

Cytokeratin expression in human fetal tongue and buccal mucosa

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Expression of cytokeratins (CK), a subset of intermediate filament (IF) proteins in epithelia, is developmentally regulated. CK expression may also change after malignant transformation. Our earlier studies on CK expression in human oral tumours and pre-cancerous lesions have shown specific changes in CK expression. We analysed CK expression in human tongue and buccal mucosa (BM) in fetuses in the embryonic age group of 16 to 27 weeks using biochemical and immunohistochemical techniques to find out whether there is any similarity in CK expression in human oral squamous cell carcinomas (SCC) and fetal oral tissues. CK 1, 8 and 18 were detected in a majority of samples using both techniques. Our earlier studies had shown aberrant expression of CK 1 and 18 in many of the oral SCC and leukoplakias. Studies by immunohistochemistry showed that these different CK antigens were expressed in different cell layers. CK 1(2) were present in the stratified epithelial layers whereas CK 8 and 18 were restricted to glandular epithelium. Till 27 weeks of gestation, both tongue and BM expressed CK 1, 8 and 18 along with CK 6 and 16. Thus, fetal tissues showed some similarities in CK pattern with their respective SCC.

1. Introduction

Cytokeratins (CK), a group of intermediate filaments (IF), are specifically expressed by epithelial tissues (Anderton 1981). There are 20 different polypeptides of CK expressed by human epithelia that have been catalogued on the basis of their molecular weights and isoelectric points (Moll *et al* 1982a, 1990). CKs are subdivided into two distinct subfamilies, type I – acidic and type II – basic or neutral (Eichner *et al* 1986). These are expressed in specific pairs comprising one member of each subfamily (Hatzfeld and Franke 1985). Expression of CK is also differentiation dependent and developmentally regulated (Franke *et al* 1982). In the human oral cavity, all the lining epithelia express the CK pair of 5 (58 kDa) and 14 (50 kDa) but expression of other CKs differ in different tissues depending upon the state of differentiation and keratinisation (Morgan *et al* 1987). Thus, buccal mucosa (BM) – a stratifying, non-keratinising tissue, expresses CK 4 and 13 along with CK 5 and 14, while dorsal tongue, which is a keratinising tissue, expresses CK 1 and 10

instead of CK 4 and 13. CKs expressed during embryonic development are not always expressed in the respective adult tissues. CK expression patterns during fetal development have been studied in human tissues such as trachea, breast, lung, stomach, intestinal epithelium, skin, and enamel organs and were found to be different from those in adults (Moll *et al* 1982b; Dale *et al* 1985; Reagur *et al* 1985; Broers *et al* 1989; Kasper *et al* 1989; Stosiek *et al* 1991).

CK expression may also change after malignant transformation. Our earlier studies on CK expression in human oral pre-cancerous lesions and squamous cell carcinomas (SCC) have shown aberrant expression of some CKs (Vaidya *et al* 1989, 1996, 1998). To find out whether these CKs expressed aberrantly in cancers are also seen during human fetal development, we analysed CK expression in human tongue and BM in the embryonic age group of 16 to 27 weeks using both biochemical and immunofluorescence techniques. We report on the expression of CK 1, 8 and 18 in both human fetal tongue and BM up to 27 weeks of gestation.

Keywords. Cytokeratins; fetal buccal mucosa; tongue

2. Materials and methods

2.1 Primary antibodies

Monoclonal antibodies (MAb)-RPN 1161 against CK 1 (67 kDa), and CK 2 (63–65 kDa), and RPN 1164 against CK 8 (52.5 kDa) were procured from Amersham International, Bucks., England. MAb to CK 18 (45 kDa) was purchased from Boehringer Mannheim Biochemica, Ottweiler, Germany. Since RPN 1161 reacted with both CK 1 and 2, both these CKs are mentioned in the results of immunofluorescence while these CKs are identified by their number on the basis of 2-D gel electrophoresis. A pan-keratin antibody raised in rabbits in the laboratory was used for immunoblotting (Vaidya *et al* 1989).

2.2 Secondary antibodies

Goat anti-rabbit IgG-horse radish peroxidase (HRPO) conjugate was obtained from Lupin Laboratory, Bhopal. FITC conjugated goat anti-mouse IgG, biotinylated goat anti-mouse IgG, and Streptavidin-HRPO were purchased from Sigma Chemical Company, MO, USA.

Chemicals were purchased from the following sources. Nonidet-P-40 from Fluka Laboratories, Buchs, Switzerland. Ampholines pH range 5.0–8.0 and 3.0–10.0, phenylmethylsulphonyl fluoride (PMSF), Antipain, Pepstatin A and diaminobenzidine tetra hydrochloride, mounting medium Citifluor from Sigma Chemical Company, MO, USA.

Thirty-two human abortuses were collected from Nowroji Wadia Maternity Hospital, Mumbai, and 32 tongue and 31 BM samples from these were used for histology, immunofluorescence and gel electrophoretic analysis. The age of the fetuses was determined by measuring crown-rump length and confirmed by history of pregnancy. The fetuses ranged in embryonic age from 16 to 27 weeks. None of the fetuses showed any obvious malformations.

Immediately after obtaining the fetus, the tissues were dissected and divided into two lots, one for microscopic studies and the other for biochemical analysis. Frozen unfixed tissues were cut on cryostat. Serial 5 to 6 µm thick sections were cut at –20°C. From each sample, one section was stained with haematoxylin and eosin for histology. The other sections were immediately used for indirect immunofluorescence staining or stored at –20°C till further processing.

2.3 Immunofluorescence analysis

Sections were fixed with chilled methanol at –20°C for 5 min before staining. After two 10 min washes with phosphate buffered saline (PBS), the sections were incubated with the appropriate MAb for 1 h at room tempera-

ture. After three washes with PBS, the slides were incubated with normal goat serum for 30 min at room temperature and the sections were then incubated with FITC conjugated goat anti-mouse IgG for 1 h at room temperature in the dark. After three more 10 min washes with PBS, the slides were mounted with coverslips using Citifluor mounting medium. In the negative controls, the primary antibody was replaced with PBS. The sections were then examined using a Zeiss epi-fluorescence microscope.

2.4 Gel electrophoresis

CK isolation, one-dimensional separation by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), immunoblotting and two-dimensional gel electrophoresis using isoelectric focussing (IEF) as first dimension and SDS-PAGE as second dimension as well as immunoblotting were performed on samples as described earlier (Vaidya *et al* 1989). The immunoblots with the pankeratin antibody were carried out on all the samples studied by SDS-PAGE alone.

3. Results

Cytokeratin expression was studied in human fetal tongue and BM in 16 to 27 weeks old fetuses by immunofluorescence or gel electrophoresis or both simultaneously.

3.1 Tongue

Normal human adult tongue expresses CK 1, 2, 4, 5, 6, 10, 13, 14 and 16 and occasionally 17. CK 5 and 14 are found only in the basal layer of both fungiform and filiform papillae. CK 1 and 10 are seen in spinous and granular layers of fungiform and filiform papillae. CK 4 and 13 are expressed in the spinous and granular layers of filiform papillae only. CK16 is found in spinous, granular and cornified layers of fungiform papillae and all the layers of filiform papillae, although the intensity varies from layer to layer (Sawaf *et al* 1990).

3.1a Histology: At 16 weeks, the tongue epithelium appeared thin with three epithelial cell layers. Their cytoplasm was clear and superficial cells did not show flattening. The epithelial cell layers became thicker from 18 weeks onwards (figure 1). Cytoplasm of the suprabasal cells showed higher eosinophilia. The number of flattened superficial cells increased with age. From 20 weeks onwards, the number of papillae increased and the mesenchymal tissue was better developed with well structured muscles. In all the fetal tissues studied, the basal cell layer was deeply stained.

3.1b *Immunofluorescence*: Twenty-one tongue samples were analysed using immunofluorescence in the embryonic age group of 16 to 23 weeks (table 1). CK 1(2), 8 and 18 were detected in the majority of the samples studied. CK 1(2) was seen in the suprabasal epithelial layers while it was not

seen in the basal layer or in the subepithelium (figure 2a). Both CK 8 and CK 18 were detected in the glandular and ductal cells in the sub-epithelial tissue but not seen in the lining epithelia (figure 2b, c). There was no correlation between the staining intensity and the embryonic age of the fetus.

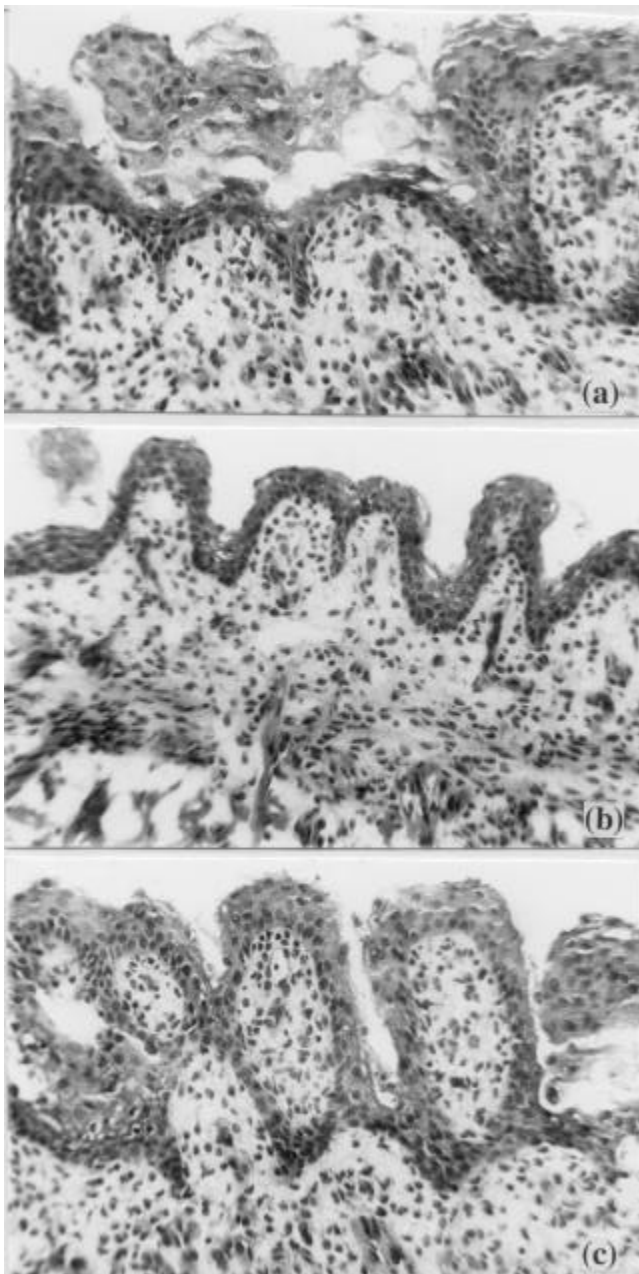


Figure 1. Histology of human fetal tongue showing progressive increase in cell layers of the lining epithelia at (a) 16 weeks, (b) 18 weeks and (c) 20 weeks ($\times 240$).

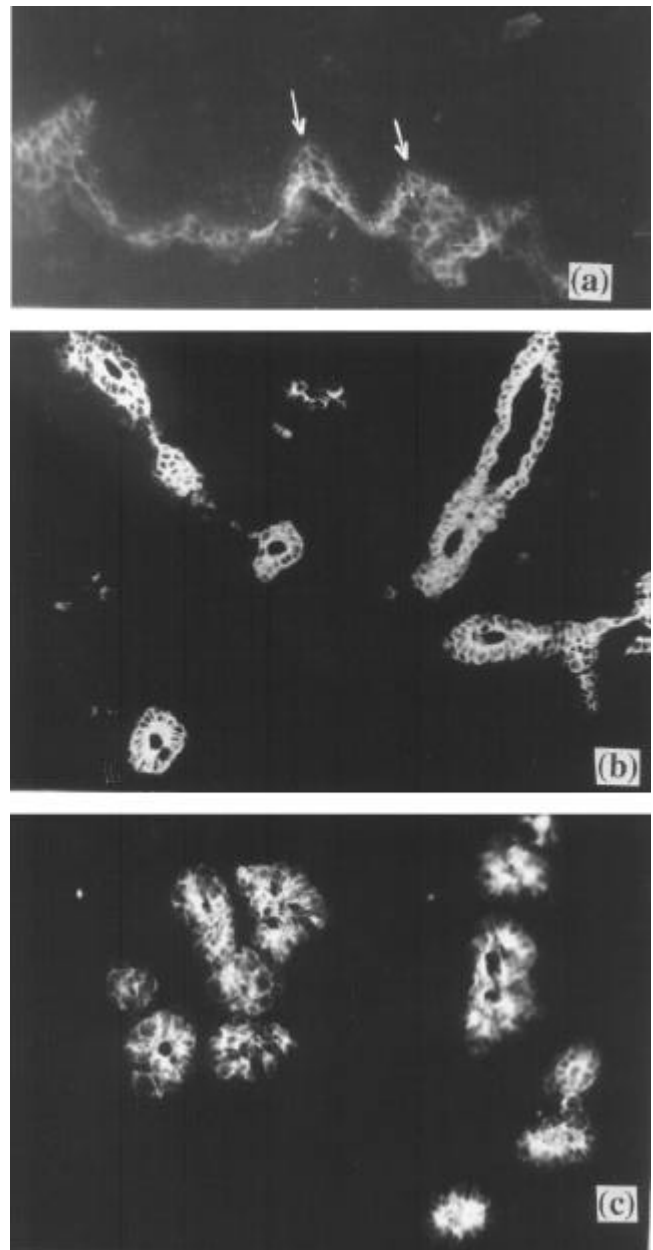


Figure 2. Immunofluorescence staining of human fetal tongue with MAb to CK. (a) MAb to CK 1(2), staining is seen only in the lining epithelium indicated by arrow. MAb to CK 8 (b) and MAb to CK 18 (c) show staining of sub-epithelial glandular structures ($\times 240$).

3.1c *Gel electrophoresis*: A total of seventeen tongue samples were analysed using SDS-PAGE, immunoblotting and 2-D gel electrophoresis. Eight of these were also analysed by immunofluorescence with MAb to CK 1(2), 8 and 18 (table 1). Our results of biochemical analyses and immunofluorescence showed good correlation (table 1). CK 1, 8 and 18 were detected in all the samples studied. CK 6 and 16 were detected in 11 samples. CK 10 was not detected till 18 weeks in any of the samples. It was seen in 4 samples above 20 weeks. CK 2 was detected in all the samples after 24 weeks except in a 25 week old fetal tongue. In all the samples studied, CK 18 stained darker in 2-D gels as compared to its normal counterpart CK 8. Representative 2-D maps of CK expression in human fetal tongue are shown in figure 4a, b.

3.2 Buccal mucosa

Normal human adult BM expresses CK 4, 5, 13, 14 and

possibly 19 (Vaidya et al 1989). In the basal layer, CK 5 and 14 are expressed while CK 4 and 13 appear in the upper layers. The staining intensity of CK 5 and 14 is progressively reduced in the upper layers (Clausen et al 1986).

3.2a *Histology*: At 16 weeks, the BM epithelium appeared thin with few epithelial layers. The superficial cells were not flattened and a number of mucous glands were seen. Histology of 18 week old fetal BM was similar to that of its 16 week counterpart. In 20 and 21 week old fetal BM, the epithelial cell layers were thicker, had more flattened cells and their cytoplasm showed higher eosinophilia. In all the samples, the basal cell layer was deeply stained.

3.2b *Immunofluorescence*: Twenty BM samples were analysed using immunofluorescence in the embryonic age

Table 1. CK expression in human fetal tongue using immunofluorescence and 2-D gel electrophoresis.

Sample No.	Age (weeks)	Monoclonal antibody			2-D gel electrophoresis
		CK-1-2	CK-8	CK-18	CK No.
1	16	++	+++	+++	1, 6, 8, 16, 18
2	16	+	+++	+++	X
3	16	+	++	+++	X
4	16	++	+++	++	1, 2, 6, 8, 16, 18
5	16	++	++	+++	X
6	16	++	+	+++	X
7	16	ND	ND	++	X
8	16	+++	+++	++	X
9	16	ND	++	++	X
10	18	ND	+++	+++	X
11	18	++	ND	ND	X
12	18	+	++	++	1, 8, 18
13	20	+	+++	+++	1, 2, 6, 8, 16, 18
14	20	ND	+++	+++	X
15	20	ND	+++	+++	X
16	20	++	+++	+++	1, 6, 8, 10, 16, 18
17	20	++	+++	+++	1, 2, 5, 8, 16, 18, 19
18	20	+	++	++	1, 6, 8, 16, 18
19	20	++	ND	ND	X
20	20	X	X	X	1, 6, 8, 16, 18
21	21	++	ND	ND	X
22	21	+++	++	++	1, 8, 18
22	22	X	X	X	1, 8, 10, 18
23	22	X	X	X	1, 6, 8, 16, 18
24	24	X	X	X	1, 2, 8, 18
25	24	X	X	X	1, 2, 6, 8, 16, 18, 19
26	25	X	X	X	1, 8, 18
27	26	X	X	X	1, 2, 6, 8, 16, 18
28	26	X	X	X	1, 2, 8, 10, 18
29	27	X	X	X	1, 2, 6, 8, 10, 16, 18

ND, Not detected; +, weak staining; ++, moderate staining; +++, intense staining; X, not done.

group of 16 to 23 weeks (table 2). CK 1(2), 8 and 18 were detected in the majority of samples studied. CK 1(2) was seen in the suprabasal epithelial layers with low staining intensity in the basal layer, but there was no staining in the subepithelium (figure 3a). Both CK 8 and CK 18 were detected in the glandular and ductal cells in the subepithelial tissue and were not seen in the stratified epithelia (figure 3b, c). There was no correlation between the staining intensity and the embryonic age of the fetus.

3.2c Gel electrophoresis: Sixteen BM samples were analysed using SDS-PAGE, immunoblotting and 2-D gel electrophoresis. Seven of these samples were also analysed using immunofluorescence with MAb to CK 1(2), CK 8 and CK 18 (table 2). Nine samples were analysed using only gel electrophoresis (table 2). Our results from biochemical analysis and immunofluorescence showed good correlation. CK 1, 8 and 18 were detected in all the samples studied. CK 6 and 16 were detected in 13 samples. CK 10, the normal counterpart of CK 1, was not

observed in any of the samples. CK 2 was detected in 5 samples while CK 19 was detected in 6 samples. CK 7 was seen in one sample each from 16, 20 and 24 week old fetuses. It was not seen in any of the samples above 24 weeks. Concentration of CK 8 was much lower compared to other CK in all the samples. Representative 2-D maps of CK expression in human fetal BM are shown in figure 4c and d.

4. Discussion

The CK profile in tissues has been shown to vary with cell type (Moll *et al* 1982a), developmental stage (Banks-Schlegel 1982; Moll *et al* 1982b), differentiation status (Moll *et al* 1982a; Sun *et al* 1984; Clausen *et al* 1986), and in pathological conditions (Bosch *et al* 1989). CK expression pattern in a tissue changes over a period of time during development and at a certain stage, adult expression begins. CK expression during human fetal development has been reported for various tissues. Moll *et al*

Table 2. CK expression in human fetal BM using immunofluorescence and 2-D gel electrophoresis.

Sample No.	Age (weeks)	Monoclonal antibody			2-D gel electrophoresis
		CK-1-2	CK-8	CK-18	CK expression
1	16	++	+++	+++	1, 6, 7, 8, 16, 1819
2	16	+	+++	+++	X
3	16	ND	++	+++	X
4	16	++	+++	++	1, 6, 8, 16, 18, 19
5	16	+	++	+++	X
6	16	++	+	++	X
7	18	++	ND	ND	X
8	18	++	+++	+++	1, 2, 8, 18
9	18	ND	++	++	X
10	20	+	+++	+++	1, 6, 7, 16, 18, 19
11	20	++	+++	+++	1, 6, 8, 16, 18
12	20	ND	+++	+++	X
13	20	++	ND	ND	X
14	20	++	+++	+++	1, 6, 8, 16, 18, 19
15	20	++	+++	++	X
16	20	X	X	X	1, 6, 7, 8, 16, 18, 19
17	21	++	ND	ND	X
18	21	++	++	++	1, 6, 8, 16, 18
19	22	X	X	X	1, 2, 6, 8, 16, 18
20	22	X	X	X	1, 6, 8, 16, 18
21	24	X	X	X	1, 2, 6, 7, 8, 16, 18
22	24	X	X	X	1, 6, 8, 16, 18
23	25	X	X	X	1, 6, 8, 16, 18, 19
24	26	X	X	X	1, 8, 18, 19
25	26	X	X	X	1, 6, 8, 16, 18
26	27	X	X	X	1, 2, 8, 18

ND, Not detected; +, weak staining; ++, moderate staining; +++, intense staining; X, not done.

(1982b), studied CK expression in fetal epidermis from the 10th week of gestation till birth and observed CK polypeptides typical of simple epithelia (CK 8, 18 and 19) till 24 weeks after which these gradually disappeared and were absent in adult epidermis. Broers *et al* (1989) used MAb to CK 7, 8, 18 and 19 to study CK expression in human fetal lung using immunocytochemistry. They found that different adult CKs appeared at different times during development and that the pattern became progres-

sively complex. Stosiek *et al* (1991) have suggested oncofetal behaviour of CK 7 due to its presence in human fetal stomach and in dysplastic and metaplastic gastric mucosa.

Adult CK expression has been shown to alter under some pathological conditions like psoriasis and after malignant transformation. We had earlier reported aberrant expression of simple epithelial CK 18 in SCC of BM and tongue (Vaidya *et al* 1989, 1996). Expression of CK 8 and 18 in SCC of different human tissues has also been demonstrated (Ermich *et al* 1989; Markey *et al* 1991; Su *et al* 1994). We observed such expression of CK 8 and 18 in fetal BM from 16 to 27 weeks but it was restricted to glandular cells in sub-epithelial region and not in the stratified epithelia. It is not clear why these CK are transiently expressed.

We also observed CK 1 expression in stratified epithelial layers in fetal BM up to 27 weeks but not in the adult tissue. CK 10 which is coordinately expressed with CK 1 was not detected in any of these samples. We have observed down regulation of CK 1 and the presence of CK 4, 5, 13 and 14 in two samples of BM from stillbirths (data not shown) which suggests that the adult pattern of expression begins sometime after the 27th week of fetal development but before birth.

We observed that the fetal tongue had 2–3 epithelial cell layers till 16 weeks. Till this time, CK 1 was expressed in the stratified epithelia and CK 8 and 18 were seen in the glandular tissue. Sawaf *et al* (1991) have shown that the simple epithelial CK 8 and 18 are expressed in the lining epithelia in tongue till 14 weeks where the tissue is not multilayered. We detected CK 8 and 18 in the sub-epithelial region from 16 weeks onwards. At this age, CK 1(2) expression was detected in the lining epithelia and it continued till 23 weeks. CK 1(2) expression was also seen in the adult stratified epithelium of the tongue. This may be the point for initiation of change over from fetal to adult CK expression. In fetal tongue CK 1(2) expression was seen only in suprabasal layers but CK 10 which is coordinately expressed with CK 1 was not detected in fetal tongue and BM tissues studied. This is similar to our report on non-expression of CK 10 in SCC of BM and tongue (Vaidya *et al* 1989, 1996). Co-expression of specific CK pairs is generally tightly regulated. In studies involving transfection of CK 1 and 10 genes in SCC derived cell lines, Kartasova *et al* (1982) detected that CK 1 expression started prior to that of CK 10 and a proliferative block occurred after CK 10 transfection. The absence of CK 10 in oral SCC and fetal tissues may be related to such a role of CK 10.

Aberrant expression of CK 8 and 18 and non-expression of CK 10 was a common feature in human fetal BM and tongue and in their SCCs. These observations suggest

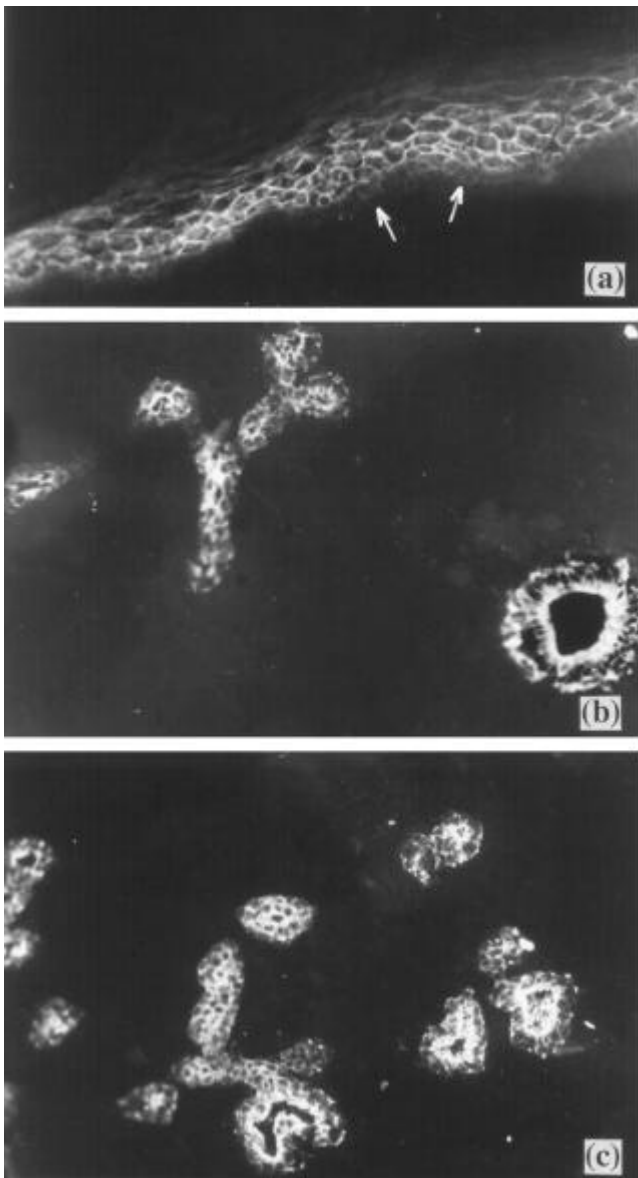


Figure 3. Immunofluorescence staining of fetal BM with MAb to CK. (a) MAb to CK 1(2) stains all the layers except the basal layer cells indicated by arrow. MAb to CK 8 (b) and to CK 18 (c) show staining of sub-epithelial glandular structures ($\times 240$).

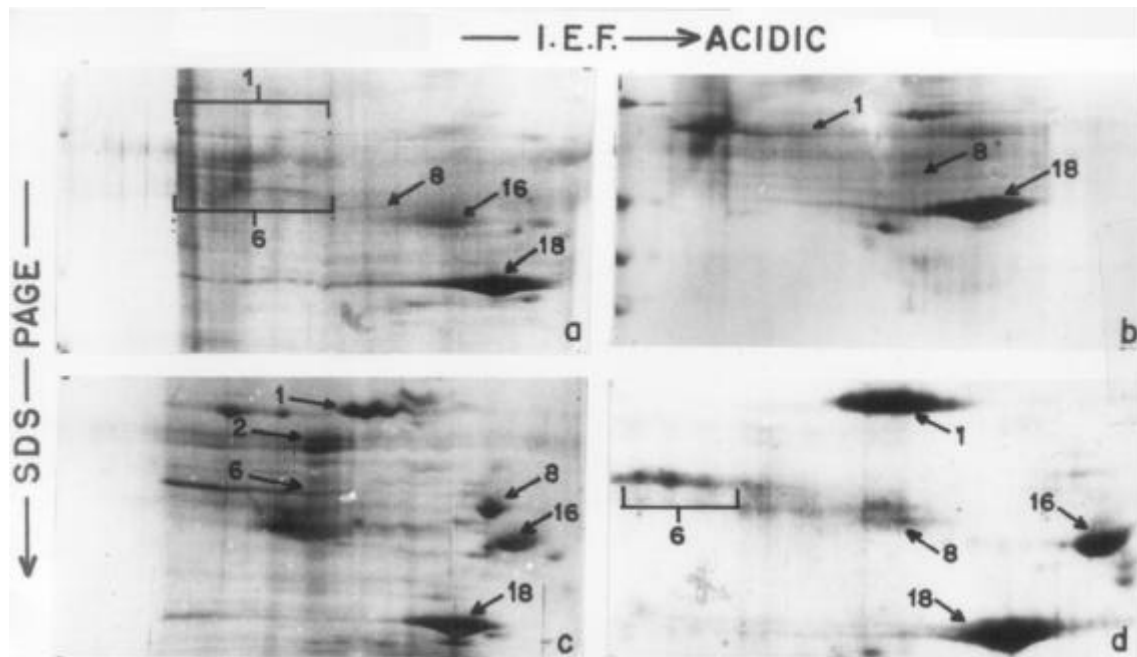


Figure 4. 2-D gel electrophoresis patterns of CK in fetal tongue and BM. (a) 22 week old tongue, sample No. 23 (table 1). (b) 18 week tongue, sample No.12 (table 1). (c) 22 week BM, sample No.19 (table 2). (d) 20 week BM, sample No. 11 (table 2). Numerals indicate CK numbers.

that tumours recapitulate fetal patterns of cytokeratin expression.

References

- Anderton B H 1981 Intermediate filaments: a family of homologous structures; *J. Muscle Res. Cell. Motil.* **21** 141–166
- Banks-Schlegel S P 1982 Keratin alterations during embryonic epidermal differentiation. A prestage of adult epidermal maturation; *J. Cell. Biol.* **93** 551–559
- Bosch F X, Ouhayoun J P, Bader B L, Collin C, Grund C, Lee I and Franke W W 1989 Extensive changes in cytokeratin expression patterns in pathologically affected human gingiva; *Virchows Arch. B. Cell Pathol.* **58** 59–77
- Broers J L V, deLeij L, Klein Rot M, ter Haar A, Lane E B, Leigh I M, Wagenaar S Sc, Vooijs G P and Ramackers F C S 1989 Expression of intermediate filament proteins in fetal and adult human lung tissues; *Differentiation* **40** 119–128
- Clausen G, Moc D, Buschard K and Dabelstein E 1986 Keratin proteins in human oral mucosa; *J. Oral. Pathol.* **15** 36–42
- Dale B A, Holbrook K A, Kimball J R, Hoff M and Sun T T 1985 Expression of epidermal keratins and filaggrin during human fetal skin development; *J. Cell. Biol.* **101** 1251–1270
- Eichner R, Sun T T and Aebi U 1986 The role of keratin subfamilies and keratin pairs in the formation of epidermal intermediate filaments; *J. Cell. Biol.* **102** 1767–1777
- Ermich T, Schuiz J, Raabe G and Schmann D 1989 Pattern of oral cytokeratins III; SDS electrophoretic analysis and immunoblotting of cytokeratins in leukoplakias and squamous cell carcinomas of the oral mucosa; *Biomed. Biochem. Acta* **48** 393–401
- Franke W W, Schmid E and Schiller D L 1982 Differentiation related patterns of expression of proteins of intermediate filaments in tissues and cultured cells; *Cold Spring Harbor Symp. Quant. Biol.* **46** 431–453
- Hatzfeld M and Franke W W 1985 Pair formation and promiscuity of cytokeratin: formation *in vitro* of heterotypic complexes and intermediate sized filaments by homologous and heterologous recombinations of purified polypeptides; *J. Cell. Biol.* **101** 1826–1841
- Kartasova T, Roop D R and Yuspa S H 1992 Relationship between the expression of differentiation specific keratin 1 and 10 and cell proliferation in epidermal tumors; *Mol. Carcinogenesis* **6** 18–25
- Kasper M, Karsten U, Stosiek P and Moll R 1989 Distribution of intermediate filament proteins in the human enamel organ: unusually complex pattern of expression of cytokeratin polypeptides and vimentin; *Differentiation* **40** 207–214
- Markey A C, Lane E B, Churchill L J, MacDonald M and Leigh I M 1991 Expression of simple epithelial keratins 8 and 18 in epidermal neoplasia; *Invest. Dermatol.* **97** 763–770
- Moll R, Franke W W, Schiller D L, Geiger B and Krepler R 1982a The catalog of human cytokeratin: Patterns of expression in normal epithelia, tumors and cultured cells; *Cell* **31** 11–24
- Moll R, Moll I and Wiest W 1982b Changes in the pattern of cytokeratin polypeptides in epidermis and hair follicles during skin development in human fetuses; *Differentiation* **23** 170–178
- Moll R, Schiller D L and Franke W W 1990 Identification of protein IF of the intestinal cytoskeleton as a novel type I cytokeratin with unusual properties and expression patterns; *J. Cell Biol.* **111** 567–580
- Morgan P R, Shirlow P J, Johnson N W, Leigh I M and Lane

- E B 1987 Potential applications of anti-keratin antibodies in oral diagnosis; *J. Oral Pathol.* **171** 212–222
- Reagur S, Franke W W and Virtanen I 1985 Intermediate filament cytoskeleton of amnion epithelium and cultured amnion epithelial cells: Expression of epidermal cytokeratins in cells of a simple epithelium; *J. Cell. Biol.* **100** 997–1009
- Sawaf M H, Ouhayoum J P, Shabana A H M and Forest N 1990 Cytokeratin expression in human tongue epithelium; *Am. J. Anat.* **189** 155–166
- Sawaf M H, Shabana A H M, Pelissier A, Forest N and Ouhayoum J P 1991 Characterization of cytokeratin patterns in the developing human tongue; *Int. J. Dev. Biol.* **35** 91–100
- Stosiek P, Brautigam E and Kasper M 1991 Expression of cytokeratin 7 in human glandular epithelium of fetal stomach; *Acta. Histochem.* **91** 21–23
- Su L, Morgan P R and Lane E B 1994 Protein and mRNA expression of simple epithelial keratins in normal, dysplastic and malignant oral epithelia; *Am.J. Pathol.* **145** 1349–1357
- Sun T T, Eichner R, Schermer A, Cooper D, Nelson W G and Weiss R A 1984 Classification, expression and possible mechanisms of evolution of mammalian epithelial keratins: A unifying model; in *The transformed phenotype: Cancer cells* (eds) A J Aeisne, G F Van der Woued, W C Topp and J E Watson (New York: Cold Spring Harbor Laboratory) vol. 1, pp 169–176
- Vaidya M M, Borges A M, Pradhan S A, Rajpal R M and Bhisey A N 1989 Altered keratin expression in buccal mucosal squamous cell carcinoma; *J. Oral. Pathol. Med.* **18** 282–286
- Vaidya M M, Borges A M, Pradhan S A and Bhisey A N 1996 Cytokeratin expression in squamous cell carcinomas of tongue and alveolar mucosa; *Oral. Oncol. Eur. J. Cancer* **32** 333–336
- Vaidya M M, Sawant S S, Borges A M, Ogale S B and Bhisey A N 1998 Cytokeratin expression in precancerous lesions of the human oral cavity; *Oral. Oncol. Eur. J. Cancer* **34** 264–267

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