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Prenatal Development of Human Major Salivary Glands. Histological and Immunohistochemical Characteristics with Reference to Adult and Neoplastic Salivary Glands.

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In order to investigate the histogenesis of salivary glands and their tumours, exploration of stem cells is regarded to be a fundamental issue. There is little doubt that the parotid gland arises from the stomodeum and is therefore of ectodermal derivation. However, the sublingual and submandibular glands remain somewhat obscure as to their derivation, but probably are ectodermally based, contrary to lingual glands (including von Ebner's glands) which are undoubtedly endodermal derivatives (Avery, 1987 Batsakis, 1980).

The frequency of occurrence of salivary gland tumors has been shown to greatly vary among major salivary glands and histopathological type of tumors. Those differences are probably related to the histologic varieties of prenatal developing salivary glands. Compared to the development of rat or mouse salivary glands, which are differentiated predominantly postnatally (Takai et al., 1985), the development of human salivary glands has not been elucidated in detail.

1. Development of salivary glands and histological scoring

In order to estimate the developmental stage of fetal salivary glands, incremental scores for glandular structures from each salivary gland were made (Lee et al. 1990 a, b). A total of 70 aborted human fetuses, confirmed as normal by histopathological examination, were obtained from the files of the RCM (Registration of Congenital Malformation, Department of Pathology, Seoul National University Children's Hospital, Seoul, Korea). Their gestational ages ranged from 10 weeks to 40 weeks. The tissue bank included 57 parotid, 60 submandibular and 51 sublingual glands, fixed in 10% neutral formalin. Serial paraffin sections of 4 μm were used for immunohistochemical studies and to identify histologic features using HE and PAS stain. The excretory ducts, striated ducts, intercalated ducts and acinar cells were observed in different developmental stages, each exhibiting characteristic histology. Two early stages in fetal salivary gland development were added for the purpose of descriptive representation.

The development stages of salivary glands were estimated using the criteria for developmental scoring of tissue elements of salivary glands listed in Table 1.

Excretory ducts: In the excretory duct, score 1 is for the initial stage of growth of cord-like strands in salivary gland mesenchyme. Score 2 is for the stage of luminalization with the appearance of inner ductal cell arrangemet in the main stalk of the salivary epithelium. Score 3 is the appearance of inner ductal cell arrangement in the main stalk of the salivary epithelium. Score 3 is for the stage of ductal development in the extra-lobular space. Score 4 is for the thickened excretory ductal epithelium composed of 3 to 4 cell layers(stratification) and shows prominent vascular proliferation around the excretory duct. Score 5 is for the stage of the differentiation of vascular proliferation around the excretory duct. Score 5 is for the stage of the differentiation of inner duct cells into columnar cells with abundant apical cytoplasm. Score 6 is for the stage of maturation of

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Table 1. Histological scores for the observation of developing major salivary glands.

A. Excretory duct
1. Cord-like epithelial strands grow deeply into the salivary gland mesenchyme making several branches.
2. Luminalization begins from the distal tubules, and there appears the arrangement of an inner ductal cell layer.
3. A 2-cell layer ductal structure composed of inner columnar cell layer and outer cuboidal cell layer usually prominent in the extra-lobular space.
4. The ducts thicken and become 3 to 4-cell layer ductal structure in the interlobular space. Abundant vascularity appears in the vicinity of the duct.
5. Inner ductal cells show columnar nuclei and abundant apical cytoplasm, and the outer ductal cell is cuboidal in shape.
6. Mature excretory ducts show a well aligned columnar inner cell layer and a slightly columnar basal cell layer with a conspicuous basement membrane. The distal side of the excretory duct discloses and increased multi-cell layer of ductal epithelium compared to the proximal side excretory duct which is usually located in the interlobular space.

B. Striated duct
1. Cord-like epithelial strands grow deeply into salivary gland mesenchyme making several branches.
2. Luminalization begins from the distal tubules and there appears the arrangement of an inner ductal cell layer.
3. A 2-cell layer ductal structure composed of inner and outer cuboidal cell seen in the intralobular space.
4. The duct becomes elongated and convoluted. Abundant vascularity appears in the vicinity of the duct.
5. Inner ductal cells show columnar nuclei and abundant apical cytoplasm, and the outer cuboidal ductal cells decrease in number.
6. Mature striated ducts lined by a layer of tall columnar cells with definite basement membrane. Occasionally polyhedral cells found in the basal area.

C. Intercalated duct
1. Club-like epithelial strands grow deeply into salivary gland mesenchyme making several terminal epithelial bulbs.
2. Luminalization begins from the distal tubules and there appears the arrangement of an inner ductal cell layer.
3. A 1-cell layer ductal structure in direct connection with acini.
4. Single layered inner ductal cells are cuboidal in shape and infrequently surrounded by flattened basal cells.
5. A 2-cell layer ductal structure composed of inner cuboidal cells and outer flattened cells.
6. Mature intercalated ducts consist of a definite cuboidal cell layer and frequent outer polyhedral cells. The basement membrane becomes conspicuous.

D. Acinar cells
1. Knob-like bulging of the proximal epithelium.
2. Several terminal bulb-like structure formed.
3. The primitive acini obtain an alveolar pattern.
4. Initial acinic cell differentiation.
5. Moderate acinic cell differentiation.
6. Complete acinic cell differentiation.

the excretrory duct including the presence of a conspicuous basement membrane.

Striated ducts: In the striated duct, score 1 is for the initial stage of cord-like epithelial strands. Score 2 is for the stage of luminalization with the appearance of an inner ductal cell arrangement, usually in the branched ductal epithelium. Score 3 is for the stage of a 2-cell layer ductal structure, usually in the intralobular space. Score 4 is for the stage of elongation and convolution of the striated duct and abundant capillary proliferation. Score 5 is for the stage of differentiation of inner ductal cells into tall columnar cells, resulting in the outer basal cells becoming reduced in number. Score 6 is for the maturation of the striated duct which consists of almost 1-cell layer with a conspicuous basement membrane. There were infrequently found polyhedral cells in the basal area of the duct.

Intercalated ducts: In the intercalated duct, score 1 is for the initial stage of club-like epithelial proliferation in the terminal branches. Score 2 is for the stage of luminalization with the appearance of an inner ductal cell nests. Score 3 is for the stage of 1-cell layer ductal structure connected with the terminal bulging of the acini. Score 4 is for the stage of proliferation of the ductal cells, resulting in the inner ductal cells being surrounded by flattened outer cells. Score 5 is for the stage of differentiation of duct cells into inner luminal cells of cuboidal shape and outer basal cell of a flattened shape. Score 6 is for the stage of maturation of the intercalated duct which is composed of cuboidal luminal cells and flattened basal cells with a conspicuous basement membrane.

Acinar cells: In the acinar compartment score 1 is for the stage of initial knob-like bulging of the proximal epithelium. Score 2 is for the stage of multiple bulb-like formations in terminal epithelium.

Score 3 is for the stage of occurrence of primitive acini having an alveolar arrangement. Score 4 is for the stage of initial acinar cell differentiation in the glandular lobules. Score 5 is for the stage of moderate acinar cell differentiation in the glandular lobules. Score 6 is for the stage of almost complete differentiation of acinar cells.

In summary, the excretory ducts were characterized by the most rapid maturation compared with others. Excretory duct maturation was identified predominantly by the stratification of ductal epithelium and by the cytodifferentiation of luminal epithelium. Excretory duct maturation took place from about 24 to 25 weeks of gestation in all major salivary gland. The striated ducts were principally composed of 2 cell layers in the early fetal period ; inner luminal cells and outer basal cells. A characteristic of striated duct maturation was that the luminal ductal cells gradually developed into tall luminal cells occupying the whole ductal thickness and forming a basement membrane along the basal border. With maturation, the number of ductal basal cells gradually decreased. The striated ducts of sublingual glands began to mature from 26 weeks of gestation. From 31 weeks of gestation, the striated duct was dilated similar to the excretory duct and became indistinguishable in histologic preparations. The striated ducts in submandibular and parotid glands also began to mature from 28 to 29 weeks of gestation and were characterized by a tall columnar single cell layer. The intercalated ducts continued to proliferate until 31 weeks of gestation in the sublingual glands, 38 weeks in submandibular gland, and 39 weeks in parotid gland. The acinar development was remarkably comparable with the PAS stainability. The sublingual gland, composed of mucous acini, showed a maximum reaction with PAS staining from 27 weeks of gestation forth. The sublingual gland consisted of short striated ducts, intercalated ducts, and relatively dilated excretory ducts. The submandibular gland partially consisted of mucous acini, and contained abundant PAS positive material from 31 weeks of gestation. The parotid gland was an entire serous gland showing abundant PAS reaction from 36 weeks of gestation.

Using the histological scoring system described above for the 4 tissue elements, the growth state of developing major salivary glands could be assesed by their growth curve (Fig. 1, Table 2, 3). Our study

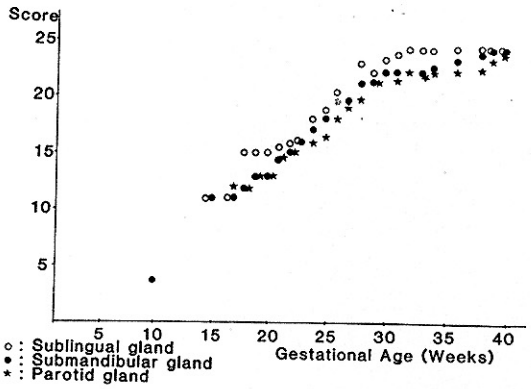

Fig. 1. Growth curve by the sum of histological scoring. The growth stage of developing major salivary gland assessed by the total score of 4 tissue elements(excretory ducts, striated ducts, intercalated ducts, and acinar cells).

disclosed that the development of the sublingual gland was the most rapid among the 3 major salivary glands, and the submandibular and parotid glands had a similar rate of development. However, the submandibular gland tended to develop slightly faster than the parotid gland. The growth curve of major salivary glands indicated that their growth rate was characteristically accelerated from the 15th week to 32nd week of gestation, and at about 35 weeks of gestation complete maturation of major salivary glands was accomplished.

2. Developmental stages of fetal salivary glands

As illustrated on the growth curve (Fig. 1), fetal salivary glands could be divided into 4 stages. The 1st is the early development stage (EDS) from 10 weeks to 18 weeks of gestation. The 2nd is the early intermediate development stage (EIDS) from 19 weeks to 24 weeks. The 3rd is the late intermediate development stage (LIDS) from 25 weeks to 32 weeks, and the 4th is the late development stage (LDS) or mature stage from 33 weeks to full term. During the EDS, glandular epithelial cells proliferated rapidly producing luminalization and forming several branches in the epithelial cords. Generally, the intermediate development stage (IDS) of fetal salivary glands coincided with the period of most rapid proliferation and cytodifferentiation. In the EIDS, glandular ducts proliferated to form a typical ductal

Table 2. Histological scoring of the developing major salivary glands in human fetuses

G.A. (weeks)	Cases (No)	Types of salivary glands											
		Parotid gland				Submaxillary gland				Sublingual gland			
		A	B	C	D	A	B	C	D	A	B	C	D
10	1	-	-	-	-	2.0	1.0	1.0	-	-	-	-	-
15	1	-	-	-	-	3.0	3.0	3.0	2.0	3.0	3.0	3.0	2.0
17	2	3.0	3.0	3.0	3.0	3.0	3.0	3.0	2.0	-	-	-	-
18	2	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	4.0	4.0	4.0	4.0
19	1	4.0	3.0	3.0	3.0	4.0	3.0	3.0	3.0	4.0	4.0	3.0	4.0
20	3	4.0	3.0	3.0	3.0	4.0	3.0	3.0	3.0	4.5	3.5	3.5	3.5
21	3	4.0	4.0	3.7	3.0	4.0	4.0	3.5	3.0	4.0	4.0	3.5	4.0
22	2	4.0	4.0	4.0	3.0	4.0	4.0	4.0	3.0	4.0	4.0	4.0	4.0
23	4	4.3	4.0	4.0	3.7	4.5	4.0	4.0	3.8	4.0	4.0	4.0	4.0
24	5	4.5	4.0	4.0	4.0	5.0	4.0	4.3	4.0	4.8	4.0	4.5	4.8
25	5	5.0	4.0	4.0	4.3	5.0	4.0	4.7	4.3	5.0	4.0	5.0	4.8
26	8	5.1	4.1	4.6	5.0	5.3	4.1	5.0	5.0	5.1	4.9	5.1	5.1
27	2	5.0	4.0	5.0	5.0	5.5	4.0	5.0	5.0	6.0	6.0	5.5	5.5
28	3	5.7	4.0	5.0	5.0	6.0	5.0	5.0	5.0	6.0	5.7	5.0	5.0
29	5	6.0	5.0	5.0	5.0	6.0	5.0	5.0	5.0	6.0	6.0	5.5	5.5
30	1	-	-	-	-	6.0	6.0	5.0	5.0	-	-	-	-
31	3	6.0	5.0	5.0	5.0	6.0	6.0	5.0	5.0	6.0	(6.0)	5.5	6.0
32	1	6.0	6.0	5.0	5.0	-	-	-	-	6.0	(6.0)	6.0	6.0
33	3	6.0	5.7	5.0	5.0	6.0	6.0	5.0	5.0	6.0	(6.0)	6.0	6.0
34	2	6.0	6.0	5.0	5.0	6.0	6.0	5.0	5.5	6.0	(6.0)	6.0	6.0
36	1	6.0	6.0	5.0	5.0	6.0	6.0	5.0	6.0	6.0	(6.0)	6.0	6.0
38	5	6.0	6.0	5.0	5.0	6.0	6.0	5.1	6.0	6.0	(6.0)	6.0	6.0
39	2	6.0	6.0	5.0	6.0	6.0	6.0	6.0	6.0	-	-	-	-
40	5	6.0	6.0	5.0	6.0	6.0	6.0	5.0	6.0	6.0	(6.0)	5.0	6.0
Total	70												

Abbreviation : GA gestation age, A excretory duct, B striated duct, C intercalated duct, D acinus, (,) illdefined observation of striated duct of sublingual gland

Table 3. Sum of the histological scoring of the developing major salivary glands in human fetuses.

G.A. weeks	Cases [No.]	Types of salivary glands		
		Parotid gland	Submaxillary gland	Sublingual gland
10	1	-	4.0	-
15	1	-	11.0	11.0
17	2	12.0	11.0	11.0
18	2	12.0	12.0	15.0
19	1	13.0	13.0	15.0
20	3	13.0	13.0	15.0
21	3	14.7	14.5	15.5
22	2	15.0	15.0	16.0
23	4	16.0	16.3	16.0
24	5	16.5	17.3	18.0
25	5	17.3	18.0	18.8
26	8	18.0	19.4	20.1
27	2	19.0	19.5	23.0
28	3	19.7	21.0	21.7
29	5	21.0	21.0	23.0
30	1	-	22.0	-
31	3	21.0	22.0	23.5
32	1	22.0	-	24.0
33	3	21.7	22.0	24.0
34	2	22.0	22.5	24.0
36	1	22.0	23.0	24.0
38	5	22.0	23.5	24.0
39	2	23.0	24.0	-
40	5	24.0	24.0	24.0
Total	70			

structure, that is, the excretory ducts became multiple layered, and the striated and intercalated ducts became elongated, consisting of 2 cell layers. This was contrary to acinar cell development which developed into lobular structures and began to differentiate into mature acinar cells. In the LIDS, ductal cells gradually differentiated into mature ductal cells, and the multi-cell layer of the excretory duct differentiated into columnar luminal cells for inner layer and cuboidal basal cells for outer layer. Luminal cells of striated duct became tall columnar cells with abundant apical cytoplasm, and intercalated duct cell became a 2-cell layer structure composed of cuboidal luminal cells and polyhedral basal cells. The acinar cells showed maturation with abundant PAS positive material in the lumen. In the LDS, salivary gland elements gradually matured until full term of gestation, and PAS positive material was present in the acini and occasionally in the ductal lumen. The salivary gland lobules were separated by connective tissue septa, and intercalated and striated ducts usually occupied intralobular position; whereas, excretory ducts were usually located in an

interlobular position. Striated ducts were quite prominent in parotid and submandibular glands while they were relatively rare in sublingual and minor salivary glands. The excretory, striated and intercalated ducts were distinguished by histological scores 4 and 5, but it was almost impossible to delineate the definite borders between these ducts in prenatal development stages.

The sublingual gland, mostly composed of mucous acini, disclosed different features of glandular development from the parotid and submandibular glands. Sublingual glands in the IDS possessed typical striated ducts according to the histological scores. However, as mucous acini matured rapidly in the LIDS, striated ducts became dilated and indistinguishable from the excretory duct. Intercalated ducts of sublingual glands were shortened and became isthmus-like structures connected with the acinar cells. On the other hand, serous acini, which matured later than mucous acini, were gradually compressed to the periphery of acinar alveoli and became a serous demilune structure. Gibson(1983), using salivary gland tissue from 6 human fetuses 13.5 to 16 weeks, observed that the development of the submandibular gland was morphologically equivalent to the development stage of the glands seen in the newborn rat or mouse, and stated that the human submandibular gland would likely reach a mature state by birth, because there was ample time remaining in a normal gestation for the maturation process to be completed. Many authors have stated that human salivary glands could mature before birth because of the length of human gestation compared with the rat or mouse, and that early secretory function is possible at the beginning of the 2nd trimester (Otis and Brent, 1954; Leeson and Forman, 1981; Gibson, 1983).

3. Prenatal developmental of the myoepithelial cell of the human submandibular gland observed by immunohistochemistry of smooth muscle actin and rhodamine-phalloidin fluorescence

The myoepithelial cell, as a contractile dendritic cell, plays an important role in the secretory function of the salivary gland. Its heterogenic differentiation has been the source of the variable complexity of the salivary gland tumors (Caselitz et al., 1986). Despite the importance as a cellular component the precise patterns in the development and distribution of salivary gland myoepithelial cells are still a matter of conjecture. Explorations on the myoepithelial cells in animal models and adult human salivary glands have elucidated several important facts. As one of the detectable probes for the myoepithelial cell, rhodamine-phalloidin was introduced and has been frequently used for its actin-specific binding property. The monoclonal antibody (MoAbs) of actin and myosin were also widely used to demonstrate the myoepithelial cells.

MoAb smooth muscle actin is very reliable to detect the early development of myoepithelial cells in human fetus, while phalloidin, which is generally specific for the actin filaments of both muscular and non-muscular actin. As markers for the cytoplasmic actin of the myoepithelial cells we used MoAb smooth muscle actin and phalloidin and studied 100 fetal submandibular glands (Lee et al., 1993a,b). By this method we found that the primitive myoepithelial cells, showing irregular fibrillar deposition, appeared in the salivary gland epithelium between 15-16 weeks of gestation, when the cytodifferentiation of acinar and ductal luminal cells had not yet taken place. These immature myoepithelial cells gradually increased in number as well as in intensity for actin staining, and they were closely apposed to the basal layer of the tubulo-alveolar structure of the salivary glands. During the early developmental stage (EDS) the phalloidin fluorescence became condensed in the basal layer. On the other hand in the previous study (Lee et al., 1991) we reported that during the EDS the primitive acinar cells of developing salivary glands were negative for MAM-3, MAM-6 antigens and epithelial membrane antigen, and the ductal luminal cells were barely stained for different MoAbs to keratins. From these findings, we could surmise that myoepithelial cells in the fetal salivary gland express the actin filaments before the acinar cells and ductal luminal cells start to mature. In other words the myoepithelial cells antedate the secretory epithelium during embryogenesis.

As the acinar cell developed further to form alveolar structure during the EIDS, we found the cytoplasmic elongation of the myoepithelial cells became evident by phalloidin fluorescence. The myoepithelial cells took place in the basal portions of the acini and intercalated ducts, and finally they

were extruded in the excretory and striated ducts. The immunostaining of MoAb actin demonstrated basaloid arrangement of polyhedral myoepithelial cells during the EIDS, and it was of interest considering the acinar and ductal cells started to express cytoplasmic epitopes such as cytokeratins, MAM-3, and MAM-6 antigens at this developmental stage. This type of myoepithelial cell was also frequently found in various salivary gland tumors as plasmacytoid or polyhedral myoepithelial cells (Caselitz et al., 1986). In the late intermediate developmental stage (LIDS) the myoepithelial cells became further flattened and dendritic at the basal portions of the acini and intercalated ducts that were reminiscent of the adult salivary glands.

This sequence of cellular events in humans is of interest. In rats, myoepithelial cells and secretory glandular cells develop at the same time during the fetal period, and it is the myoepithelial cell which subsequently differentiates asynchronously with the secretory cells. In our study conspicuous dendritic myoepithelial cells appeared during 25-26 weeks of gestation, when glandular acini were immature, and thereafter they increased in number through LIDS and LDS in conjunction with differentiation and maturation of acinar cells. Therefore in the human fetus, it is probably important for the myoepithelial cells to mature earlier in order to push the secretory materials from the acini out toward the excretory ducts, against the amniotic fluid pressure.

The difference of the myoepithelial cell distribution in the striated and excretory ducts between fetal and adult salivary glands (Table 4, 5) strongly suggested the existence of a dynamic aging process in the formation and distribution of myoepithelial cells. Based on spindle shape and dendritic processes it appears that the myoepithelial cells are contractile and first develop in the acini and intercalated ducts, and subsequently move toward the excretory ducts through the basal portions of the ducts. The latter process may usually take place postnatally but seems to be negligible prenatally. However, with the fact of the general aging process of the epithelial cells, that the atrophic cells are pushed out superficially and eventually extruded from the epithelium, the present findings may indicate that myoepithelial cells develop from intercalated duct reserve cells and mature in the acini. One may also say that contrary to the degenerating acinar and ductal cells which are able to be exfoliated directly into glandular lumen, the degenerating myoepithelial cells in the acini move toward the stratified epithelium of excretory ducts where cellular mobility as well as cellular turn-over are relatively activated.

Regarding the morphological changes of the myoepithelial cells during the fetal development, from polyhedral shape to dendritic form, the primitive polyhedral myoepithelial cells are similar to the

Table 4. Myoepithelial cell development in different stages of fetal submandibular glands observed by immunostaining of MoAb smooth muscle actin and phalloidin fluorescence.

Stages (weeks)	Cases	Acinus	Intercalated duct	Striated duct	Excretory duct
EDS(10-18)	13	1	0-1	0	0
EIDS(19-24)	21	2	1	0	0
LIDS(25-32)	38	3-4	2	0	0
LDS(33-40)	28	4	2-3	0	0
Total	100				

Degree of developmental scores of myoepithelial cell :
Score 0 : negative reaction, Score 1 : primitive myoepithelial cell,
Score 2 : immature polyhedral myoepithelial cells,
Score 3 : flattened or spindle-shaped myoepithelial cell,
Score 4 : abundant dendritic myoepithelium
EDS : early developmental stage
EIDS : early intermediate developmental stage
LIDS : late intermediate developmental stage
LDS : late developmental stage

Table 5. Myoepithelial cell distribution of adult human submandibular glands observed by the immunostaining of MoAb smooth muscle actin and phalloidin fluorescence

Age (decade)	Cases	Acinus	Intercalated duct	Striated duct	Excretory duct
2nd	2	+++	++	+(rare)	+(rare)
3rd	1	+++	++	+(rare)	+(rare)
4th	3	+++	++	+(occasional)	+(rare)
5th	2	+++	++	+(occasional)	+(occasional)
6th	2	+++	++	+(frequent)	+(occasional)
Total	10				

Degree of developmental scores of myoepithelial cell :
- : no myoepithelial cell,
+ : atrophic polyhedral myoepithelial cell,
++ : flattened or spindle-shaped myoepithelial cell,
+++ : abundant dendritic myoepithelium

plasmacytoid myoepithelial cells of salivary gland tumors. The fact that the myoepithelial cells contain contractile actin filaments could explain the probable mobility of the myoepithelial cells in the salivary gland tumors as well as in the normal salivary glands. Especially in pleomorphic adenoma the transitional or transformed myoepithelial cells gradually infiltrate into glandular mesenchyme to produce typical myxoid degeneration, and they show slight immunoreactivity of MoAb actin and different cytokeratins. Actually in our extended study on the salivary gland tumors the transformed myoepithelial cells were occasionally positive for the MoAb actin and showed reduced phalloidin fluorescence. Therefore we presume that the neoplastic myoepithelial cells are also mobile but they overgrow in abnormal directions and subsequently transform into different tumor cells exhibitig cytologically different phenotypes. However, in order to get to know the more precise life-cycle mode of normal and neoplastic myoepithelial cells further study should be recommended with other biological methods.

4. Immunohistochemical detection of S-100, S-100α, S-100β proteins, glial fibrillary acidic protein, and neuron specific enolase in the prenatal and adult human salivary glands

Although studies on the markers of the myoepithelial cells have been controversial in the literature, S-100 protein has been frequently introduced as a useful marker. But recent studies using polyclonal antibody (PoAb) to S-100 protein have shown negative result for the myoepithelium of the normal adult salivary glands (Mori et al., 1986; Dardick et al., 1991). The occasional positive reaction of S-100 protein for the myoepithelium was attributed to the staining of unmyelinated nerve endings, and it was also suggested that the unmyelinated nerve endings made direct contact with plasma membranes of the myoepithelial and acinar cells (Huang et al., 1992). Nevertheless, it is accepted that the myoepithelial derived cells of pleomorphic adenoma show intense reaction for S-100 protein (Crocker et al., 1985), and that the modified myoepithelial cells of pleomorphic adenoma may display positive reaction for vimentin, glial fibrillary acidic protein (GFAP), and sometimes neuron specific enolase (NSE) (Mori et al., 1989a,b). Therefore, it may be claimed that the neoplastic myoepithelial cells in pleomorphic adenoma represent undifferentiated phenotypes of salivary gland cells of neurogenic origin.

Many authors supposed that progenitor cells or reserve cells may be present either in intercalated ducts or in other ductal segments of the salivary gland, and may have the potential to transform either to myoepithelial or ductal epithelial cells. They presumed that the great variation in the expression of S-100, S-100α, and S-100β, GFAP, and NSE in pleomorphic adenomas (Mori et al., 1989 a, b ; Ninomiya et al., 1989 ; Huang et al., 1992) suggested the possibility of the transformation of such progenitor cells toward various directions. We have examined a hundred developing human fetal salivary glands of gestational age from 10 to 40 weeks (n=100) and normal adult glands (n=10) for immunoreactivity to S-100 protein and its subunits S-100α, S-100β, GFAP and neuron specific enolase (NSE) (Lee et al., 1993b). In the early intermediate developmental stage (19-32 weeks) some acinar basal cells showed immunoreactivity to S-100 protein which rapidly disappeared in the late developmental stage (33-40 weeks). Adult salivary glands were negative for S-100 protein (Table 6). The S-100α subunit was strongly positive in the glandular ducts and acini of both fetal and adult glands. S-100β, although present in some acini and ductal cells during the late intermediate developmental stage, was rarely seen in the adult glands. GFAP and NSE were positive in the developing salivary epithelium in the early developmental stage (15-18 weeks). Above findings indicated that the developing salivary epithelia show transient neuronal phenotype during active cytodifferentiation stage of glandular acini and ducts. Our study (Lee et al., 1993b) also demonstrated early expression of GFAP and NSE during 15-18 weeks of gestation when the cytodifferentiation of salivary gland epithelium begins. Weak positive reaction was localized not only in the presumed myoepithelial cells but usually in the proliferating acinar and ductal cells(Table 6). Therefore, we presumed that the actively proliferating and differentiating salivary gland epithelium during the early fetal period displays transient neural characteristics.

Classical description of myoepithelial cells in relation to their presence in the acini and inter-

Table 6. Immunohistochemical detection of various antibodies in the developing salivary glands of human fetuses and adults.

Developmental stages(weeks)	number	S-100	S-100α	S-100β	GFAP	NSE
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

calated ducts of salivary glands has been well mature myoepithelial cells which are localized at the acini and intercalated ducts of prenatal human developing salivary glands, whereas they show wide distribution in the ductal system of the adult human salivary glands.

Our study (Lee et al., 1993 b,c) also showed that the immunostaining of PoAb S-100 protein was not specific for the myoepithelial cells, and was expressed in some serous acinar cells of fetal salivary gland. The presence of S-100 protein was most intense during the period of EIDS and LIDS at which the cytodifferentiation of serous acinar cell was most active. These findings indicated that the S-100 protein plays a role during the differentiation and maturation of serous acinar cells. It was reported that regenerating intercalated ductal cells or serous acinar cells of adult salivary gland showed a strong reaction for PoAb S-100 protein. This fact can be correlated with a marked reduction of S-100 protein expression following duct ligation of the submandibular glands in rats(Hashimoto et al., 1992; Shinohara et al., 1992). These findings imply a significant role of S-100 protein in the alternative metabolic or cytodifferentiation processes. However, the stainability for S-100 proteins in neoplastic myoepithelium still remains to be confirmed.

In our study (Lee et al., 1993 b) normal adult salivary glands showed diffuse positivity for S-100α in serous acinar cells and ductal cells. On the other hand S-100β was known not to be found in acinar cells of the salivary gland. Molin et al.(1984) also stated that the ductal segments, including convoluted tubules, striated and excretory ducts of rat salivary glands expressed S-100α, while S-100β was found only in the intercalated duct. Although Hara et al.(1984) observed that S-100β protein was diffusely positive in the cytoplasm of normal myoepithelial cells as well as tumor cells of pleomorphic adenoma, we could not find any dendritic myoepithelium which was positive for PoAb S-100 or MoAb S-100β protein. What we found was that some serous acinar cells and intercalated duct cells of fetal salivary glands were strongly positive for MoAb S-100β as condensed granular pattern in the EIDS and LIDS, whereas the immunostaining was almost absent in adult salivary glands.

Therefore, after evaluation of normal developmental and neoplastic transformation of the salivary glands a suggestion could be made that neuronal differentiation of ductal reserve cells is responsible for the production of modified myoepithelial cells in both normal developmental salivary gland and neoplastic transformation is made.

5. Lysozyme, lactoferrin, α1-antichymotrypsin and α1-antitrypsin localization during development

A biological role of the salivary glands is to secrete mucous materials, enzymes, and antibacterial substances. In the parotid gland of normal human adults, the lactoferrin staining has been seen in some acinar cells scattered among negatively stained glandular cells. Intercalated duct cells were positive, but excretory duct cells were negative for lactoferrin (Korsrud and Brandtzaeg, 1982; Mitani et al., 1989) Lysozyme has been demonstrated in acinar cells and some intercalated ducts in mature glands (Tsukitani et al., 1985; Mitani et al., 1989). In our study (Lee et al., 1990a, b), lysozyme staining was observed begining from the EIDS, and was strongly positive in serous acinar cells more intensely than lactoferrin during the fetal period.

Our study (Lee et al., 1990a, b) revealed that lactoferrin (LF) staining was weak in the fetal salivary gland. Only a slight immunoreaction was observed in serous acinar cells and ductal cells during the LIDS. Positive lactoferrin staining in duct cells almost disappeared in the LIDS, but a slight positive LF staining in the serous acinar cell persisted. It could

be suggested that LF, which has a higher relative molecular mass (M_r-80000) than (M_r-14000), was not concentrated enough to detect immunohistochemically in fetal salivary glands compared to mature cells.

Reitamo and Konttinen(1980) observed that in all mature salivary glands, LF staining was seen in intralobular ducts but never in interlobular ducts and acini. LF staining was detected in most serous demilune cells of mixed glands and in some but not all acinar cells of pure serous glands, but never in pure mucous glands. They also examined 7 human fetuses from 11 to 21 weeks of age, and reported LF staining in mononuclear cells, presumably granulocytes, in various organs beginning at 13 weeks of gestation, and in granular cells of the tongue from 20 weeks. Caselitz et al. (1981a) described the most intense LF reaction in salivary gland adenocarcinoma, and interpreted LF production as a "marker" of glandular of acinar differentiation in parotid tissue.

Positive staining of lysozyme(LY) and LF in salivary gland tumours has also been observed. Luminal cells of pleomorphic adenomas were usually positive, whereas malignant tumour cells were negative or rarely positive (Caselitz et al., 1981a,b; Mitani et al., 1989). Others reported the frequency of a positive LF reaction in pleomorphic adenoma, was higher than that of lysozyme(LY) (Mitani et al., 1989). Confirming those results, the frequency of a positive reaction to LY and LF in the study of mature salivary glands and benign tumours was relatively higher than that in fetal salivary glands.

Alpha-1-antitrypsin (α1-AT) is a major plasma protease ihibitor and a product of metabolism in adult and fetal liver. α1-ACT and inhibits trypsin, chymotrypsin, and kallikrein (Glaudie et al., 1980). Sehested et al. (1985) and Murase et al. (1985) reported that α1-AT and α1-ACT were found in luminal epithelial tumour cells of pleomorphic adenomas but not in modified myoepithelial cells. In adult salivary glands, α1-AT was positive in the ductal segment, and alpha-1-antichymotrypsin (α 1-ACT) was usually negative in all glandular cells. Our study(Lee et al., 1990b) showed that in the LIDS, which represented the fetal period for active proliferation and cytodifferentiation of glandular epithelium, staining of α1-ACT and α1-AT increased rapidly. And in the LDS, which represented the advanced stage of fetal salivary gland development, the α1-AT reaction continuously increased in ducts and serous acini. However, α1-ACT was slightly reduced in glandular ducts. We concluded that the presence of α1-ACT and α1-AT in developing salivary gland epithelium is a characteristic of differentiation. We also presumed that the immunohistochemical demonstration of LY, LF, α1- ACT, and α1-AT in developing salivary gland tissue suggests that they might participate in a protective mechanism, particualrly in serous cells and ductal segments in the human fetus.

6. Immunohistochemical findings of lymphoid tissue in developing salivary glands

A close relationship between developing salivary gland and lymphoid tissue have been described. During the process of their simultaneous development, salivary gland acini and ducts may be embedded in the lymphoid tissue (intralymph node salivary gland) or vice versa(intra salivary gland lymphoid tissue). The development of lymphoepithelial lesions may be related to this phenomenon.

We studied 79 fetal salivary glands from the early developmental stage(EDS) to the late developmental stage(LDS)(Lee et al., 1993c). Histological examination and various immunohistochemical stains were made.

Lymphoid tissue aggregations were observed near the major excretory ducts of parotid and submandibular glands in 17 week fetus. However, localized proliferation of lymphoid tissue in the sublingual gland was seen only after 38 week of gestational age. At 20 week in the parotid and submandibular glands, the aggregations were completely separated from the glandular tissue by connective tissue septa. Numerous salivary ducts with single layer of cells were found embedded in the medulla of the lymph nodes, and at 38 week in the parotid and submandibular glands, the embedded ducts were seen disintegrating after an increase of mononuclear cell infiltration. The histologic features of ductal inclusions in all three types of salivary glands were similar.

The immunohistochemical findings of salivary glands ducts included in the lymphoid tissue and the lymphoid infiltration in all the three salivary gland were similar. In the EDS, mononuclear cells in the newly formed lymphoid tissue and connective tissue

stroma showed strong lysozomal activity. The mononuclear cells in the connective tissue stromal decreased in number with increasing gestational age. The glandular epithelial cells, especially the serous acinar cells showed increased staining for lysozome with increasing gestational age. In the LDS, immunostaining for lysozyme was intense in serous cells but negative in the mucous cells. The ductal cells were negative throughout the entire gestational period. No lactoferrin reactivity was observed in lymphoid tissue of the developing salivary gland. It was only during LIDS and LDS that the serous acini occasionally showed positive lactoferrin staining. The basal cells at the border of the intralobular striated ducts in the LIDS showed a positive LF staining, but largely in the LDS.

The immunohistochemical reaction of α1-AT was negative in the lymphoid tissue and infiltrating mononuclear cells in the connective tissue stroma of developing salivary gland. In the EDS, immunostaining was infrequent in the glandular ducts, however it became moderately positive in the LIDS. The immunoreactivity of acinar cells was sparse during the fetal period. The immunostaining of α 1-ACT in lymphoid aggregation was negative except for a mild degree during the LDS. The stromal mononuclear cells, however, showed an intense reaction to α1-ACT during all stages of development. In the EDS, the glandular epithelium showed a slight positive α1-ACT staining in the ducts. Beginning from the EIDS, α1-ACT staining became intense in the ducts and serous acini, but mucus cells had no immunostaining. However, the reactivity gradually reduced by the beginning of LDS, and only minimal reactivity remained in the glandular ducts by late LDS. In the EDS, a few S-100 protein positive cells were scattered in the lymphoid tissue.

In the EDS and EIDS a mild KL1 and K8.12 reaction in ductal cells embedded in lymph nodes were observed. The reactivity decreased with increasing gestational age and was negative in LDS. PKK1 reactivity was negative in LIDS and LDS except for a rare positive reaction in EDS and EIDS. However, in LDS, ductal cells in the vicinity of lymph nodes were strongly positive for KL1 and PKK1. Immnostaining of glandular ducts embedded in lymphoid tissue were negative to MoAbs 67D11 (Anti-MAM-3), 115D (Anti-MAM-6) and to epithelial membrane antigen throughout the gestational age studied.

The subepithelial accumulation of lymphoid tissue in the respiratory, alimentary and genitourinary tracts constrained by a connective tissue capsule, are called mucosal-associated lymphoid tissue (MALT). Although the histology and dynamics of MALT have been well characterized in the gastrointestinal tract and in the lung, there are no reports of a systemic search for MALT in the salivary glands. Our study (Lee et al., 1993b) showed that lymphoid cell aggregation is a unique, if not critical, feature in developing human salivary gland. This feature seemed to be a physiological process during development as the lymphoid aggregations were found in the great majority of the normal fetal salivary glands studied.

In conclusion, it is suggested that the early appearance of mononuclear cells in intralobular lymphoid tissue could be an immunologic response to the intraluminal antigenic material in the developing excretory ducts. This may be either endogenous or exogenous, and in turn they pose further antigenic stimulation to induce retrogressive change of the embedded salivary gland epithelium during fetal period. A high frequency of intralobular lymphoid tissue aggregation in the fetal salivary gland is therefore, a normal developing process, and the exogenous infiltration of mononuclear cells play a role to produce lymphoid tissue in salivary glands. However, the explanation for histogenesis of lymphoepithelial lesions from these inclusions need further investigation.

7. Myoepithelial cell and modified myoepithelial cells in normal and neoplastic salivary gland.

The role of myoepithelial cells(MECs) in salivary 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; Kahn, et al., 1985; Dardick et al., 1989a,b; Batsakis et al., 1992). Various monoclonal and polyclonal antibodies have been used to elucidate the nature of the cell population in normal and neplastic salivary glands with particular reference to myoepithelial cells. However, data on the markers of the in normal and neoplastic glands have not been consistent (Mori et al., 1990; Dardick et al., 1991; Morinaga et al., 1992)

Cytokeratins are expressed in normal salivary glands. 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., 1989a, b; 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 differentiated 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 was 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 papers(Lee et al., 1993a, b) we have reported that all the developing MECs were localized in 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. 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 regressing MECs in the excretory ducts of older adult salivary glands. However, in our study increased number of desmosomes between developing MECs during the EIDS and LID suggested 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 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 ultrastructrual features with the MECs. 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 combinaton of ductal, myoepithelial, myoepithelial-like and undifferentiated cells (Shear, 1966; Gibson, 1983).

A complex organization of actin and intermediate filaments in normal MECs of human parotid glands has been reported by Noberg 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). 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 detction of normal MECs. 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. 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 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 the vimentin postive cells were different in number and shape from the true MEC usually detected by CK-14 and α-SMA, we presumed that these cells are of 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 interchangeably 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(Caselitz et al., 1981a,b; Caselitz et al., 1984; Azumi and Batifora, 1987; 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 occurence 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. (1991) 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., 1991a,b). And its significance could be found in fetal salivary glands. In our study by double immunostaining method(Lee et al., 1993a) we observed predominant staining of CK-14 during the EIDS, and predominant staining of α-SMA during the LIDS and the EIDS, and predominant staining of α-SMA during the LIDS and LDS. And also ultrastructurally there were increase of intermediate filaments during the EIDS, and increase of myofibril in the LIDS and LDS. There was also 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. The outer layer cells of tubulo-ductal structure of pleomorphic adenoma showed a strong positivity of vimentin. These facts support 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., 1993a,b). 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 phenotype related to neural crest and/or the ductal basal structures. Although the embryonal ductal basal cells eventually develop 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., 1981a,b; Kahn et al., 1985; Dardick et al., 1989b). 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). We could only partly agree with this opinion. Rather we believe that many pleomorphic adenomas arise from the intercalated duct cells, and suggested that the so-called modified MECs of salivary gland tumors originate from the ductal basal cells of not only the intercalated ducts but also of the striated and excretory ducts. It is important to reevaluate the origin of modified MECs in pleomorphic adenoma and allied diseases of salivary glands. We have suggested that the 'modified MECs' in the salivary gland tumors are not of myoepithelial origin, but are rather modified basal cells of the ductal system.

References

Avery JK. *Oral development and histology : Development, structure, and function of salivary glands. Baltimore : Williams & Wilkins, 1987.*

Azumi N, Battifora H. *The distribution of vimentin and keratin in epithelial and nonepithelial neoplasms. Am J Clin Path 1987 ; 88 : 286-96.*

Batsakis JG, el-Naggar AK, Luna MA. *Epithelialmyoepithelial carcinoma of salivary glands. Ann Otol Rhinol 1992 ; 101 : 540-2.*

Batsakis JG. *Salivary gland neoplasia : an outcome of modified morphogenesis and cytodifferentiation. Oral Surg 1980 ; 49 : 229-32.*

Caselitz J, Becker J, Seifert G, Weber K, Osborn M. *Coexpression of keratin and vimentin filaments in adenoid cystic carcinomas of salivary glands. Virchows Arch 1984 ; 403 : 337-44.*

Caselitz J, Jaup T, Seifert G. *Lactoferrin and lysozyme in carcinomas of the parotid gland. Virchows Arch 1981a ; 394 : 61-73.*

Caselitz J, Osborn M, Hamper K, et al. *Pleomorphic adenomas, adenoid cystic carcinomas and adenolymphomas of salivary glands analysed by a monoclonal antibody against myoepithelial basal cells ; An immunohistochemical study. Virchows Arch 1986 ; 409 : 805-16.*

Caselitz J, Osborn M, Seifert G, Weber K. *Intermediate-sized filament proteins(prekeratin, vimentin, desmin) in the normal parotid gland and parotid gland tumours. Virchows Arch 1981b ; 393 : 273-86.*

Chomette G, Auriol M, Vaillant JM, Kasai T, Okada Y, Mori M. *Basaloid carcinoma of salivary glands, a variety of undifferentiated adenocarcinoma ; Immunohistochemical study of intermediate filament proteins in 24 cases. J Pathol 1991a ; 163 : 39-45.*

Chomette G, Auriol M, Vaillant JM, Kasai T, Okada Y, Mori M. *Heterogeneity and co-expression of intermediate filament proteins in adenoid cystic carcinoma of salivary glands. Pathol Biol 1991b ; 39 : 110-6.*

Crocker J, Jenkins R, Campbell J, Fuggle WJ, Sha V. *Immunohistochemical demonstration of S-100 protein in salivary gland neoplasms. J Pathol 1985 ; 146 : 115-21.*

Dardick I, Cavell S, Boivin M, Hope D, Parks WR, Stinson J, Yamada S, Burns BF. *Salivary gland myoepithelioma variants: Histological, ultrastructural, and immunocytological features. Virchows Arch 1989a ; 416 : 25-42.*

Dardick I, Strais M, Parks WR, DeBardi GF, Kahn HJ. *S-100 protein antibodies do not label normal salivary gland myoepithelium ; Histologic implications for salivary gland tumors. Am J Pathol 1991 ; 138 : 619-28.*

Dardick I, Thomas MJ, van Nostrand AW. *Myoepithelioma-new concepts of histology and classification : a light and electron microscopic study. Ultrastruct Pathol 1989b ; 13 : 187-224.*

Gibson MHL. *The prenatal human submandibular gland : A histological, histochemical and ultrastructural study. Anat Anz 1983 ; 153 : 91-105.*

Glaudie J, Lamonatagne L, Horsewood P, Jenkins E. *Immunohistochemical localization of α1-antitrypsin in normal mouse liver and pancreas. Am J Pathol 1980 ; 101 : 723-35.*

Gustafsson H, Kj rell U, Eriksson A, Virtanen I, Thornell LE. *Distribution of intermediate filament proteins in developing and adult salivary glands in man. Anat Embryol 1988 ; 178 : 243-51.*

Hara K, Ito M, Takeuchi J, Iijima S, Endo T, Hidaka H. *Distribution of S-100α protein in normal salivary glands and salivary glands tumors. Virchows Arch 1984 ; 401 : 237-49.*

Hishimoto J, Yamada K, Ogata K, Takai Y, Mori M. *Immunoreaction of keratin, actin, S-100 protein and rat-EGF in duct-ligated rat salivary glands. J Oral Pathol Med 1992 ; 21 : 214-20.*

Huang JW, Hashimura K, Sakamoto F, Yuba R, Mori M, Yoneda K, Yanagihara M, Mori S. *Heterogeneity and multiple expression of intermediate filament proteins, S-100 protein and neuron specific enolase in skin mixed tumor. Anticancer Res 1992 ; 12 : 1107-14.*

Kahn HJ, Baumal R, Marks A, Dardick I, van Nostrand AWP. *Myoepithelial cells in salivary gland tumors ; A immunohistochemical study. Arch Pathol Lab Med 1985 ; 109 : 190-5.*

Korsrud FR, Branditzaeg P. *Characterization of epithelial elements in human major salivary glands by functional markers : Localization of amylase, lactoferrin, lysozyme, secretory component, and secretory immunoglobulins by parotid immunofluorescence staining. J Histochem Cytochem 1982 ; 30 : 657-66.*

Lee SK, Hwang JO, Chi JG, Yamada K, Mori M. *Prenatal development of myoepithelial cell of human submandibular gland observed by immunohitochemistry of smooth muscle actin and rhodamine-phalloidin fluorescence. Pathol Res Pract 1993a ; 332-41.*

Lee SK, Kim EC, Chi JG, Hashimura K, Mori M. *Immunohistochemical detection of S-100, S-100α, S-100β proteins, glial fibrillary acidic protein, and neuron specific enolase in the prenatal and adult human salivary glands. Pathol Res Pract 1993b ; 1036-43.*

Lee SK, Lim CY, Chi JG, Hashimura K, Yamada K, Kunikata M, Mori M. *Immunohistochemical study of lymphoid tissue in human fetal salivary gland. J Oral Pathol Med 1993c ; 23-9.*

Lee SK, Lim CY, Chi JG, Yamada K, Hashimura K, Kunikata Mori M. *Prenatal development of human major salivary glands and immunohistochemical detection of keratins using monoclonal antibodies. Acta Histochemica 1990a ; 213-35.*

Lee SK, Lim CY, Chi JG, Yamada K, Kunikata M, Hashimura K, Mori M. *Immunohistochemical localization of lysozome, lactoferrin, α1-anti-chymotrypsin, and α1-antitrypsin in salivary gland of human fetuses. Acta Histochemica 1990;201-11.*

Lee SK, Lim CY, Chi JG, Yamada K, Mori M, Tsubura A, Mori S, Hilgers, Govindarajan M. *Immunohistochemical distribution of MAM-3 and MAM-6 antigens in developing salivary glands of the human fetus. Acta Histochemica 1991 ; 355-60.*

Leeson CL, Forman DE. *Postnatal development and differentiation of the secretory elements of the rabbit parotid and submandibular glands. Anat Anz 1981 ; 149 : 210-25.*

Mitani H, Murase N, Mori M. *Immunohistochemical demonstration of lysozyme and lactoferrin in salivary pleomorphic adenomas. Virchows Arch 1989 ; B57 : 257-65.*

Molin SO, Rosengren L, Haglid K, Baudier J, Hamberger A. *Differential localization of "Brain-specific" S-100 and its subunits in rat salivary glands. J Histochem Cytochem 1984 ; 32 : 805-14.*

Mori M, Minomiya T, Okada Y. *Myoepitheliomas and myoepithelial adenomas of salivary gland origin. Immunohistochemical evaluation of filament proteins. S-100α and S-100β, glial fibrillary acidic proteins, neuron-*

specific enolase, and lactoferrin. Pathol Res Pract 1989a ; 184 : 168-78.

Mori M, Murase N, Hosaka M, Orito T. *Immunohistochemical expression of S-100 protein in reactive and neoplastic myoepithelial cells of variant salivary pleomorphic adenoma. Acta Histochem Cytochem 1986 ; 19 ; 231-40.*

Mori M, Ninomiya T, Okada Y. *Myoepitheliomas and myoepithelial adenomas of salivary gland origin, immunohistochemical evaluation of filament proteins, S-100α and β, glial fibrillary acidic proteins, neuron-specific enolase, and lactoferrin. Pathol Res Pract 1989b ; 184 : 168-78.*

Mori M, Yamada K, Tanaka T, Okada Y. *Multiple expression of keratins, vimentin, and S-100 protein in pleomorphic salivary adenomas. Virchows Arch 1990 ; B58 : 435-44.*

Morinaga S, Hashimoto S, Tezuka F. *Epithelialmyoepithelial carcinoma of the parotid gland in a child. Acta Pathol Jpn 1992 ; 42 : 358-63.*

Murase N, Kobayashi K, Mitani H, Mori M. *Immunohistochemical localization of α1-antichymotrypsin in salivary pleomorphic adenomas. Virchows Arch 1985 ; 408 : 107-16.*

Ninomiya T, Naito R, Okada Y, Kobayashi K, Mori M, Tsukitani K. *Immunohistochemical localization of the α and β subunits of S-100 protein in pleomorphic adenoma of the salivary glands. Virchows Arch Cell Biol 1989 ; B57 : 63-75.*

Norberg L, Dardick I, Leung R, Burford-Mason AP. *Immunogold localization of actin and cytokeratin filaments in myoepithelium of human parotid salivary gland. Ultrastruct Pathol 1992 ; 16 : 555-568.*

Otis EM, Brent R. *Equivalent ages in mouse and human embryos. Anat Rec 1954 ; 120 : 33-63.*

Reitamo S, Konttinen M. *Distribution of lactoferrin in human salivary glands. Histochemistry 1980 ; 6 : 285-91.*

Sehested M, Barfoed C, Krogdahl A, Bretlau P. *Immunohistochemical investigation of lysozyme, lactferrin, α1-antitrypsin, α1-antichymotrypsin and ferritin in parotid gland tumor. J Oral Pathol 1985 ; 14 : 459-65.*

Shear M. *The structure and function of myoepithelial cells in salivary glands. Arch Oral Biol 1966 ; 11 : 679-780.*

Shinohara M, Oka M, Yamada K, Hashimura K, Yuba K, Mori M. *Immunohistochemical and electron microscopic studies of obstructive lesions in salivary glands. J Oral Pathol Med 1992 ; 21 : 370-75.*

Takai Y, Noda Y, Sumitomo S, Kawamura K, and Mori M. *Immunohistochemical detection of keratin proteis in salivary gland ducts of mammals. Acta Histochem Cytochem 1985 ; 18 : 353-61.*

Tsukitani K, Nakai M, Tatemoto Y, Mori M. *Histochemical studies of obstructive adenitis in human submandibular gland : I. Immunohistochemical demonstration of lactoferrin, lysozyme and carcinoembryonic antigen. J Oral Pathol 1985 ; 14 : 631-38.*

Zarbo RJ, Bacchi CE, Gown AM. *Muscle-specific protein expression in normal salivary glands and pleomorphic adenoma : an immunocytochemical study with biochemical confirmation. Mod Pathol 1991 ; 4 : 621-26.*