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Keratin 14 immunoreactive cells in pleomorphic adenomas and adenoid cystic carcinomas of salivary glands

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Abstract Our recent study of developing myoepithelial cells (MECs) in rat salivary glands demonstrated that developing MECs begin to express α-smooth muscle actin (αSMA) first and, thereafter, keratin 14. Therefore, it is unlikely that duct basal cells expressing keratin 14 alone are immature or undifferentiated MECs. In this study we carried out immunohistochemistry of pleomorphic adenomas and adenoid cystic carcinomas including normal salivary glands using monoclonal antibodies to keratin 14, smooth muscle proteins and keratin 19. The smooth muscle proteins examined included αSMA, h-caldesmon and h1-calponin; h1-calponin was observed in keratinocytes and nerve fibers, indicating that the protein is not specific to smooth muscle, whereas αSMA and h-caldesmon turned out to be highly specific markers for smooth muscle cells in normal tissues. In normal glands, MECs were positive for both keratin 14 and smooth muscle proteins (αSMA and h-caldesmon). Non-MEC cells were essentially devoid of smooth muscle proteins. Non-MEC duct basal cells expressed keratin 14 with or without keratin 19, and luminal cells keratin 19 with or without keratin 14. This suggests that the keratin 14-positive, smooth muscle proteins-negative duct basal cells are luminal cell progenitors. Luminal cells in tubular structures of both tumors were positive for keratin 19 with or without keratin 14. Nonluminal peripheral cells of pleomorphic adenomas were mostly positive for keratin 14, and a small fraction of them expressed smooth muscle proteins. Conversely, peripheral cells of adenoid cystic carcinomas were mostly positive for smooth muscle proteins, and some of them expressed keratin 14. These results strongly suggest (1) that the luminal cell progenitors transform into major constituents of pleomorphic adenoma cells with keratin 14 but not smooth muscle proteins, and (2) that the peripheral cells of adenoid cystic carcinoma are derived from undifferentiated MECs. Solid structures of pleomorphic adenomas were formed by proliferation of the peripheral cells. MECs were observed only occasionally in the periphery. Solid and cribriform structures of adenoid cystic carcinomas were formed by proliferation of the luminal cells. MECs were observed in the periphery and around the pseudocyst.

Key words Salivary gland tumor · Keratin · Alpha-smooth muscle actin · Calponin · Caldesmon

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Introduction

Identification of the myoepithelial cell (MEC) is the fundamental step in understanding the developmental process and formation of histoarchitecture of salivary gland tumors. The MEC is a unique hybrid of epithelial and smooth muscle cells. Therefore, coexpression of keratin 14 and α-smooth muscle actin (αSMA) has been the most widely used marker for identification of MECs [6–8, 14, 21, 22, 29, 31, 48, 50, 55]. Tumor cells with keratin 14 are more abundant than are those with αSMA in certain tumors, such as pleomorphic adenoma and adenoid cystic carcinoma [6, 8, 22, 31, 34, 48, 55, 56, 60]. By definition, the neoplastic cells expressing keratin 14 alone (without αSMA) are not necessarily derived from MECs. Indeed, keratin 14 is found in MECs as well as non-MEC large duct cells in normal salivary glands [6–8, 21, 22, 31, 55]. Nevertheless, recent researchers mostly consider the keratin 14-positive tumor cells without αSMA to be immature or undifferentiated MECs. This assumption is derived from the proposed mechanism of the MEC ontogeny; i.e., both basal cells of mature ducts and MECs are derived from a common primordia, the undifferentiated basal cells extending from the ducts to the oral epithelium [45]. In other words, the MECs and the basal duct cells are the proximal and distal components,

respectively, of the same basal cell/MEC continuum that extends form the acinus to the distal most duct. This appears to account for the presence of keratin 14 in both the basal duct cells and the MECs.

Our recent study on developing rat salivary glands, however, demonstrated that keratin 14 expression by MECs and basal cells was preceded by the expression of the smooth muscle proteins, such as αSMA and h1-calponin [42]. The first identifiable MECs, which appear in the basal layer of the terminal buds and the adjacent cell cords, express h1-calponin and αSMA but not keratin 14. These MECs with the smooth muscle proteins fully invest the terminal secretory apparatus and then begin to express keratin 14. Therefore, the above concept of MEC/basal cell continuum may not apply, at least to the rat salivary glands. Alternative explanation for the basal cell lineage implies that basal cells differentiate to luminal cells through a process including changeover from keratin 14 to keratin protein(s) specific to mature luminal cells [21, 24, 28].

The aim of the present study was to gain insights into the exact nature of the tumor cells with keratin 14 in pleomorphic adenoma and adenoid cystic carcinoma. To learn the extent of contributions of cells of the MEC and luminal cell origin to these tumor cells, immunohistochemistry was carried out using specific monoclonal antibodies against keratin 14 [44], αSMA [50], h1-calponin [26], h-caldesmon [26] and keratin 19 [33]. H1-calponin and h-caldesmon are newly discovered proteins with putative regulatory function in smooth muscle contraction [51, 58]. Keratin 19 is a major component of simple epithelia [4, 38], and is expressed by luminal cells and some basal cells in the salivary gland [22, 28].

Table 1 Clinical features of tumors

Case no.	Sex	Age	Site
Pleomorphic adenoma			
1	Female	25	Palatine gland
2	Female	38	Parotid gland
3	Female	46	Palatine gland
4	Male	31	Submandibular gland
5	Male	54	Parotid gland
6	Male	46	Submandibular gland
7	Female	37	Parotid gland
8	Female	61	Palatine gland
9	Female	27	Submandibular gland
10	Female	57	Submandibular gland
11	Male	29	Parotid gland
12	Male	38	Palatine gland
Adenoid cystic carcinoma			
1	Female	41	Palatine gland
2	Male	61	Submandibular gland
3	Male	27	Palatine gland
4	Male	35	Submandibular gland
5	Female	75	Palatine gland
6	Female	54	Submandibular gland
7	Male	59	Palatine gland
8	Male	56	Submandibular gland

Materials and methods

We collected 12 cases of pleomorphic adenoma and 8 cases of adenoid cystic carcinoma (Table 1). All cases were retrieved from the files of the Clinical Laboratory at the Osaka University Dental Hospital from 1993 to 1998. Normal salivary glands were included in 12 cases (4 parotid, 3 submandibular and 5 palatine glands). All the patients gave their informed consent prior to their inclusion in this study. The tissues were obtained during surgery, fixed in 10% neutral buffered formalin and embedded in paraffin. Sections (2–4 μm) were cut, mounted on silane-coated glass slides, deparaffinized and rehydrated to distilled water. One section from each tissue block was stained with hematoxylin and eosin to assess the histology, and the others were used for immunohistochemistry.

Because proteins excepting αSMA were not detected satisfactorily in the formalin-fixed, paraffin-embedded sections, we carried out a pilot study to seek the most effective way of antigen retrieval. We compared several methods, i.e. trypsin digestion [13] and microwave heating in zinc sulfate solution, lead thiocyanate solution [49] or citrate buffer [9]. Among these, microwave heating in citrate buffer was most effective; normal salivary glands processed by this method yielded immunohistochemical results similar to those obtained from unfixed frozen sections (data not shown).

In the following description, if no temperature is specified for a procedure this means it was carried out at room temperature. Sections were incubated for 5 min with 3% H_2O_2 to block endogenous peroxidase and washed in distilled water. The sections for αSMA were washed in 0.01 M phosphate-buffered saline (PBS; pH 7.2), and the next step of immunohistochemistry followed directly without antigen retrieval. Those for the other proteins were placed in a Coplin jar filled with 0.01 M citrate buffer (pH 6.0) and heated for 10 min (two 5-min cycles) at 700 W in a household microwave oven. After heating they were allowed to cool for 20–30 min, rinsed in distilled water and then in PBS, and subjected to the immunostaining procedure.

Immunohistochemistry was carried out by indirect immunoperoxidase method. All the sections were incubated for 30 min with 10% normal rabbit serum in PBS containing 1% bovine serum albumin (PBS-BSA) to block nonspecific binding. The serum was wiped away, and the sections were incubated overnight at 4°C with one of the primary antibodies which had been diluted with PBS-BSA (Table 2). They were then thoroughly washed with PBS and incubated for 30 min with peroxidase-conjugated rabbit anti-mouse IgG (DAKO, Glostrup, Denmark) which had been diluted 1:200 with PBS-BSA containing 1% normal human serum of AB blood type. After three 10-min washes with PBS, the sections were incubated for 5 min with 3,3'-diaminobenzidine tetrahydro-

Table 2 Monoclonal antibodies used for immunohistochemistry

Antibody	Specificity	Dilution	Source
LL002	Keratin 14	1:100	Ylem, Roma, Italy
1A4	α-Smooth muscle actin	1:1,000	Dako, Glostrup, Denmark
hCP	H1-calponin	1:120,000	Sigma Chemical Co., St. Louis, Mo.
hHCD	H-caldesmon	1:500	Sigma Chemical Co.
A53-B/A2	Keratin 19	1:500	Sigma Chemical Co.

chloride-H_2O_2 solution to visualize immunoreaction sites. They were washed with distilled water, counterstained briefly with Mayer's hematoxylin, dehydrated, and coverslipped with Permount.

Negative controls for immunostaining were performed by substituting the primary antibodies with PBS and normal mouse IgG.

Results

The results are summarized on Tables 3–5. All the immunostaining were cytoplasmic except for some nuclear staining of h1-calponin (see below).

Normal salivary glands (Table 3)

The results are summarized in Table 3. We excluded large excretory duct from the analysis. The smaller ducts distal to the intercalated portion were classified into intralobular and interlobular categories. The intralobular and interlobular ducts were striated in the major salivary glands, while striation was only occasionally observed in the distal ducts of the palatine salivary gland (see [5]). In this study, we distinguished two cell groups; the peripheral cells and the luminal cells. In the acinus and the intercalated duct, the peripheral cells consisted almost exclusively of highly flattened MECs. In the distal ducts, the peripheral cell population was a mixture of relatively bulky basal epithelial cells and occasional MECs.

Both acini and intercalated ducts of all glands examined were invested with MECs which expressed immunoreactivity for keratin 14 and smooth muscle proteins (Fig. 1a–d; Table 3), but not keratin 19. Immunoreaction products of h-caldesmon occupied somewhat narrower areas in the cytoplasm than did those of the other proteins. Occasional MECs loaded with keratin 14 and smooth muscle proteins were also observed in the periphery of the larger intralobular and interlobular ducts

Table 3 Staining patterns of normal salivary glands[a]

Antigen	Peripheral cell				Luminal cell			
	Acinar	Intercalated ductal	Intralobular ductal[b]	Interlobular ductal[b]	Acinar	Intercalated ductal	Intralobular ductal[b]	Interlobular ductal[b]
Keratin 14	0/0/0/12	0/0/0/12	0/0/4/8	0/0/0/12	12/0/0/0	10/2/0/0	7/2/3/0	7/2/3/0
α-Smooth muscle actin	0/0/0/12	0/0/0/12	10/1/1/0	11/1/0/0	12/0/0/0	12/0/0/0	12/0/0/0	12/0/0/0
H-caldesmon	0/0/2/10	0/0/0/12	11/1/0/0	11/1/0/0	12/0/0/0	12/0/0/0	12/0/0/0	12/0/0/0
H1-calponin	0/0/0/12	0/0/0/12	10/1/1/0	11/1/0/0	12/0/0/0	12/0/0/0	12/0/0/0	12/0/0/0
Keratin 19	12/0/0/0	12/0/0/0	0/0/6/6	0/0/7/5	12/0/0/0	0/0/0/12	0/0/0/12	0/0/0/12

[a] Reactivity patterns are scored as follows: no cell stained/less than 25% of cells stained/25–75% of cells stained/more than 75%f cells stained
[b] In parotid and submandibular glands, these denote striated ducts

Table 4 Staining patterns of pleomorphic adenoma cells[a]

Antigen	Tubular		Solid		Squamous metaplasia	Plasmacytoid	Myxoid	Chondroid
	Luminal	Peripheral	Luminal	Nonluminal				
Keratin 14	0/4/6/2	0/0/3/9	0/2/8/2	0/0/5/7	0/1/3/0	4/0/0/0	1/11/0/0	5/0/0/0
α-Smooth muscle actin	12/0/0/0	0/10/2/0	12/0/0/0	0/12/0/0	4/0/0/0	4/0/0/0	0/12/0/0	5/0/0/0
H-caldesmon	12/0/0/0	10/2/0/0	12/0/0/0	9/3/0/0	4/0/0/0	4/0/0/0	11/1/0/0	5/0/0/0
H1-calponin	0/7/5/0	0/0/5/7	0/5/7/0	0/0/6/6	0/1/3/0	0/0/0/4	0/0/11/1	2/3/0/0
Keratin 19	0/0/0/12	12/0/0/0	0/0/1/11	10/2/0/0	3/1/0/0	4/0/0/0	12/0/0/0	5/0/0/0

[a] Reactivity patterns are scored as follows: no cell stained/less than 25% cells stained/25–75% cells stained/more than 75% cells stained

Table 5 Staining patterns of adenoid cystic carcinoma cells[a]

Antigen	Tubular		Cribriform			Solid
	Luminal	Peripheral	Luminal	Pseudocyst	Nonluminal Nonpseudocyst	
Keratin 14	0/0/7/1	0/4/3/1	0/1/6/1	0/6/1/1	0/2/5/1	0/2/1/1
α-Smooth muscle actin	8/0/0/0	0/0/0/8	8/0/0/0	0/0/5/3	0/8/0/0	0/4/0/0
H-caldesmon	8/0/0/0	0/3/5/0	8/0/0/0	0/3/5/0	1/7/0/0	0/4/0/0
H1-calponin	6/2/0/0	0/0/0/8	6/2/0/0	0/0/5/3	0/8/0/0	0/2/1/1
Keratin 19	0/0/1/7	8/0/0/0	0/0/0/8	7/1/0/0	0/3/5/0	0/1/2/1

[a] Reactivity patterns are scored as follows: no cell stained/less than 25% cells stained/25–75% cells stained/more than 75% cells stained

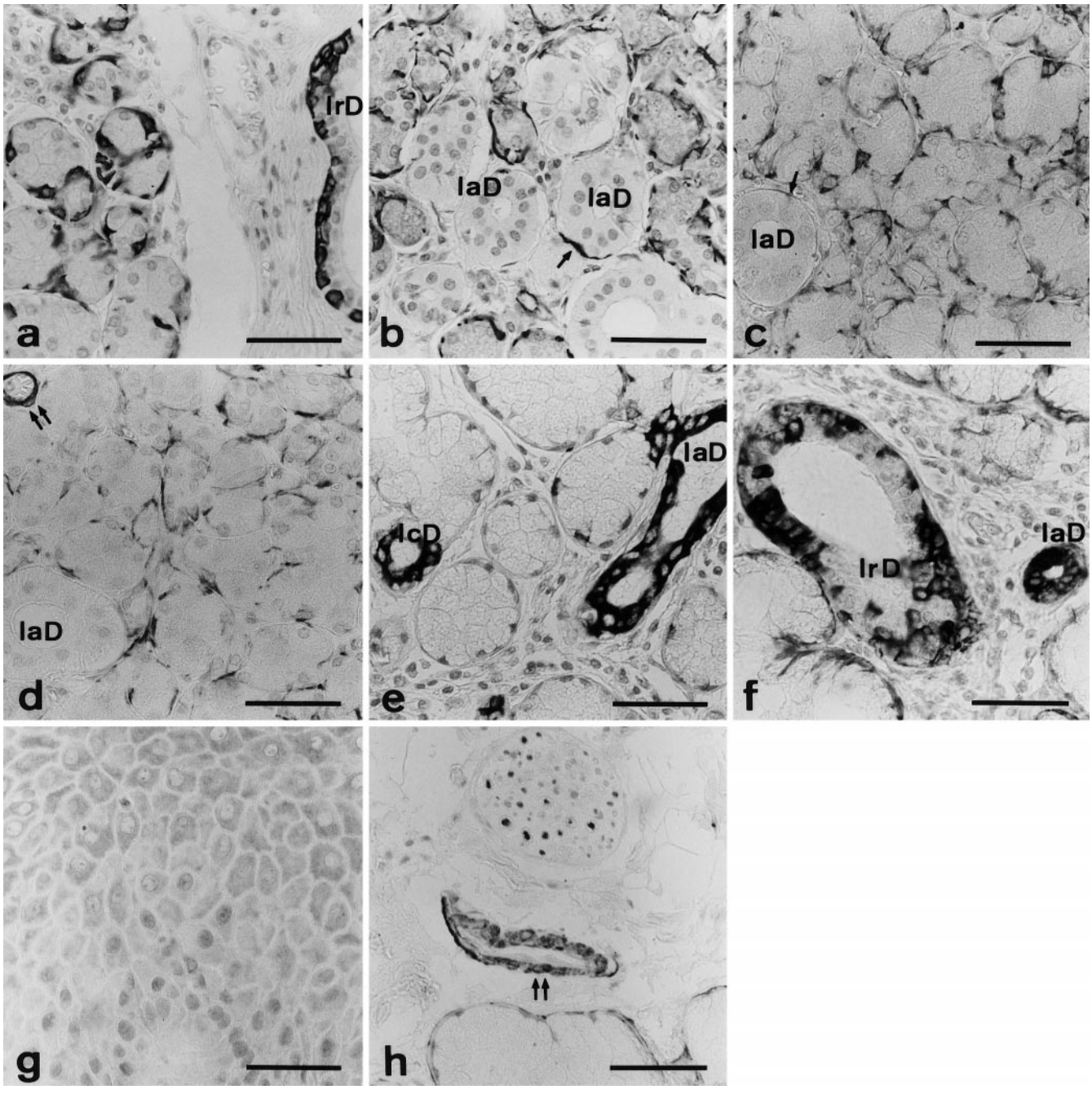

Fig. 1 Immunoperoxidase histochemistry of normal submandibular (**a–d**) and palatine (**e, f, h**) glands. Keratin 14 (**a, f**); αSMA (alpha-smooth muscle actin, **b**); h1-calponin (**c, g, h**); h-caldesmon (**d**); keratin 19 (**e**). Sections were counterstained with hematoxylin. Myoepithelial cells (MECs) around acini and intercalated ducts express immunoreactivity for keratin 14 (**a**), αSMA (**b**); h1-calponin (**c**); h-caldesmon (**d**); they are less frequently observed in the periphery of intralobular ducts (*arrows* in **b** and **c**). Keratin 14 is also expressed by basal cells of interlobular duct (**a**). Note immunoreaction products of h-caldesmon show somewhat narrower distribution in the cytoplasm of MECs (**d**). Keratin 19 immunoreactivity is apparent in luminal cells of ducts (**e**). It is never observed in acinar cells. Keratin 14 is expressed by some luminal cells in palatine gland ducts (**f**). Keratinocytes in palatine mucosa express weak immunoreactivity for h1-calponin in their cytoplasm and/or nuclei (**g**). H1-calponin immunostaining is occasionally seen in peripheral nerve cells (**h**). *Double arrows* in **d** and **h** indicate positive reaction of vascular walls for smooth muscle proteins (*IcD*, intercalated duct, *IaD* intralobular duct, *IrD* interlobular duct). *Bars:* 50 μm, ×300

(Fig. 1b, c). The peripheral or basal cells in these ducts generally took flattened or triangular configuration and lacked immunoreactivity for smooth muscle proteins. These cells were positive for keratin 14 and often for keratin 19 (Fig. 1a, f; Table 3). Keratin 19 immunoreactivity was usually seen in all luminal cells of all duct system, but not in the acinar cells (Fig. 1e; Table 3). In palatine glands, but not in major salivary glands, the luminal cells of the ducts sometimes also expressed immunoreactivity for keratin 14 (Fig. 1f).

Keratins 14 and 19, and smooth muscle proteins were expressed by cells other than the glandular epithelial cells. Oral mucosae, which were included in the palatine materials, expressed immunoreactivity for keratin 14. The immunoreactivity was strongest in the basal layer. The basal keratinocytes sometimes also expressed keratin 19. Immunoreactivity for smooth muscle proteins were seen in vascular smooth muscle cells (Fig. 1d, h) and myofibroblasts in the connective tissue. The myofibroblasts were rare in normal tissues and pleomorphic adenomas, but were numerous in adenoid cystic carcinomas (Fig. 3b). Here again immunoreaction products of h-caldesmon showed a limited distribution in the cytoplasm of these cells.

Among the smooth muscle proteins examined, h1-calponin immunostaining was unique; i.e., it was observed in some keratinocytes in the palatine epithelia and occasional nerve fibers (Fig. 1g, h). Calponin immunostaining in these cells was sometimes seen in their nuclei (Fig. 1g). Unique and broader calponin staining was also seen in the neoplastic tissues. Therefore, we describe calponin immunostaining separately from the rest of the smooth muscle proteins, αSMA and h-caldesmon.

Pleomorphic adenomas (Table 4)

All the cases included tubular, solid and myxoid structures. The tubular structure was a two-cell layered structure surrounding a central lumen. The luminal layer was made up of a monolayer of cells with immunoreactivity for keratin 19 (Fig. 2c). These keratin 19-positive luminal cells often coexpressed various intensity of immunoreactivity for keratin 14 (Fig. 2a; Table 4). Tumor cells in the peripheral layer were negative for keratin 19, but were mostly positive for keratin 14 (Fig. 2a, c). The keratin 14-positive peripheral cells only occasionally exhibited immunoreactivity for αSMA (Fig. 2b; Table 4). The peripheral layer cells often piled up, and consequently the tubular structure was made up of more than two cell layers, at least in some parts (Fig. 2a, c). In the solid structure, tumor cells surrounding the lumen were similar to the luminal cells in the tubular structure, i.e., positive for keratin 19 (not shown for solid structure, but see Fig. 2c for a form transitional from a tubular structure) and often coexpressed keratin 14 immunoreactivity (Table 4). The rest of the tumor cells or nonluminal cells in this structure rarely expressed immunoreactivity for keratin 19 (Table 4). They were often positive for keratin 14 (Fig. 2d), and some of these keratin 14-positive cells expressed aSMA. The cells coexpressing keratin 14 and αSMA were spindle- or stellate-shaped and were encountered in the periphery of the solid structure (Fig. 2e). In the myxoid structure, cells with keratin 14 and smooth muscle proteins were observed only occasionally (Fig. 2d, e; Table 4). H-caldesmon immunoreactivity was occasionally expressed by the cell types that expressed αSMA (Fig. 2f; Table 4).

Squamous metaplasia was seen in 4 cases (1 in a parotid gland, 2 in the submandibular gland and 1 in the palatine gland). Tumor cells in the squamous metaplasia sometimes exhibited immunoreactivity for keratin 14 (Fig. 2g). Clusters of plasmacytoid cells were seen in 4 cases (1 in the parotid gland, 1 in the submandibular gland and 2 in the palatine gland), and chondroid structure in 5 cases (1 in the parotid gland, 3 in the submandibular gland and 1 in the palatine gland). They were devoid of keratin 14, keratin 19, αSMA and h-caldesmon (Table 4).

H1-calponin immunoreactivity was seen in all the tumor cell types (Table 4). Calponin immunoreactivity was seen in some luminal and many nonluminal cells in the epithelial structure. In the mesenchymal structure, many myxoid and occasional chondroid cells also expressed the immunoreactivity. In the foci of plasmacytoid cells and squamous metaplasia which were seen in the limited cases, tumor cells were positive for calponin.

Adenoid cystic carcinomas (Table 5)

Tubular and cribriform structures were seen in all cases, whereas solid structure was in 4 cases (2 in the submandibular gland and 2 in the palatine glands). The luminal layer of the tubular structure was made up of keratin 19-positive cells, most of which were also positive for keratin 14 (Fig. 3a, c; Table 5). The luminal cells were negative for αSMA or h-caldesmon. On the other hand, the peripheral cells were mostly positive for αSMA and, less frequently, for h-caldesmon (see Fig. 3b for example of αSMA; Table 5). Some peripheral cells were also positive for keratin 14 (Fig. 3a; Table 5). The tubular structure was often made up of more than two cell layers. This was mostly due to an increase in luminal rather than in peripheral cells (cf. Fig. 3b, c).

In the cribriform structure, tumor cells surrounding the true lumina (luminal cells) were immunoreactive for keratin 19, whereas those surrounding the pseudocysts were not (Fig. 3f). Most pseudocyst cells were immunoreactive for αSMA and less frequently for h-caldesmon (Fig. 3e for example of h-caldesmon; Table 5), whereas the luminal cells were immunonegative for these smooth muscle proteins. Keratin 14 was expressed by many luminal cells. The pseudocyst cells sometimes expressed this keratin (Fig. 3d). In many cases, the rest of the tumor cells (nonluminal nonpseudocyst cells) in this structure were positive for keratin 14 and/or keratin 19 (Fig. 3d, f; Table 5). Some nonluminal nonpseudocyst cells in the periphery of the tumor also expressed smooth muscle proteins (Fig. 3e). The solid structure was frequently made up of keratin 19- and/or keratin 14-positive cells (Fig. 3g, h). Some solid structure cells were positive for αSMA and less

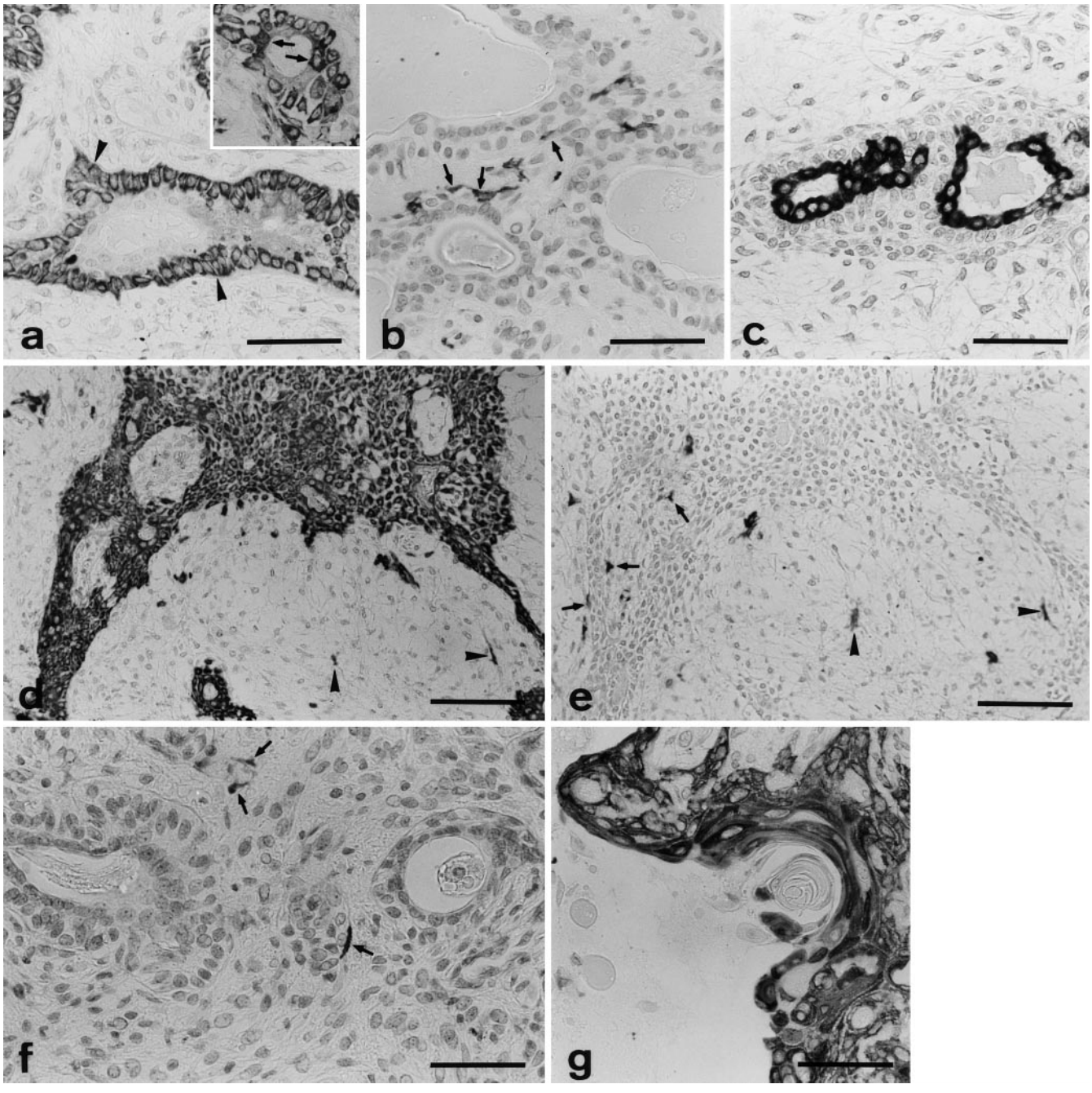

Fig. 2 Immunoperoxidase histochemistry of pleomorphic adenomas in parotid (**a, c, g**), submandibular (**d, e**) and palatine (**b, f**) glands. Keratin 14 (**a**, **d, g**); αSMA (**b, e**); keratin 19 (**c**); h-caldesmon (**f**). Sections were counterstained with hematoxylin. In tubular to solid structures, luminal cells are positive for keratin 19 (**c**). They often also express immunoreactivity for keratin 14 (*arrows* in *inset* in **a**). Peripherally located cells are negative for keratin 19 (**c**), but positive for keratin 14 (**a**). These cells sometimes exhibit immunoreactivity for αSMA (*arrows* in **b**). When the structure consists of more than two-cell layers and begins to generate solid structure, the increases are always in the peripheral cells (*arrowheads* in **a**; compare with **c**). **d**, **e** Semiserial sections showing solid and myxoid structures. In solid structures, many tumor cells are positive for keratin 14 (**d**). Some peripherally located cells also express αSMA (*arrows* in **e**). In myxoid structures, cells with keratin 14 and αSMA are observed only occasionally (*arrowheads* in **d** and **e**). Immunoreactivity for h-caldesmon is less frequent than that for αSMA (**f**). Occasional cells in the periphery of solid structure and those in mesenchymal structure are positive for this protein (*arrows* in **f**). Tumor cells in squamous metaplasias express immunoreactivity for keratin 14 (**g**). *Bars:* **a–c, f, g** 50 μm, ×300; **d, e** 100 μm, ×150

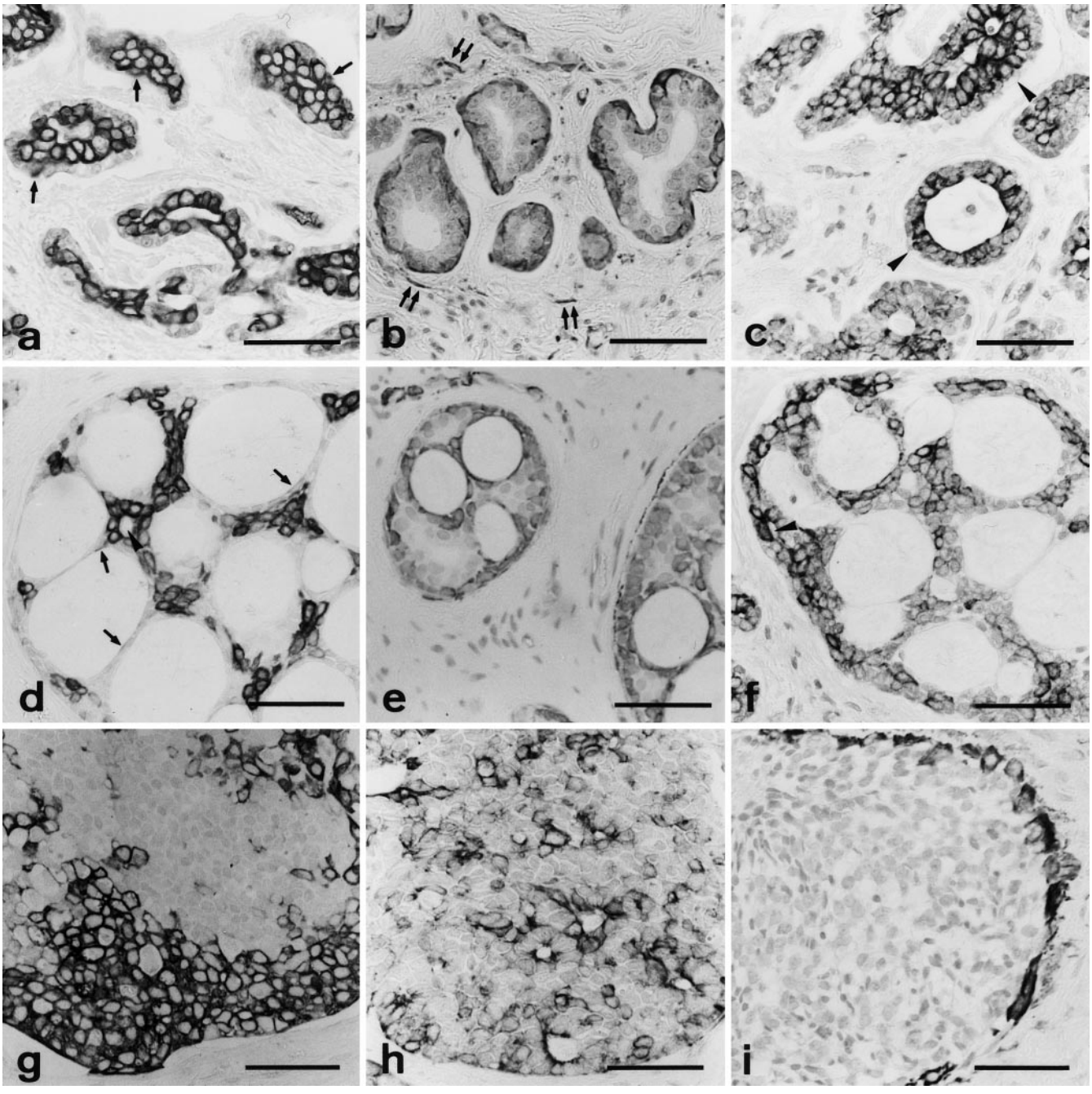

Fig. 3 Immunoperoxidase histochemistry of adenoid cystic carcinomas in submandibular (**a–f**) and palatine (**g–i**) glands. Keratin 14 (**a, d, g**); αSMA (**b, i**); keratin 19 (**c, f, h**); h-caldesmon (**e**). Sections were counterstained with hematoxylin. In tubular structures, keratins are expressed mainly by luminal cells (**a, c**), while αSMA only by peripheral cells (**b**). Peripheral cells occasionally express keratin 14 (arrows in **a**) but not keratin 19. When the structure consists of more than two-cell layers, the increased are mostly the luminal cells (*arrowheads* in **c**; compare with **b**). *Double arrows* in **b** indicate myofibroblasts positive for αSMA. In cribriform structures, tumor cells surrounding true lumina (luminal cells) are positive for keratins 14 and 19 (**d** and **f**), but not for h-caldesmon (**e**). *Arrowheads* in **d** and **f** indicate true lumina. H-caldesmon-positive cells are seen around pseudocysts and in the periphery (**e**). Pseudocyst-lining cells (pseudocyst cells) are mostly negative but sometimes positive for keratin 14 (*arrows* in **d**). Nonluminal nonpseudocyst cells are often positive for keratin 14 (**d**) and/or keratin 19 (**f**). In solid structures, tumor cells are often positive for keratin 14 (**g**) and/or keratin 19 (**h**). Cells with αSMA are sometimes seen in the periphery (**i**). *Bars*: 50 μm, ×300

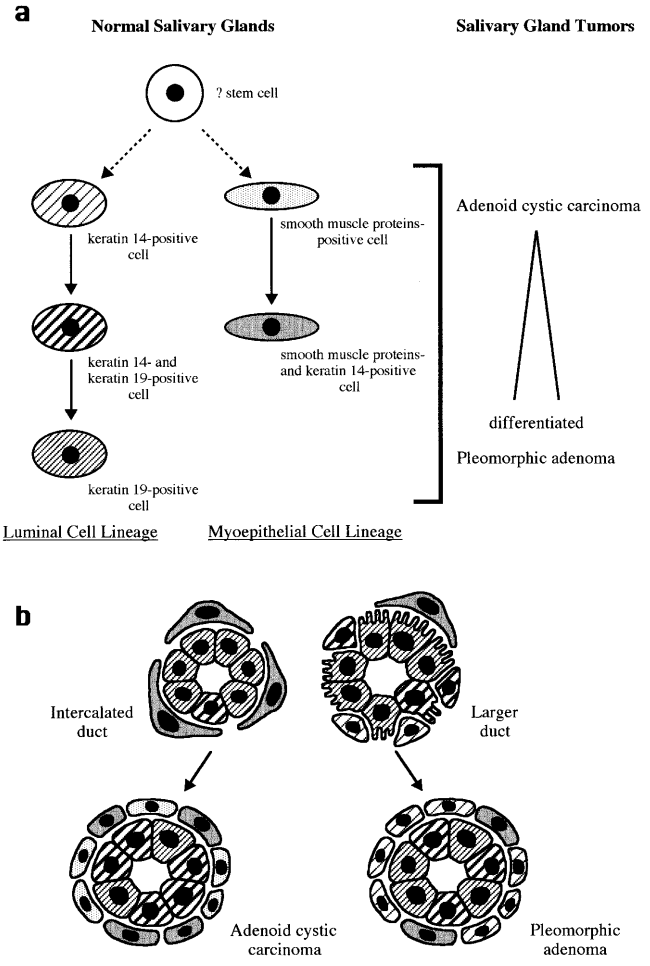

Fig. 4 Schematic diagrams showing **a** two cell lineages of normal salivary glands and **b** cell types which make up normal ducts and tubular structures of pleomorphic adenoma and adenoid cystic carcinoma. Both pleomorphic adenoma and adenoid cystic carcinoma are made up of cells of both luminal and myoepithelial cell lineages. In general, the cells in adenoid cystic carcinoma are less differentiated than those in pleomorphic adenoma (**a**). Cells in myoepithelial cell lineage are more prominent in adenoid cystic carcinoma (**b**)

frequently h-caldesmon. They were localized to the periphery of the solid structure (Fig. 3i; Table 5).

H1-calponin was seen in at least a few cells of all the above cell types (Table 5). In two cases, many tumor cells in the solid structure were immunoreactive for this protein.

Discussion

Despite a minor difference in detection sensitivity, h-caldesmon and αSMA immunoreactivities were seen in similar cell types in normal and neoplastic tissues. In normal tissues, these proteins were detected only in smooth muscle cells and MECs. On the other hand, h1-calponin immunoreactivity was distributed in a broader range of cell types than the other two smooth muscle proteins. In normal tissues, calponin immunoreactivity was seen not only in MECs and vascular smooth muscle cells but also in nerve fibers and the palatine mucosal epithelium. Non-smooth muscle tissue cells including nerve tissue cells [2, 35, 57] and keratinocytes [27, 36] are also known to express the h2 and acidic isoforms of calponin. Hence, the term ‘smooth muscle proteins’ includes αSMA and h-caldesmon but not h1-calponin. In neoplastic tissues, the calponin immunoreactivity was detected in wider range of cells than h-caldesmon and αSMA. This tendency was particularly exemplified in pleomorphic adenomas. The calponin immunoreactivity in tumor cells negative for h-caldesmon and αSMA may reflect the occurrence of tonofilaments [10, 12, 18, 19, 23, 43] and/or nerve proteins such as S-100 protein and glial fibrillary acidic protein [1, 3, 6, 11, 23, 30, 32, 39, 40, 56, 59]. Therefore, it is safe to conclude so far that the calponin immunoreactivity in the tumor cells both in the previous [48] and the present studies does not necessarily represent the exact distribution of MEC-related cells.

The present results do not support the notion that all the keratin 14-positive cells in pleomorphic adenomas and adenoid cystic carcinomas originate from MECs [8, 17, 20]. The keratin 14-positive tumor cells should not be considered to be MEC-related cells only because of their basal position. As already mentioned, the expression of keratin 14 by any kinds of developing salivary gland cells is preceded by expression of smooth muscle markers by developing MECs [42]. Therefore, the keratin 14 expression without smooth muscle proteins do not appear to be a marker for undifferentiated MECs. In normal palatine glands, a substantial subpopulation of duct luminal (therefore non-MEC) cells express keratin 14. These cells are essentially negative for smooth muscle markers. Because virtually all normal duct luminal cells express keratin 19, these keratin 14-positive cells are positive for keratin 19. Therefore, the tumor cells with keratin 14 but not smooth muscle markers are strongly suggested to be luminal cell progenitors (Fig. 4a).

The keratin 14 expression by normal duct luminal cells was detected in palatine glands but not in large salivary glands. This would also support the above argument that the keratin 14-positive duct basal cells are progenitors of luminal cells. In the palatine gland, the intralobular and interlobular ducts are less differentiated, and the luminal cells rarely acquire typical basal striation [5]. The intercalated duct of palatine gland exhibits a high degree of structural pleomorphism [5], and relatively undifferentiated cells appear to persist. Because cell migration appears to be an indispensable phenomenon involved in histogenesis and continuous turnover of luminal cells [21, 24, 28] (see also [15, 46] for reviews), it is well accepted that basal cells move to the luminal position. The changeover from keratin 14 to keratin 19 may be a fundamental event normally occurring in both developing and mature salivary gland ducts.

Both in pleomorphic adenoma and adenoid cystic carcinoma, tubular growth of the tumor cells is the basics from which various growth structures develop [17]. Almost all luminal cells in the tubular structure of both tu-

mors appeared to have been derived from luminal cell lineage because they mostly expressed keratin 19, and less extensively keratin 14, but never expressed smooth muscle proteins (Fig. 4b). As for the peripheral cells, there was a considerable difference in the cell marker expression between the two tumors. In adenoid cystic carcinomas, most peripheral cells expressed smooth muscle proteins but never expressed keratin 19. Therefore, they are likely to be derived from MEC lineage (Fig. 4b). Absence of keratin 14 from at least a part of these smooth muscle protein-positive cells may be explained by a hampered differentiation of MEC lineage cells due to malignancy. In pleomorphic adenomas, on the other hand, the smooth muscle protein-negative peripheral tubular cells outnumbered the positive cells. These peripheral cells often expressed keratin 14 and appeared to be derived from the luminal cell lineage (Fig. 4b). The predominance of the MEC-related cells may suggest that adenoid cystic carcinoma arises from the intercalated ducts, while the rarefaction of MEC-related cells and the abundance of cells of the luminal cell lineage may suggest that pleomorphic adenoma arises from larger ducts (Fig. 4b). Because squamous metaplasia is more frequent in larger ducts [24, 47], occurrence of squamous metaplasia in pleomorphic adenoma may support this notion. The peripheral and the luminal cells in the tubular pleomorphic adenoma appeared more primitive than the cells in corresponding layers of normal salivary gland ducts: the peripheral keratin 14-positive tumor cells lacked keratin 19, and the luminal keratin 19-positive cells more frequently coexpressed keratin 14 (Fig. 4b). As in MEC-related tumor cells in adenoid cystic carcinomas, the differentiation might have been arrested in tumor cells of the luminal cell lineage.

Increase of cell layers in tubular structure is the early step in the development of solid and cribriform structures [17]. In pleomorphic adenomas, the increase was usually seen in the peripheral layer lacking keratin 19 immunoreactivity. Consequently the solid structure was composed mainly of the basal cells with keratin 14. Some MECs were observed only in the periphery. In the myxoid structure, the smooth muscle proteins and keratins currently under scrutiny were expressed by only a small number of tumor cells. The markers included keratin 14, αSMA and h-caldesmon but not keratin 19, suggesting that these tumor cells were derived from the MEC lineage. This observation supports the notion that MECs play a part in the formation of myxoid structure [16, 18, 19, 54] by their active production of extracellular matrix [52, 53]. However, the present immunohistochemical study did not shed any light on the controversy about the MEC origin of the chondroid and plasmacytoid cells [19, 23, 25, 37, 39, 41]. In adenoid cystic carcinomas, on the other hand, the luminal layer increased more frequently than the peripheral layer. Consequently, both the cribriform and the solid structures were frequently made up of luminal cells with keratin 19. The occasionally observed basal cells with only keratin 14 should be taken as a reflection of less differentiated state of the luminal cells in these malignant tumors. Undifferentiated MECs without keratin 14 could be also seen in this malignancy. Both differentiated MECs with keratin 14 and undifferentiated MECs without keratin 14 were sometimes the major constituent of the cribriform and solid structures. They usually surrounded the pseudocysts and/or localized to the periphery of both structures. Here again, MECs are thought to be engaged in the production of extracellular matrix [10, 12, 16, 43, 54], but their abundance suggests that they have a more prominent role in adenoid cystic carcinomas than do pleomorphic adenomas.

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