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Fetal Myoepithelial Cells of Submandibular Glands and Modified Myoepithelial Cells in Pleomorphic Adenoma : Immunohistochemistry and Ultrastructure

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= Abstract = **To elucidate the divergent expression of tissue markers for myoepithelial cells (MECs) of submandibular glands, fetal submandibular glands (n 100), adult submandibular glands (n 10) and pleomorphic adenoma (n 20) are examined for cytokeratin 14 (CK-14), vimentin, and α-smooth muscle actin (-SMA) by double immunohistochemical methods. In addition fetal submandibular glands (n 20) and pleomorphic adenoma (n 3) are studied ultrastructurally to correlate with the immunohistochemical findings. The polyhedral MECs found in the early intermediate developmental stage (EIDS, 19-24 weeks) of fetal submandibular gland showed strong CK-14 positivity, weak α-SMA positivity, and occasional vimentin positivity. These polyhedral MECs matured into wedge shaped, spindle or dendritic MECs. A large number of dendritic MECs seen in the late developmental stage (LDS, 33-40 weeks) showed a mild CK-14 positivity, strong α-SMA positivity, and negative vimentin. The CK-14 was also expressed strongly for some basal cells of the excretory ducts and rarely of striated ducts, while these ductal basal cells were negative for α-SMA and vimentin. Electron microscopic examination of fetal submandibular glands disclosed abundant intermediate filaments in the polyhedral MECs during the EIDS. The intermediate filaments gradually decreased in amount as MECs matured into dendritic MECs in the LDS, and the dendritic MECs became filled with myofibrils. In pleomorphic adenomas the plasmacytoid or modified MECs usually seen in periductal portions showed coexpression of CK-14 α-SMA and vimentin α-SMA in double immunostaining.**

Key words : *Cytokeratin 14, Vimentin, α-Smooth muscle actin, Myoepithelial cell, Fetus, Adult, Human, Salivary gland, Pleomorphic adenoma*

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INTRODUCTION

The role of myoepithelial cells (MECs) in sub-

mandibular gland tumors has been a matter of conjecture for a long time. The participation of the MECs is an important consideration in histogenetic approach and the classification of the salivary gland tumors (Batsakis,1980; Batsakis et al. 1992; Dardick et al. 1989; Dardick et al. 1989b; Kahn et al. 1985). Various monoclonal and polyclonal antibodies have been used to elucidate the nature of the cell population in normal and neoplastic salivary glands with particular reference to MECs. However, data on the markers of the MECs in normal and neoplastic glands have not been consistent (Dardick et al. 1991; Mori et al. 1987; Mori et al. 1990; Morinaga et al. 1992; Morinaga et al. 1987; Nisen et al. 1981; Zarbo et al. 1991).

Cytokeratins are expressed in normal salivary glands (36, 41). Among them cytokeratin 14 (CK-14) is expressed selectively in MECs associated with acini and intercalated ducts. It is not expressed in acinar cells or ductal luminal cells (Dardick et al. 1989; Dardick et al. 1988; Mori et al. 1990), but is strongly positive for the epithelial basal cells. However, as the salivary gland develops from the stomodeal ectoderm to produce tubulo-alveolar structures which have a continuous basal cell layer from oral mucosa epithelium to acini, it is also known that a number of basal cells of acini and intercalated ducts differentiate into MECs with close interaction of salivary mesenchyme. Meanwhile the basal cells of excretory ducts and striated ducts are not clearly defined for their role. Therefore it is important to know if CK-14 is expressed in ductal basal cells and dendritic MECs in the early developmental stage of human fetal salivary glands, adult salivary glands and salivary gland tumors. In our previous paper (Lee et al. 1993a) we have reported that all the developing MECs were localized at the acini and intercalated ducts, and suggested that aged MECs could migrate along the ductal basal layer to form a longitudinally elongated MEC in the striated and excretory ducts of adult salivary glands. In this study we used the monoclonal antibody CK-14 for the coexpression on MEC and the ductal basal cell. And if the ductal basal cells had similar developmental characteristics as the MECs, one may presume that the ductal basal cells have close relation as the modified MECs in pleomorphic adenoma.

Present study is to define the developmental sequence of MECs in human salivary glands by immunohistochemistry and electron microscopy. Furthermore if CK-14 and vimentin, together with α-SMA could be detected for the markers of the normal developing MECs and neoplastic modified MECs, its participation as a tumor element in pleomorphic adenoma should also be important.

MATERIALS AND METHODS

One hundred human fetal submandibular glands from 15 weeks to 40 weeks of gestation, ten adult human submandibular glands and surgically resected specimens from twenty cases of pleomorphic adenoma were included in this study. The fetal submandibular glands were obtained from the files of normal embryos and fetuses of Seoul National University Department of Pathology. These fetuses were confirmed as normal after thorough dissection and measurements. The gestational age of each fetus was deduced from the crown-rump length or maternal records. The specimens were fixed in Bouin's solution or methanol/acetic acid (95/5) for 3-5 hours, embedded in paraffin, and sectioned at 4 m. Paraffin sections were treated with monoclonal antibodies (MoAbs) of cytokeratin 14 (CK-14, 50 KD; Biogene), vimentin (V9, 57KD; Dako), and α-smooth muscle actin (α-SMA, M851, Dako) by indirect immunohistochemical method using labelled streptavidin-biotin (K681; Dako) staining technique. Simultaneously fetal lip tissue sections were utilized for the positive and negative control stainings. For the electron microscopic observation twenty fetal submandibular glands, from gestational age of 17 weeks (1), 20 weeks (2), 23 weeks (1), 25 weeks (3), 30 weeks (2), 32 weeks (2), 33 weeks (3), 35 weeks (2), 37 weeks (1), 38 weeks weeks (2), 39 weeks (1), were fixed in 5% buffered glutaraldehyde. Immunohistological methods, histologic details and immunohistochemical findings of individual cell type of the developing submandibular glands of human fetuses are basically same with our previous study (Lee et al. 1990), and additionally double immunohistochemical stainings were

also employed by peroxidase-antiperoxidse (PAP) and alkaline phosphate-antialkaline phosphatase (APAAP) methods, using MoAbs of CK-14 / α-SMA and vimentin / α-SMA. Although the available primary antibodies of the CK-14, Vimentin and α-SMA were mouse antibodies, we have done the denaturation procedure for the first primary antibody, dipping in the 95% ethanol for 60 minutes. And in order to prevent the biotin cross-reactivity the APAAP method was used for the second primary antibody.

In the present study the developmental landmarks of Lee et al (Lee et al. 1991) for fetal submandibular gland are used; The terminal glandular epithelium makes acinar structure in the early developmental stage (EDS; from 10 to 18 weeks of gestation), and the proliferation and cytodifferentiation of glandular epithelium are most active in the early intermediate developmental stage (EIDS; from 19 to 24 weeks of gestation), and glandular development for cytodifferentiation takes place in the late intermediate developmental stage (LIDS; from 25 to 32 weeks of gestation), and the submandibular glands are almost in full maturation in the late developmental stage (LDS; from 33 to 40 weeks of gestation).

RESULTS

Immunohistochemistry of cytokeratin 14, vimentin, and α-smooth muscle actin

Fetal and adult submandibular glands

Cyotkeratin 14; In the EDS the primitive MECs found in the basal layer of the terminal ducts and acini showed a slight immunoreactivity for CK-14 in the EDS (Fig. 4a), whereas the polyhedral or wedge-shaped MECs showed a strong immunostaining for CK-14 in the EIDS (Fig. 4b). A few basal cells in the striated ducts proximal to the ductal orifice were also positive for CK-14, and the number of these positive basal cells increased through the excretory duct. Near the glandular orifice almost all the basal cells showed strong positivity for CK-14 with the same intensity in oral mucous epithelium. The spindle/dendritic MECs around the basal layer of acini and intercalated ducts gradually encompassed the whole acini as the salivary gland matured into the LIDS (Fig. 4c). In the LIDS most dendritic MECs were consistently positive, but less strongly than in EIDS (Fig. 4d). All basal cells of the oral mucosa at this time of development were positive for CK-14 (Fig. 4f). In the adult salivary glands the dendritic MECs particularly around the acini and intercalated ducts were stained for CK-14, and a few spindle-shaped MECs in the basal layer of the striated and excretory ducts showed a weak positivity. Meanwhile many cuboidal basal cells of the excretory ducts and all basal cells of oral mucosa showed strong positivity for CK-14 (Table 1, 2).

Vimentin: A few basal cells in the terminal ducts and acini showed a weak immunostaining for vimentin in the EDS. In the EIDS basal cells of acini showed scattered intense staining. No positive cells were present in the striated or excretory ducts at this time (Fig. 5e). Some po-

Table 1. Immunohistochemical Detection of MoAb CK-14, Vimentin, and -SMA in the Submandibular Glands of Human Fetuses and Adults

Developmental stages (weeks)	Numbers of cases	CK-14	Vimentin	-SMA
EDS (10-18)	13	±/+		
EIDS (19-24)	21	+++	+	+
LIDS (25-32)	38	++	+	++
LDS (33-40)	28	+/++	±	+++
Adult	10	+	–	++/++
Total	110			

EDS; early developmental stage, EIDS; early intermediate developmental stage.

LIDS; late intermediate developmental stage, LDS; late developmental stage.

Degree; – : negative, ±:: trace, + : slight, ++ : moderate, +++ : strong.

Table 2. Comparison of CK-14, vimentin, and α-SMA reactivity in the submadibular glands and oral mucosa epithelia of human fetuses

	Acinus	I.D.	S.D.	E.D.	B.L.O.M.
CK-14	+++	++	±	+	+++
Vimentin	+	±	−	−	−
α-SMA	+++	++	−	−	−

I.D.; intercalated duct, S.D.; striated duct, E.D.; excretory duct

B.L.O.M.; basal layer of oral mucosa

lyhedral or wedge-shaped basal cells of the acini and intercalated ducts were positive for vimentin. They remained positive until in the LIDS. With the double immunostainings of vimentin and α-SMA some vimentin positive cells expressed α-SMA conspicuously. However, these positive cells decreased rapidly in the LDS, and no dendritic MECs were positive for vimentin (Table 1, 2). Whereas no reaction for vimentin was seen in the normal adult submandibular gland epithelium (Table 1, 2).

Alpha-smooth muscle actin: The primitive MECs were weakly positive for α-SMA in the EDS, whereas the polyhedral or wedge-shaped MECs showed a moderate positivity in the EIDS (Fig. 5a). When MECs formed spindle to dendritic shape, they showed a strong positivity. With double immunostainings of CK-14 and α-SMA the immature MECs in the EDS and EIDS showed predominant CK-14 positivity over α-SMA (Fig. 5a). On the other hand as the MECs matured through the LIDS and LDS, α-SMA positivity overwhelmed the CK-14 staining (Fig. 5b, 5c). Through the entire developmental stages of fetal submandibular glands α-SMA positive cells were not found in the striated and excretory ducts. In the adult submandibular glands the MECs around the acini and intercalated ducts were strongly immunostained for α-SMA, and a few spindle shaped MECs in the basal layer of the striated and excretory ducts were also stained moderately. In double immunostainings of α-SMA and CK-14, the dendritic MECs were stained more intensely for α-SMA than for CK-14 (Table 1, 2).

Pleomorphic adenoma

The findings were studied by different tumor elements such as tubulo-ductal structure, plasmacytoid cell aggregation, myxo-chondroid area, and mesenchymal metaplastic area. The ductal cells of tubulo-ductal structure expressed a strong CK-14 positivity, but they were negative for vimentin or α-SMA. Meanwhile the outer cells of tubuloductal structure were diffusely positive for vimentin. Some plasmacytoid cells aggregated toid cells aggregated at the peripheral basal layer were strongly positive for CK-14 and vimentin, but slightly positive for α-SMA. The transformed or neoplastic MECs in the areas of myxochondroid or mesenchymal metaplasia showed irregular coexpression of CK-14, vimentin and α-SMA. However, in the double immunostainings of CK-14/α-SMA and vimentin/α-SMA the modified or transformed MECs were strongly widely positive for CK-14 and vimentin, but they showed only focal coexpression of α-SMA (Fig. 5d, 5f).

ELECTRON MICROSCOPIC OBSERVATION

Fetal and adult submandibular glands

In the late EDS (15-18 weeks) basally located epithelial cells in the terminal acini were seen with abundant intermediate filaments and a few primitive myofibrils in their cytoplasm (Fig. 1). In the early EIDS (19-21 weeks) the developing MECs were distinctive from the acinar cells with prominent marginated dense chromatin. The MECs were polyhedral or wedge-shaped, and contained abundant intermediate filaments, smooth endoplasmic reticulum, and occasionally myofibrils in their cytoplasmic processes. The polyhedral MECs usually formed a compact basal layer arrangement (Fig. 2). As the acinar cells proliferated and differentiated in the EIDS, the MECs became wedge shaped to form elongated cytoplasmic processes in the outer surface of the acini. In the late EIDS the MECs became spindle or dendritic in shape and showed abundant myofibrils and intermediate filaments. The

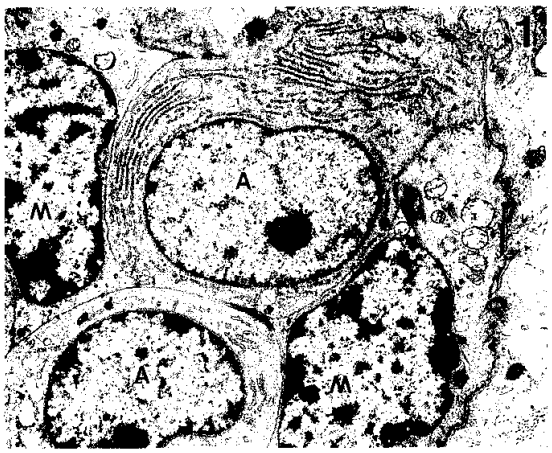

Fig. 1. Developing submandibular gland epithelium, 18 weeks fetus. Myoepithelial cells (M) containing primitive myofibrils and intermediate filaments are distinguishable from acinar cells (A) (× 6000)

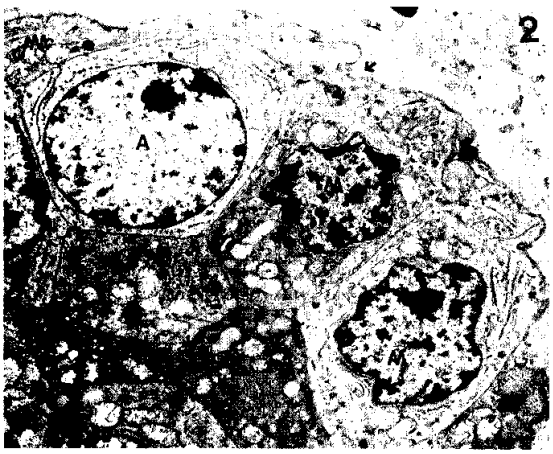

Fig. 2. Acinus of 24 weeks fetus. Basally located polyhedral myoepithelial cells (M) are directly connected with dendritic cytoplasmic processes (arrow) of adjacent myoepithelial cells (× 6000)

cytoplasmic processes gradually encircled the outer surface of the acini. In the LIDS the MECs formed several dendritic processes to surround the whole acini. These cytoplasmic processes

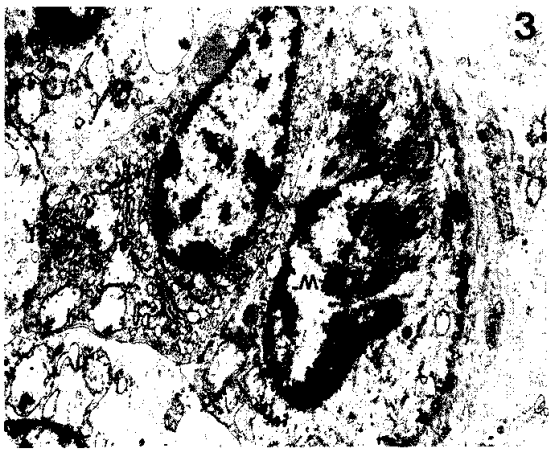

Fig. 3. Maturing myoepithelial cell of 36 weeks fetus. Abundant myofibrils and a few intermediate filaments are noted. Comparing with acinar cell (A) with plentiful rough endoplasmic reticulum the myoepithelial cell shows scanty cytoplasmic organelles (× 8000)

contained abundant myofibrils and had numerous desmosomes with adjacent MECs. There were very sparse desmosomes between the MECs and the acinar cells. The intermediate filament gradually reduced in amount as the MECs matured. Subsequently the MECs were further compressed to be typical dendritic shape. In the LDS the MECs had abundant myofibrils and sparse intermediate filaments in their cytoplasmic processes (Fig. 3). As the acinar cells reached maturation, the MECs formed tight dendritic network at the outer surface of the acini. However, there still appeared a few polyhedral or spindle MECs in the intercalated ducts, but not in the striated or excretory duct. In the adult submandibular glands the dendritic MECs encircled the acini completely, and these cells contained abundant myofibrils and a small amount of intermediate filament. Numerous desmosomes were noted between the dendritic cytoplasmic processes of MECs. A few desmosomes were also found between the MECs and acinar cells. The striated and excretory ducts showed spindle-shaped MECs in the basal layer.

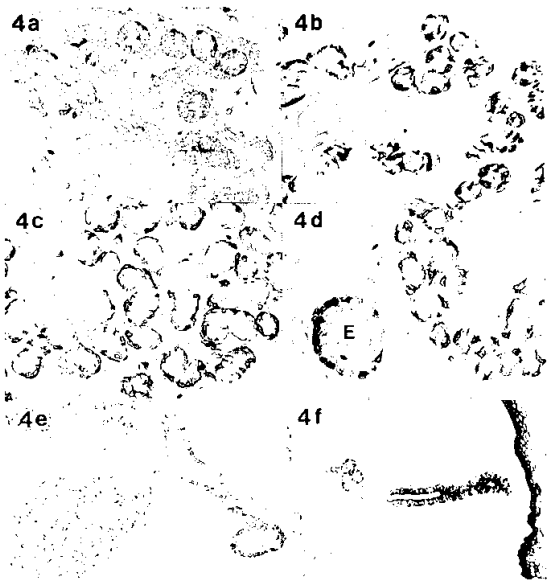

Fig. 4. No counter staining. Cytokeratin 14: a; 16 weeks fetus. Some basal cells of the terminal acini show moderate staining. b ; 22 weeks. Polyhedral or wedge shaped myoepithelial cells are intensely stained. c; 28 weeks. Spindle to dendritic myoepithelial cells are stained (× 200). d; 36 weeks. Dendritic myoepithelial cells are positive, as well as some basal cells of excretory duct (E) (× 200). e; 30 weeks. Many basal cells of the excretory duct proximal to the ductal orifice are positive, whereas distal striated duct show no positive reaction (× 100). f; 32 weeks. All basal cells of oral mucosa are strongly positive (× 100)

The MECs contained a small amount of myofibril, intermediate filaments, and smooth and rough endoplasmic reticulum.

Pleomorphic adenoma

Variable features of tumor cells, i.e., ductal cells, intermediate cells, squamous cells, modified MECs, tranformed MECs, were seen with their

Fig. 5. No counter staining. 5a-5d; double immunostainings of CK-14 (DAB, brown) / α-SMA (fast red). 5a-5c; fetal human submandibular gland. 5a; 18 weeks fetus, CK-14 staining is predominant overwhelming the α-SMA (× 400). 5b; 28 weeks fetus, α-SMA staining is increased (× 200). 5c; 36 weeks fetus, α-SMA staining is predominant overwhelming the CK-14 (× 200). 5d; pleomorphic adenoma, modified MEC in plasmacytoid shape is still positive for CK-14, while the transformed MEC in dendritic mesenchymal feature showed increased α-SMA staining (× 200). 5e-5f; double immunostaining of vimentin (DAB, brown) / α-SMA (fast red). 5e; fetal human submandibular gland, 22 weeks fetus, some basal MEC in cuboidal to wedge shape coexpress the vimentin and α-SMA (× 200). 5f; pleomorphic adenoma, a lot of modified MECs in plasmacytoid shape show strong coexpression of vimentin and α-SMA (× 200)

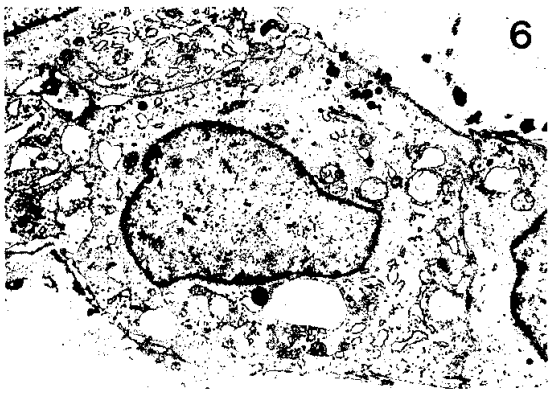

Fig. 6. Pleomorphic adenoma; typical plasmacytoid MEC, noted the abundant intermediate filaments, small amount of rough endoplasmic reticulum, and a few secretory granules (black arrows) (× 6000)

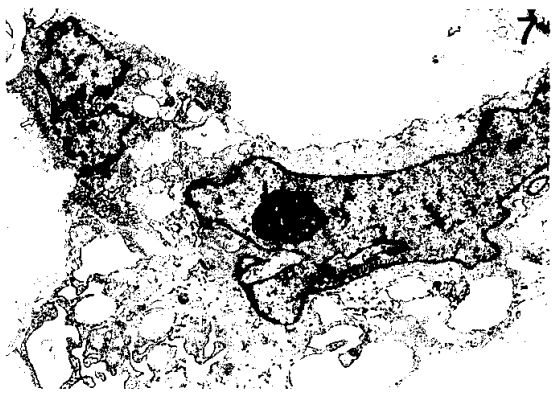

Fig. 7. Pleomorphic adenoma; typical transformed MECs, noted prominent intermediate filaments, immature myofibrils (blank arrows), well developed cytoplasmic processes, and few rough endoplasmic reticulum (× 6000)

different cytoskeletal characteristics. Among them the modified MECs, which is polyhedral in shape, usually contained abundant intermediate filaments and few myofibrils. They also showed small a amount of rough endoplasmic reticulum and a few secretory granules in their cytoplasm. The transformed MECs, which formed typical dendritic processes, showed prominent intermediate filaments, a few immature myofibrils, and rare rough endoplasmic reculum in their cytoplasms.

Table 3. Positive reaction of CK-14, vimentin, and α-SMA in the different types of myoepithelial cells in comparison to the electron microscopic findings.

	Polyhedral MEC	Wedge MEC	Spindle MEC	Dendritic MEC
[Immunostaining]				
CK-14	- + +	- - +	- +	+
Vimentin	+	±	-	-
α-SMA	+	+ +	+ + /+ + +	+ + +
[EM findings]				
myofibril	±	+	+ +	- + +
intermediate fibril	+ - -	+ + -	+ +	+

0 MEC; myoepithelial cell Legends

DISCUSSION

The myoepithelial cells (MECs) have been considered an integral part in some salivary gland tumors. Classically, the MEC of the salivary glands has been described in the acini and intercalated ducts, and this classic decription seems true only in the developing salivary glands. In adult, the MECs are also seen in relation to the striated and excretory ducts together with acini (Dardick et al. 1989a; Lee et al. 1993a; Morinaga et al. 1987). The suggestion that the contractile MECs have efficient mobility and move retrogressively from the acinus to the distal ductal structure supports the increased number of atrophying MECs in the excretory ducts of older adult salivary glands. However, in this study the presence of an increased number of desmosomes between developing MECs during the EIDS and LIDS (Fig. 2) suggests a different histogenetic progress of the MECs between the acinar cells and ductal cells. This implies that the mature dendritic MECs constitute an intimate cytoplasmic networks with each other to encircle and "compress" the acini efficiently, and that

the MECs have great mobility and a different cell-cycle from the other salivary gland elements. It is also intriguing to note that the basal cells of the excretory ducts share certain immunohistochemical and ultrastructural features with the MECs (Dardick et al. 1990). The coexpression of CK-14, α-SMA and vimentin by ductal basal cells in the normal fetal and adult salivary glands support the view that these cells represent a different combination of ductal, myoepithelial, myoepithelial-like and undifferentiated cells (Gibson, 1983; Shear, 1966). A complex organization of actin and intermediate filaments in normal MECs of human parotid glands has been reported by Noberg and Dardick (Norberg et al. 1992). And α-SMA and rhodamine phalloidin with its actin specific binding property have been identified in the MECs of the developing salivary glands (Lee et al. 1993a). In the present study we found that CK-14 was expressed by the ductal basal cells as well as MECs of salivary glands in fetuses and adults. Among various cytokeratins the CK-14 (50 KD) is an acidic (type I) keratin protein, which is predominantly distributed in the basal cells of stratified epithelia including oral mucosa, and the CK-14 together with α-SMA has been consistently used for the detection of normal MECs. In this study the polyhedral MECs in the EIDS showed prominent CK-14 staining overwhelming the α-SMA immunoreactivity. And these cells were found to contain abundant intermediate filaments and a few myofibrils ultrastructurally. Meanwhile dendritic MECs in the LDS and adulthood showed strong α-SMA staining overwhelming the CK-14 immunoreactivity. They contained reduced intermediate filaments but abundant myofibrils ultrastructurally. Although some basal cells of striated and excretory ducts of fetal salivary glands showed positivity for CK-14, they failed to show any myofibrils ultrastructurally. Therefore, we presumed that the CK-14 positive basal cells of striated and excretory ducts of normal adult salivary glands were hardly differentiated into MECs.

Gustafsson et al. (1988) reported that vimentin was expressed for the MECs and basal cells of excretory ducts of 18-22 weeks old fetal salivary glands, and suggested that the coexpression of cytokeratin and vimentin in certain salivary gland tumors may be a sign of undifferentiation in expressing the filament pattern of earlier gland development stage. In this study we confirmed that some basal cells in the acini and intercalated ducts expressed vimentin only during the EIDS and LIDS. The vimentin-positive cells were of polyhedral shape in the EIDS and wedge-shape in the LIDS, very much reminiscent of the polyhedral and wedge-shaped modified MECs of pleomorphic adenoma that showed positive reaction for CK-14, α-SMA, and vimentin (Lee et al. 1993a). Although in this study the vimentin positive cells were different in number and shape from the true MEC usually detected by CK-14 and -SMA, we presumed that these cells are in the same lineage with the MECs only in the course of myoepithelial differentiation. These findings seen in the fetal and adult salivary glands may support the fact that CK-14, vimentin and α-SMA are changeably expressed in the modified MECs of pleomorphic adenomas. The utilization of vimentin as a marker of myoepithelial derivation in the salivary gland tumor cells has been debated by many authors (Azymi et al. 1987; Caselitz et al. 1984; Caselitz et al. 1981; Zarbo et al. 1991). Vimentin is widely distributed in the mesenchymal cells, but never found in normal epithelial cells. Therefore, its positivity in salivary gland tumors suggested the occurrence of mesenchymal metaplasia of myoepithelial tumor cells.

It is generally considered that the pleomorphic adenoma and adenoid cystic carcinoma are characterized by a proliferation of modified MECs. And Dardick et al. (1990) suggested that the intermediate cells of mucoepidermoid carcinoma are the counterparts of modified MECs of pleomorphic adenoma. Co-expression of keratin and vimentin in pleomorphic adenoma and adenoid cystic carcinoma has been reported (Caselitz et al. 1984; Chomette et al. 1991; Chomette et al. 1991). And its significance could be found in fetal salivary glands. In this study by double immunostaining method we have observed predominant staining of CK-14 during the EIDS, and predominant staining of α-SMA during the

LIDSing the EIDS, and predominant staining of α-SMA during the LIDS and LDS. And also ultrastructurally it could be confirmed the increased of intermediate filaments during the EIDS, and increased of myofibril in the LIDS and LDS. Although it is still difficult to identify the exact content of intermediate filaments by ultrastructural observation or immunohistochemical detection, participation of variable amount of intermediate filaments and myofibrils in the modified MEC play an important histogenetic role in salivary gland tumors. However, the present study reveals the over-expression of CK-14 on α-SMA for the fetal MECs of EDS and EIDS and for the modified MECs of pleomorphic adenoma, and also disclosed the vimentin expression in the basal cells of terminal ductal epithelium in the EDS and some polyhedral or wedge-shaped basal cells of acini and intercalated ducts in the EIDS and LIDS. It appears that the primitive MECs of normal fetal salivary glands can express vimentin only in the early differentiation stage. The outer layer cells of tubulo-ductal structure of pleomorphic adenoma showed a strong positivity of vimentin. This fact supports the presumption that the modified MECs are related to the undifferentiated cells of ductal basal cells rather than to mature MECs of acini.

It has been repeatedly demonstrated that modified or neoplastic MECs express neural cell markers, i.e., S-100 protein, neuron specific enolase and glial fibrillary acidic protein (Lee et al. 1993b). It was also reported that some basal cells of terminal acini and ducts in the early fetal salivary gland expressed S-100 protein, and ductal cells of early fetal salivary gland expressed glial fibrillary acidic protein and neuron specific enolase, while MECs in normal glands do not (Dardick et al. 1991). The modified or neoplastic MECs in salivary gland tumors consistently express the above neural cell markers. Therefore, it is quite probable that these so-called £modified MECs£ express the phenotypes related to neural crest and/or the ductal basal structures. Although the embryonal ductal basal cells eventually developed into each salivary compartments, i.e., acini, intercalated ducts, striated ducts, and excretory ducts, it is generally agreed that the basal cells of acini and intercalated ducts have a capacity to differentiate into the true MECs. And many authors insisted that the various histologic features in pleomorphic adenoma were mainly based on a divergent histogenesis of the MEC (Caselitz et al. 1981; Dardick et al. 1989b; Kahn et al. 1985; Mori et al. 1987; Palmer et al. 1985). It was also supposed that pleomorphic adenomas arise from intercalated duct reserve cells, that are actually major precursor cells of MECs (Batsakis et al. 1992; Dardick et al. 1990; Mori et al. 1987). We can only partly agree with this opinion. Rather we believe that many pleomorphic adenomas arise from the intercalated duct cells, and would like to suggest that the so-called modified MECs of salivary gland tumors originate from the ductal basal cells of not only the intercalated ducts but also the striated and excretory ducts.

With the present immunohistochemical and ultrastructural findings in fetal, adult and neoplastic salivary glands, we believe it is important to reevaluate the origin of modified MECs in pleomorphic adenoma and allied diseases of salivary glands. We also believe the "modified MECs" are not of myoepithelial origin. They are rather modified basal cells of the ductal system in the salivary gland tumors.

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