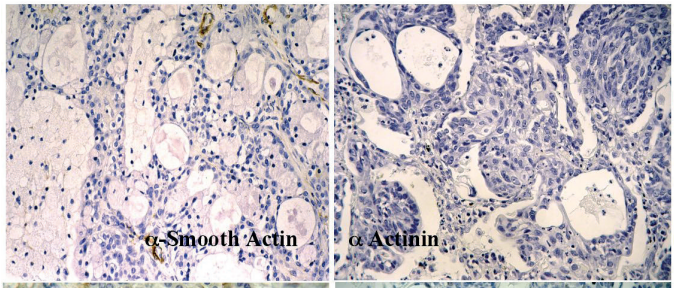



Figure 2



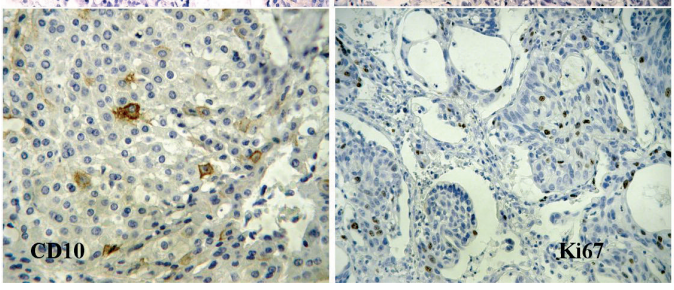
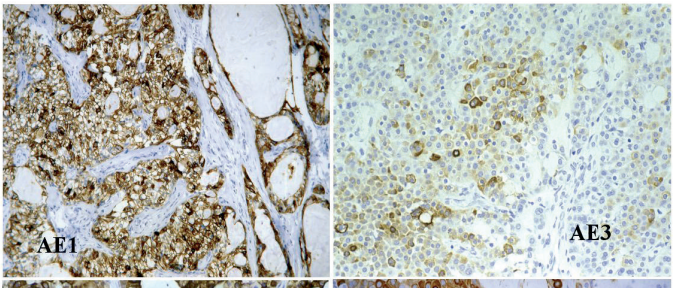




Figure 3



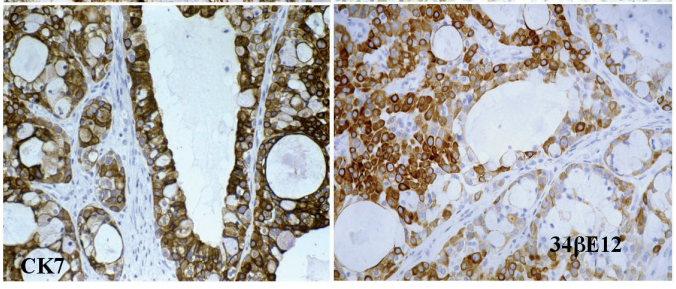




Figure 4

**Fig. 1.** Both high and low grade mucoepidernoid tumors showed solid and glandular components with cystic spaces filled with pools of mucin. **a.** Dominant glandular component, lined by goblet mucous cells in low grade tumors (H&E stain, x 20). **b.** Solid growth areas composed of transitional cells were the dominant component in high grade tumors (H&E stain, x 20).

**Fig. 2. a.** Electron photomicrograph showed classic goblet cells with abundant mucus droplets (double stain, x 4000). **b.** Electron photomicrograph showed transitional cells with nuclear indentations, a varied amount of mucus, bundles of tonofilaments, and desmosomes between cells (double stain, x 6000).

**Fig. 3.** Immunostaining for α-smooth actin and α-actinin were negative in all tumoral cell types (x 10). Immunostaining for CD10 was positive in just isolated cells in one case (x 20). Immunostaining for Ki67 showed a variable nuclear expression (x 10).

**Fig. 4.** Immunostaining for AE1 and CK7 was strongly positive in all cell types (x 20), whereas AE3 was weakly positive (x 20). Immunostaining for CK34ßE12 showed strongly stained intermediate cells, whereas luminal cells were negative (x 20).

**Table1.** Antigenic profile of bronchial mucoepidermoid tumor. Reactivity of antibody.

| TYPES OF CELLS | AE1 | AE3 | CK7 | CK8 | CK10 | CK18 | CK19 | CK20 |
|---|---|---|---|---|---|---|---|---|
| Luminal mucous cells | + | + | + | - | - | + | + | - |
| Luminal non-mucous cells | + | + | + | + | - | + | + | - |
| Intermediate cells | + | + | + | + | - | + | + | - |
| Squamous differentiated cells | + | + | + | + | - | + | + | - |
| TYPES OF CELLS | CK 8/18/19 | 34ßE12 | α-SMA actin | HHF35 actin | α-actinin | CD10 | S100 | GFAP |
| Luminal mucous cells | + | - | - | - | - | - | - | - |
| Luminal non-mucous cells | + | - | - | - | - | - | - | - |
| Intermediate cells | + | + | - | - | - | +* | - | - |
| Squamous differentiated cells | + | + | - | - | - | - | - | - |

* CD10 was focally positive in just one case.

**Table 2.** Expression ki67 and p53 in mucoepidermoid tumors.

| MUCUEPIDERMOID TUMOR | Ki67 - or <25% | Ki67>25% | p53 - or <25% | p53>25% |
|---|---|---|---|---|
| Low Grade | 11(68.8%) | 0 | 11(68.8%) | 0 |
| High Grade | 0 | 5(31.25%)* | 2(12.5%) | 3(18.75%)º |
| Total | 11(68.8%) | 5(31.25%) | 13(81.25%) | 3(18.75%) |

*p=0.0002, ºp=0.0.01

were also undifferentiated cells, which had large ovoid nucleus with indentations and absence of cytoplasmic distinguishing features. The transitional cells were similar to the undifferentiated cells but with a varied amount of mucus. Finally, we have also observed squamous cells with bundles of tonofilaments, desmosomes between cells and hemidesmosomes between the basal lamina (Fig. 2b). Myoepithelial cells were not identified, as we were not able to observe myofilaments in any of the cell types of the five cases examined.

## Discussion

The first case of mucoepidermoid tumor of the bronchus was reported by Smetana et al. in 1952. Mucoepidermoid tumors of the bronchial tree represent a minor subset of the broad group of "bronchial adenomas" and comprises less than 5% of all pulmonary neoplasms (Wilkins et al., 1963; Yousem et al., 1987). They are morphologically and clinically similar to mucoepidermoid tumors of the salivary glands (Klacsmann et al., 1979; Spencer, 1979).

The histogenesis of this tumor is still controversial. Most studies support a excretory duct cell origin (Eversole, 1971; Klacsmann et al., 1979). However, Dardick et al. (1984) suggested that both duct-luminal epithelial and myoepithelial cells are involved. Finally, the tumor originate from the surface bronchial epithelium (Sniffen et al., 1958; Guillou et al., 1994). These studies have been based mainly in histochemical and ultrastructural findings.

Identification of the myoepithelial cell is therefore the fundamental step in understanding the formation of bronchial mucoepidermoid tumors. These cells have structural features of both epithelial and smooth muscle cells. They are fusiform or stellate and are located between the basement membrane and the epithelial layer of the bronchial duct and acinus and they are not easily seen by routine haematoxylin eosin stain.

We have studied sixteen bronchial tumors using various immunohistochemical antibodies. This is advantageous, because it allows recognition of cell types that may not be easily seen by routine light microscopy and also allows the detection of antigens to speculate on the histogenesis of the tumor. There are many different types of antigens traditionally used as myoepithelial markers. The first type includes smooth muscle actin (Tsukada et al., 1987; Hirano et al., 1990; Ogawa et al., 2000; Ogawa, 2003), which is the most specific for this purpose. In a less specific way, S100 (Dardick et al., 1991), 34bE12 (Hirano et al., 1990), CD10 (Gusterson et al., 1986; Moritani et al., 2002) GFAP and actinin (Glukhova et al., 1995) have been also interpreted as myoepithelial cells markers. The markers used in this study for myoepithelial cells -α-smooth muscle actin, HHF35 actin, α-actinin, S-100 and GFAP were negative. CD 10 was focally positive in just one case, but the

positivism of only one antibody against myoepithelial cells should not be considered diagnostic, because it is not specific and it should always be evaluated in addition to the other markers (Moritani et al., 2002).

All different types of tumoral cells (mucous luminal, intermediate and squamous) showed a strong immunohistochemical stain with monoclonal antibodies against various types of the cytokeratins (AE1, AE3, CK7, CK8, CK18, CK19) that are characteristic of ductal cells of normal bronchial glands by us previously reported (Sanchez-Mora et al., 2005).

The ultrastructural myoepithelial features include the identification of numerous microfilaments that frequently show aggregation into dense, dark bodies that resemble the contractile elements of smooth muscle cells, and indeed, they do contain actin and myosin (Barsky et al., 1983; Dardick et al., 1990; Takai et al., 1994).

Our ultrastructural and immunohistochemical findings suggest a tumor arising in the duct of the submucosal bronchial gland, with lack of participation of myoepithelial cells. The mucoepidermoid tumor shows a variety of epithelial cell types from which myoepithelial filaments are absent.

In conclusion, the antigenic profile of bronchial mucoepidermoid tumors is similar to that previously reported by us in the duct of submucosal bronchial glands. These tumoral cells showed an immunohistochemical profile of epithelial differentiation and lack myoepithelial features. The ultrastructural study supports these findings.

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