

Antigenic profile of human bronchial gland

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Summary. Bronchial glands have been regarded as modified salivary glands. It is well known that there no previous reviews concerning the antigenic profile of the bronchial wall. The aim of this study is a systematic survey of the antigenic profile and to describe the histology of normal human bronchial glands. Six formalin-fixed, paraffin-embedded surgical specimens were studied using a panel of 22 polyclonal and monoclonal antibodies by the avidin-biotin-peroxidase method. Bronchial glands disclosed a tubuloacinar structure. The smallest ducts intercalated originated from a cluster of secretory acini and converge to form an excretory duct. No striated duct was observed. Acinar united is composed by mucous, serous and mixed units. Myoepithelial cells are found in relation to the intercalated ducts and secretory acinis. Secretory cells of bronchial glands reacted strongly with cytokeratin AE1 and moderately for CK7, CK18. Additionally, serous acinar cells reacted with AE3, CK19, CK5/6/8/18, CK8/18/19, and Leu7. Myoepithelial cells reacted strongly with α -smooth muscle actin, CD10 and CK34 β E12. Ductal system cells differed from acinar secretory cells in expressing CK34 β E12 and HSP27. In conclusion, the detailed knowledge of the immunohistochemical reactivities of normal cell types of normal human bronchial glands will prove useful in studies of bronchial pathology, especially of neoplastic processes.

Key words: Immunohistochemical, Bronchial glands

Introduction

The trachea and bronchial tree is endowed with a rich supply of submucosal mixed, serous and mucous glands, identical to those of the upper airways. Bronchial glands are an endodermal derivative with a very similar histological structure to salivary glands (Marchevsky, 1994). Morphologically, the mature bronchial glands closely resemble mixed salivary glands, although their

ductal system is not as structurally complex as that of the salivary glands. Although the histological structure and immunohistochemical profile of salivary tissues (Geiger et al., 1987; Morinaga et al., 1987; Burns et al., 1988; Gugliotta et al., 1988; Gustafsson et al., 1988) is well known no previous report has been found addressing both topics in human bronchial glands. We considered that the knowledge of the immunohistochemical profile of normal cells types of human bronchial glands would be useful in studies of bronchial pathology, especially to understand the histogenesis of some bronchial neoplasias as mucoepidermoid tumors.

The aim of the present work is to study the expression of intermediate filaments as well as CD10 and S-100 proteins, in human normal bronchial glands.

Materials and methods

Six formalin-fixed, paraffin-embedded bronchial samples obtained from surgical lobectomy specimens were used. Glands were considered as normal when no inflammation or hyperplasia were present. All cases showed a Reid index below 0.25 mm in spite of the fact that the specimens come from patients who smoked.

Sections 4 μ m thick, were cut up and mounted on glass slides. For immunohistochemical techniques the specimens were deparaffinized in xylene and rehydrated step by step with descending concentrations of ethanol. The sections were incubated at 37°C with 0.3% H₂O₂ in absolute methanol for 10 min, to block endogenous peroxidase. After washing with phosphate-buffered saline (PBS), for pH 7.2 for 20 min. they were incubated with primary antibodies for 45 min in a moist chamber at room temperature. Primary antibodies used were: anti-S-100 (Dako, diluted 1:1500), anti-desmin (Dako, pre-diluted 1:2), anti-chromogranin A (Dako, pre-diluted 1:2), anti-synaptophysin (Biomed, pre-diluted 1:5), anti-Leu-7 (Becton Dickonson, pre-dilution 1:20), anti-HSP27 (Biogenex, pre-dilution 1:2), anti-HSP70 (Biogenex pre-dilution 1:2), anti- α -smooth muscle actin (Enzo, pre-dilution 1:2), anti-CD31 (Novocastra, dilution 1:30), anti-CD34 (Biogenex, pre-dilution), anti-34 β E12 (Dako, dilution 1:2), anti-AE1 (Biomed, pre-dilution 1:2), anti-AE3 (Biomed, pre-dilution 1:2), anti-CK7 (Novocastra, dilution 1:50), anti-CK8 (Novocastra,

dilution 1:100), anti-CK10 (Biogenex, pre-dilution, anti-CK18 (Novocastra, dilution 1:40), anti-CK19 (Novocastra, dilution 1:150), anti-CK20 (Novocastra, dilution 1:50), anti-Citokeratins 5/6/8/18 (Novocastra, dilution 1:100), anti-Citokeratins 8/18/19 (Biogenex, pre-dilution 1:2.5), Ki67 (Dako, dilution 1:200), anti-Glial Fibrillary Acidic Protein (GFAP) (Dako, dilution 1:1000), and anti-CD10 (Novocastra, dilution 1:30). (Table 1)

The sections were subsequently incubated with biotinylated anti-mouse and anti-rabbit Ig G and LBA (DAKO) for 25 min at room temperature, rinsed in PBS for 5 min, and immersed in avidin peroxidase complex for 25 min. Finally the peroxidase was localized immersing the samples into a fresh mixture of diaminobenzidine and substrate for 10 min. After washing in distilled water, the sections were lightly counterstained with hematoxylin, dehydrated in ethanol, cleared in xylene and coverslipped using Permount.

Stained sections were evaluated by two different observers at two different times and a diagnostic consensus was obtained.

Results

Histology of the bronchial gland

Bronchial glands show a tubuloracinar structure, with mucous, serous and mixed acinar units that converge to an intercalated duct. However some terminal units drain directly into the excretory ducts without connection with the intercalated duct (Fig. 1). The ductal

system is constituted by two different levels: intercalated ducts that converge and form the excretory duct.

The smallest ducts originate from a cluster of secretory acini. These ducts are lined by a adluminal layer of cuboidal cells which rest on a discontinuous basal layer of triangular cells. The intercalated ducts join together to form a excretory duct. This shows a pseudostratified epithelium of columnar ciliated cells, which are intermingled with small basal cells and goblet cells. No striated duct is observed.

The acinar units show two different end pieces. The first one consists of groups of only mucous or serous cells. The second one is composed of a mixed cell population with groups of mucous cells near the intercalated duct and a peripheral crescent-shaped nest of serous cells. The units are surrounded by a basement membrane that encircles the entire structure.

Myoepithelial cells are demonstrable from the intercalated duct level down to the terminal secretory end pieces. These cells occupy the space between the acinar basement membrane and the basal aspect of the secretory cells.

Secretory acini and ducts are surrounded by connective tissue, containing fibroblasts, small nerves, and a variable number of lymphoid cells.

Antigenic Profile of Bronchial Gland: (results are summarized in table 2 and figure 2).

Acini

Secretory Cells. Secretory mucinous cells show a

Table 1. Reagents used in the immunohistochemical characterization.

ANTIBODY	SOURCE	DILUTION
S-100	Dako	1:1500
Desmin	Dako	1:2
Chromogranin A	Dako	Pre-diluted 1:2
Synaptophysin	Biomedica	Pre-diluted 1:5
Leu-7	Becton Dickonson	Prediluted 1:20
α -smooth muscle actin	Enzo	Pre-diluted 1:2
CD31	Novocastra	1:30
CD34	Biogenex	Pre-diluted
34 β E12(68;58;56,5;50kD)	Dako	1:2
AE1(acidic keratins: 40; 48;50;50';56,5 kD)	Biomedica	Pre-diluted 1:2
AE3(basic keratins: 65 to 67;64;59;58;56;52 kD)	Biomedica	Pre-diluted 1:2
CK7(54kD)	Novocastra	1:50
CK8(52,5kD)	Novocastra	1:100
CK10(56,5kD)	Biogenex	Pre-diluted
CK18(45kD)	Novocastra	1:40
CK19(40kD)	Novocastra	1:150
CK20(46kD)	Novocastra	1:50
CK5/6/8/18(58kD;56kD;52.5kD;45kD)	Novocastra	1:100
CK8/18/19(52.5kD;45k;40kD)	Biogenex	Pre-diluted 1:2,5
Ki67	Dako	1:200
GFAP	Dako	1:1000
CD10	Novocastra	1:30
HSP27	Biogenex	Pre-diluted
HDP70	Biogenex	Pre-diluted

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positive staining for AE1, Leu-7, and HSP 70. Immunostaining was located in the apical and lateral dominion. Secretory serous cells reacted with AE1, AE3, CK7, CK18, CK19, CK5/6/8/18, CK8/18/19, Leu-7, and HSP70.

Myoepithelial Cells. Myoepithelial cells stained for cytokeratins AE1, AE3, CK7, and 34βE12. All the other cytokeratin antibodies were negative. Myoepithelial cells were strongly immunoreactive for α-smooth muscle actin, CD10, HSP27, and HSP70. Only one specimen (16,6%) showed positive staining for S-100 protein in myoepithelial cells. Neither myoepithelial nor secretory cells reacted positively for desmin, chromogranin, or synaptophysin.

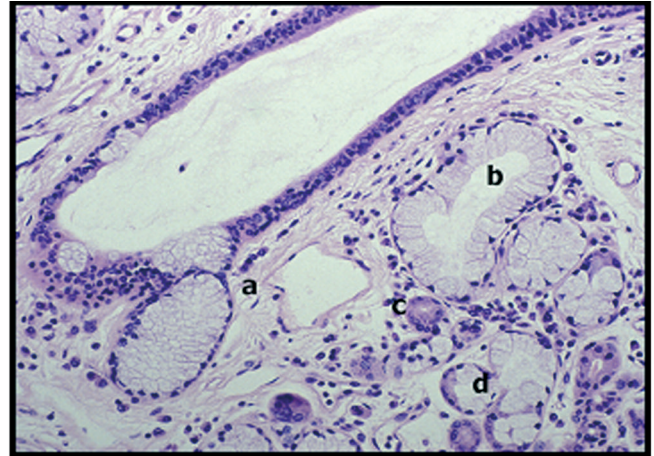


Fig. 1. Histology of the bronchial gland. Acini drainage directly into excretory duct (a), mucous acini (b), serous acini (c), mixed acini (d). (x 20)

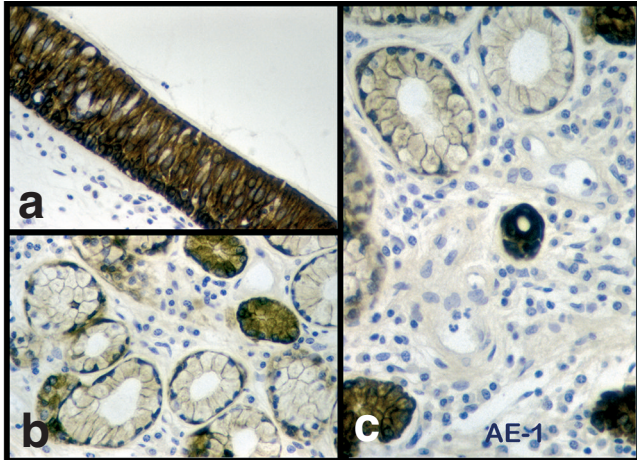


Fig. 2. AE1 stains either basal and apical cells in respiratory epithelium, as well as intercalated duct, acinis and myoepithelial cells. a, b, x 20; c, x 40

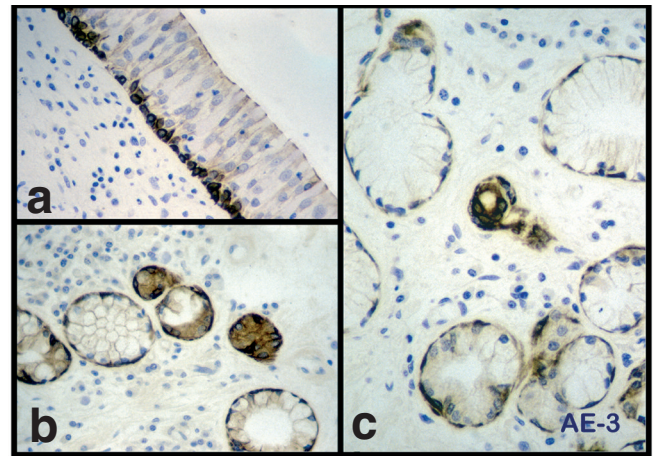


Fig. 3. AE3 stains basal cells in respiratory epithelium, intercalated duct, acinis and myoepithelial cells. a, b, x 20; c, x 40

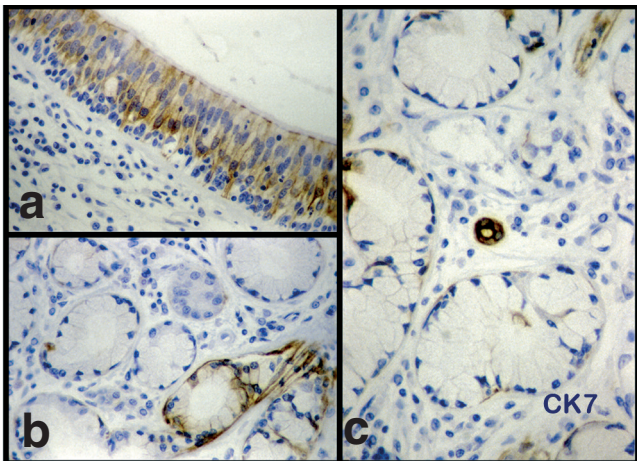


Fig. 4. CK 7 in bronchial epithelium neither stains basal cells nor acinis, stains intercalated duct and myoepithelial cells. a, b, x 20; c, x 40

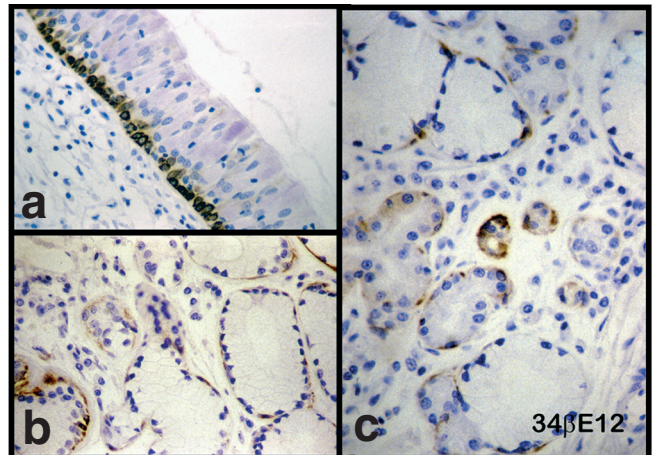


Fig. 5. 34 β E12 stains basal cells in respiratory epithelium, basal cells intercalated and myoepithelial cells. a, b, x 20; c, 40

Ductal System

Intercalated Ducts. Both the adluminal and basal cells layers of intercalated ducts reacted with AE1, AE3, CK 19, CK8/18/19, and 34 β E12. HSP 27 and HSP 70 were strongly positive in both types of cells. Adluminal cells reacted with pancytkeratin CK5/6/8/18 and CK8/18/19.

Excretory Ducts. Adluminal and basal cells showed a strong reaction for AE1, AE3, CK19 and HSP70. The basal cell layer reacted for CK34 β E12 and HSP27. Columnar adluminal cells reacted for CK7, CK18, CK5/6/8/18 and CK8/18/19. Excretory ducts showed scattered cells with a positive cytoplasmic immunoreaction for chromogranin and synaptophysin. This antigenic profile was the same as that of surface bronchial epithelium (Figs. 2-5).

Stroma. The connective tissue between the glands showed nerve fibers labeled for chromogranin, synaptophysin and Leu7. Blood vessel endothelium reacted for antibodies CD31 and CD34.

Discussion

Human parietal bronchial glands show a histological structure very similar to that of salivary and lacrimal glands. All of them are composed of a secretory part,

with mucous and/or serous acini, draining to the lumen through a ductal system, in which there are large excretory ducts and intercalated ducts. A striated duct can be seen only in salivary glands.

The excretory component is lined by a double row of cells. A basal one in contact with the external basal lamina, and an adluminal component in which tall columnar mucous and no mucin secreting cells can be seen in large ducts. A simple row of non secretory cuboidal cells can be seen in intercalated ducts. Myoepithelial cells are only present in the acinar units and as discontinuous rows on the intercalated duct.

Bronchial glands show a simpler structure than the salivary ones with no striated ducts as well as shorter intercalated ducts and even occasionally some secretory acini draining directly into the large duct lumen.

From the immunohistochemical point of view three different profiles of keratin expression have been demonstrated. Adluminal cells of large and intercalated ducts show an expression of low and high molecular weight keratins including CK8 and 34 β E12 keratins. Basal cells show a more limited spectrum of expression and do not react to staining for 7,8,19,20 keratins. The keratin profile is very similar to that found in salivary and lacrimal glands (Kahn et al., 1985; Caselitz et al., 1986; Geiger et al., 1987; Gustafsson et al., 1988). Mucin-secreting cells show a very limited expression of low molecular weight keratins but surprisingly enough serous cells show a complex profile very similar to that

Table 2. Antigenic profile of glandular elements of normal bronchial human glands

GLANDULAR ELEMENT	AE1	AE3	CK7	CK8	CK10	CK18	CK19	CK20	CK 8/18/19	CK 5/6/8/18
Secretory acini										
Mucous cells	+	-	-	-	-	-	-	-	-	-
Serous cells	+	+	+	-	-	+	+	-	+	+
Myoepithelial cells	+	+	+	-	-	-	-	-	-	-
Intercalated ducts										
Luminal cells	+	+	+	+	-	+	+	-	+	+
Basal cells	+	+	-	-	-	-	-	-	-	-
Excretory ducts										
Luminal cells	+	+	+	+	-	+	+	-	+	+
Basal cells	+	+	-	-	-	-	-	-	-	-
GLANDULAR ELEMENT	PAN CK	34 β E12	Leu7	α -actin	GFAP	CD10	S100	Cromo	SF	CD34
Secretory acini										
Mucous cells	-	-	-	-	-	-	-	-	-	-
Serous cells	+	-	+	-	-	-	-	-	-	-
Myoepithelial cells	-	+	-	+	-	+	+ ^o	-	-	-
Intercalated ducts										
Luminal cells	+	+	-	-	-	-	-	+	+	-
Basal cells	-	+	-	-	-	-	-	-	-	-
Excretory ducts										
Luminal cells	+	+	-	-	-	-	-	+	+	-
Basal cells	-	+	-	-	-	-	-	-	-	-

*: scattered cells; ^o: 16% of the specimen .

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of adluminal cells, the absence of CK8 being the main difference.

The myoepithelial cells are stellate- or spindle-shaped cells located along the basal aspect of the intercalated duct and acinus of human salivary and bronchial glands. Myoepithelial cells, as the name implies, have structural features of both epithelial and smooth muscle cells. There are three types of immunocytochemical markers of the myoepithelial cells in salivary glands. The first type of proteins expressed only by myoepithelial cells includes smooth muscle protein markers such as SMA, h-caldesmon, calponin (Kahn et al., 1985; Tsukada et al., 1987; Gugliotta et al., 1988; Hirano et al., 1990; Dardick et al., 1991). They are also expressed by stromal vasculature. The second type, expressed by myoepithelial and duct cells (luminal and/or basal cells) includes keratins 34BE12, 5 and 17 (Caselitz et al., 1986; Dardick et al., 1987; Geiger et al., 1987; Burns et al., 1988; Moll et al., 1989; Fuchs and Weber, 1994). Vimentin is the third type and, in addition to the myoepithelial cells, is expressed by the mesenchymal cells and some duct cells (Krepler et al., 1982; Geiger et al., 1987; Gustafsson et al., 1988; Hirano et al., 1990). Therefore the first proteins are the best to identify myoepithelial cells (Ogawa, 2003).

Myoepithelial cells stain strongly for α -smooth muscle actin, which therefore seems to be a sensible and specific marker in agreement with previous reports in salivary (Kahn et al., 1985; Tsukada et al., 1987; Gugliotta et al., 1988; Hirano et al., 1990; Dardick et al., 1991), lacrimal (Grossniklaus et al., 1990; Vigneswaran et al., 1990) and mammary glands (Gugliotta et al., 1988; O'Hare et al., 1991). Bronchial and blood vessel wall smooth muscle is also clearly demonstrated by this antibody.

Myoepithelial and epithelial ductal cells of bronchial gland react with CK34BE12 and CK7 as demonstrated in myoepithelial cells of salivary (Caselitz et al., 1986; Geiger et al., 1987; Tsukada et al., 1987) and lacrimal glands (Vigneswaran et al., 1990; Kivela, 1992).

Other potential markers for myoepithelial cells as S-100, GFAP and CD10 have given conflicting results regarding the expression of this markers. In previous studies S-100 protein have been used as a marker for myoepithelial cells in salivary glands (Nakazato et al., 1982; Kahn et al., 1985; Haimoto et al., 1987), although a low or absent positivity (Kahn et al., 1985; Ninomiya et al., 1989) have been reported in some studies. In our material myoepithelial cells demonstrated S-100 expression only in one case (16.6%). So S-100 protein is a highly specific marker but with a low sensitivity. Desmin is not expressed by myoepithelial cells also in agreement with previous studies (Truong et al., 1990; Zarbo et al., 1991). CD10 was reported to be present in myoepithelial cells of human breast (Gusterson et al., 1986) with a staining pattern identical to that of smooth muscle actin (SMA) (Moritani et al., 2002). In our study CD10 demonstrated a very high sensitivity and specificity with myoepithelial cells.

Chromogranin A and synaptophysin detected a few scattered cells in the surface epithelium of the bronchus and excretory ducts, and nerve fibers adjacent to the secretory acini, as in salivary glands (Kahn et al., 1985; Caselitz et al., 1986; Geiger et al., 1987; Gustafsson et al., 1988). No neuroendocrine cells can be demonstrated along the intercalated ducts or in the acinar units.

In summary the antigenic profile of the bronchial gland when compared to that of human salivary glands, show much more similarities than differences. From the histological point of view normal human bronchial glands at this level showed a similar acinar structure to mixed salivary glands, characterized by mucous, serous and mixed acinis that converge to the intercalated duct. Nevertheless in bronchus some terminal units drain directly into excretory ducts without any ductal structure. Myoepithelial cells are present from acinar units to intercalated ducts. No striated duct was observed. The detailed knowledge of the immunohistochemical profile of normal cells types of human bronchial glands will probably be useful in studies of bronchial pathology, and especially to understand the histogenesis of some tumors as mucoepidermoid carcinomas.

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