Cytokeratins & Their Expression Pattern

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Cells possess a cytoskeleton that provides the structural framework. It facilitates intracellular transport, supports cell junctions, transmits signals about cell contact and permits motility. The cytoskeleton is a complex network of filaments that influence the dynamic morphology of eukaryotic cells in their tissue environment. It maintains the structural integrity of cells, anchoring intra-cytoplasmic organelles to the cellular membranes. The three structural elements of the cytoskeleton are micro-filaments, microtubules and intermediate filaments, all are dynamic structures assembled from protein sub-unit and disassembled as cellular activities and external influences on cell changes.

Microfilaments are 6- 8 nm (60 Å) in diameter and consists of globular actin molecules polymerized in to long filaments.
Microtubules are tubular or cylindrical structures with an average diameter of 25nm (150 Å) in diameter, they consist of the protein tubulin.

Intermediate filaments are approximately 10 nm(100 Å) in diameter and have diverse protein composition. They are not contractile but are important in maintaining the cell shape, contact between adjacent cells and between the cell and extracellular matrix.

Keratins are filamentforming proteins of epithelial cells and are essential for normal-tissue structure and function. Keratin genes account for most of the intermediate filament genes in the human genome, making up the two largest sequence homology groups, type I and II, of this large multigene family. They are highly differentiation specific in their expression patterns, implying functional differences.

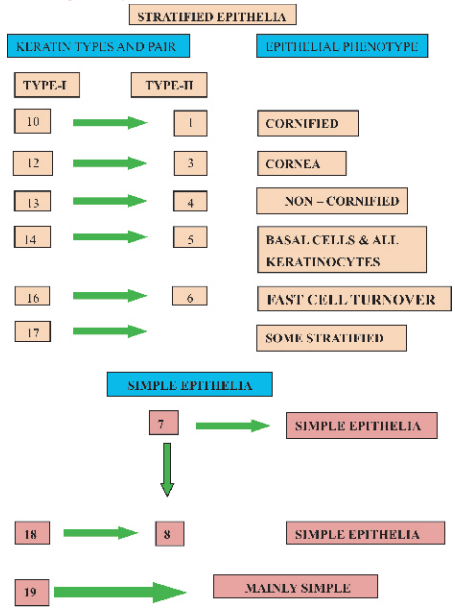
Cytokeratins, a complex multigene family of proteins, are intermediate filament keratins specifically expressed by epithelial cells. The epithelial keratins or cytokeratins (CK) constitute a family of 20 polypeptides distinguishable by their molecular weight, isoelectric point, X-ray diffraction pattern and also as hard and soft keratin. They are divided into two main groups:
Type 1 (CK 9–20), which are smaller and acidic polypeptides.
Type 2 (CK 1–8), larger and basic-neutral polypeptides.

Functions Of Cytokeratin

Keratin intermediate filaments are involved in resistance towards different types of stress. The cytokeratin cytoskeleton protects cells against mechanical stress through formation of a 3-D complex that associates with proteins of hemidesmosomes and desmosomes. Cytokeratin associations with the nuclear envelope probably play a nonstructural role. K8 and possibly other cytokeratins can protect tissue from injury by serving as a "sponge" for stress activated phosphate kinases. Evidence for this role has been obtained in hepatocytes isolated from mice synthesizing keratins that contain mutated phosphorylation sites including K8 and K18, which were mechanically stable following liver perfusion.

Epithelial sheet migration is a fundamental process in both morphogenesis and tissue repair that requires maintenance of cell–cell junctions. Cytokeratins interact with desmosomes and hemidesmosomes, thus contributing to cell to cell adhesion and to connection with the underlying connective tissue[1]

Pairing of Cytokeratins



Antibodies To Cytokeratins

In the early days of immunohi-stochemistry, most antikeratin antibodies used in surgical pathology were not reactive to a specific keratin; rather they were mixed monoclonal antibodies or polyclonal antibodies that reacted to several keratins. Currently, high quality anti-keratin monoclonal antibodies to all of the 20 keratins are commercially available.[2,3]

The commonly used antibodies are:

Pankeratin Cocktail

The cocktail antibodies contain monoclonal antibodies to AE1, AE3, CAM5.2 and 35BH11. It reacts to all epithelial tissues and their tumors.

Clone LP34 Antibody

This is a broad-spectrum monoclonal antibody that is reactive to K5. K6, K8, K17 & K19. It is positive on both simple glandular and stratified squamous epithelium. LP3 can be used as a pancytokeratin antibody to differentiate carcinoma from sarcoma, lymphoma or malignant melanoma.

Pancytokeratin Antibodies

This contains monoclonal antibodies to K5, K6, K8 & K18. They recognize all epithelial tissues and their neoplasms.

Wide Spectrum Screening Antibody

This is a rabbit anti-cow polyclonal antibody that also reacts with human keratins.

AE1 & AE3 Antibody

This antibody is a mixture of AE1 clone and AE3 clone. Monoclonal AE1 recognizes type I keratin, while AE3 recognizes type II keratins. Thus, AE1 and AE3 is a pan-specific antibodies for human keratins.

High molecular weight keratin antibody clone 34BE12

This antibody recognizes K1, K5, K10, and K14, which are expressed in complex epithelia, basal cells and myothelial cells.

CAM5.2

This antibody reacts with K8 and K18. In normal tissue, it stains simple and glandular epithelium. CAM5.2 stains most of the epithelial-derived tissues and their tumors. It is useful for the differentiation of adenocarcinoma from squamous cell carcinoma.

Anti-Cytokeratin 5/6

This antibody reacts with K5 and K6, but does not react to K1, K7, K8, K10, K13, K14, K18 and K19. This antibody recognizes basal cells. A part of stratum spinosum of epidermis and mesothelium, but is not reactive with simple epithelium or their tumors.

Expression Pattern

Normal Oral Epithelia

There are two mutually exclusive pathways along which epithelial cells of oral mucosa may differentiate and are reflected in there expression of keratin subsets. Simple epithelia, whether cornified or non-cornified or lining or secretory and including very early embryonic epithelia, express keratins 8, 18 and to lesser extent 19.[4]

Keratin gene expression is regulated in a differentiation - specific manner, creating patterns that are well conserved among mammalian species. Cornified epithelia of the attached gingival and hard palate contain the primary pair CK5 and CK14 in basal cells and sometimes in suprabasal cells, the secondary, differentiation – specific pair, CK1 & CK10, is consistently present above the level of rete processes.

As cell exit the basal layer, they switch off the transcription of CK5 and CK14 genes and switch on expression of CK1 and CK10 genes. Simple epithelial keratins CK7, CK8 & CK18 and also CK19, are present in merkel cells, notably in gingival and hard palate.[5]

Non – cornified epithelia of the alveolar and buccal mucosa and mucosa from floor of the mouth and ventral and lateral surfaces of tongue expresses the same keratin pair of CK5 and CK14, as in cornified epithelium. Subpopulation of suprabasal cells in cornified epithelium express CK4 & CK13, which are markers of non – cornification. The presence of this 'inappropriate' subpopulation appears to be due to normal variation rather than to minor pathological change. Cells of the differentiation compartment, generally all suprabasal cells, express CK4, CK13, CK6, & CK16. In addition, CK19 is distributed heterogeneously in non – cornified epithelium, predominantly in basal cells but occasionally in suprabasal cells.

CK 4 – 6(large & basic), CK13 (intermediate – sized & acidic) and CK14 – 17(small & acidic) are expressed in non keratinizing stratified squamous epithelium. The epithelia of the dorsal surface of the tongue show mixed patterns of keratin expression. In filiform and fungiform papillae, keratin expression is similar to that of cornified epithelia whereas interpapillary epithelium expresses the keratins of non – cornified epithelia.[5]

In fetal tongue CK1 expression was seen only in suprabasal layer but CK10 which is coordinately expressed with CK1, was not detected. This may be because CK1 expression started prior to CK10 and a proliferative block occurred due to CK10 transfection. Aberrant expression of CK8 & CK18 and non – expression of CK10 is a common feature in human fetal tongue and in their pathology. This suggests that tumors recapitulate fetal patterns of cytokeratin expression[6]

Expression of Keratins K1, K10 In Normal Oral Epithelia.

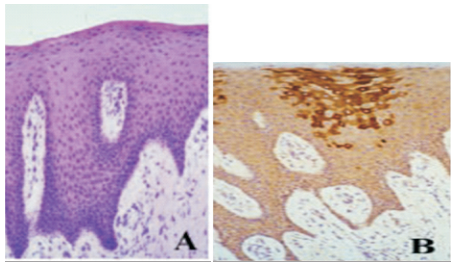


Fig. : Histological appearance of essentially normal gingiva showing para-keratinization(A). In adjacent sections, expression of K1 (B) and K10

Simple (glandular) Epithelium

CK7 and CK8 (intermediate – sized and basic) and CK18 & CK19 (smallest in size and acidic) are exclusively expressed in nearly all simple epithelia, pseudostratified respiratory epithelium and transistional epithelium. CK8 & CK18 pair together and have similar distribution. The expression of CK19 is found in broad range of epithelial tissues.

Mesenchyme

Normal mesenchyme – derived soft tissue rarely expresses keratins. Vascular endothelial cells are specialized mesenchyme – derived epithelial cells. Normal endothelial cells are positive for CK1, while endothelia of normal veins, venules and lymphatics are commonly positive for CK7 and CK18.[7]

Oral Epithelial Dysplasia

Oral lesions, both benign and premalignant, are often characterized by an increase or decrease in the degree of cornification. Dysplastic changes in oral epithelium are considered to indicate premalignancy, particularly if the changes are severe. Some characteristics of dysplastic epithelium can be interpreted as manifestations of disordered differentiation. Thus, it might be expected that disturbances of keratin expression might accompany dysplasia.[5]

Staining with antibodies to keratin 1 & 10, when compared with that to keratin 4 & 13 may, provide a sensitive indicator of shifts in the keratinization pattern. In case of dysplasia, the two pairs may be co-expressed in the same group of cells.

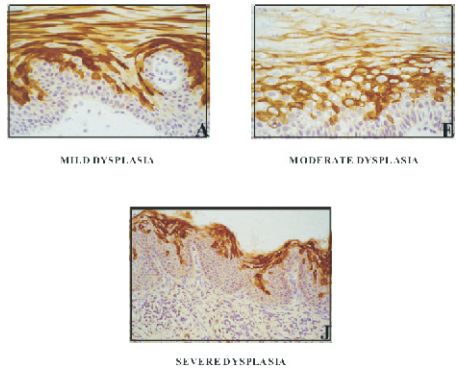
Abnormal differentiation of oral stratified epithelium was indicated by reduced expression of keratin 13 and suprabasal expression of CK14. The keratin pattern of normal mucosa was generally seen also in lesions with mild dysplasia. The differentiation – specific keratin CK13 mostly expressed by clusters of cells, apparently being lost in areas with increased degree of cellular atypia. In areas with hyperorthokeratosis and hyperparakeratosis, Ck13 is absent but CK10 is present. Single cell positivity for CK14 was also present in the parakeratinized layers. In moderate to severe dysplasia, all cell layers express CK14 but shows negativity for Ck13 and CK10. Epithelial cells at the border between normal and dysplastic epithelium showed co-expression of CK14 and CK13. Ck14 expression seems to be related to the cellular degree of pluripotency. Poorly differentiated pluripotent cells, such as embryonic basal epidermal cells, express CK14 at only low levels whereas basal cells in normal adult epithelium which have entered the differentiation program show intensified CK14 expressio[4,8].

Increased and frequent occurrence of CK16 in epithelial dysplasias has also recently been reported where CK16 was expressed in parakeratinized region by most suprabasal cells. Accordingly basal and parabasal cells in about 40% of the leukoplakias with dysplasia probably synthesize CK6 & CK16 instead of CK5 & CK14.

Simple epithelial keratins, CK8 and CK18, have also been detected in 'leukoplakia' especially from non – cornified sites and areas of severe dysplasia or microinvasion. These keratins were localized mainly to the deeper layer. Recent study suggests that expression of CK18 correlate strongly with severity of dysplasia, all severely dysplastic cases being positive.

It has previously been suggested that CK19 is a marker for premalignancy within oral keratosis and there are controversial reports of CK19 expression in oral dysplastic epithelium. Suprabasal expression of this keratin could be a marker of premalignant change but other report suggests variable expression of CK19, confined to basal and parabasal layers in dysplasia. A similar distribution has been observed in non – cornified epithelium. An immunoblotting study has shown significant synthesis of CK19 protein in Leukoplakia, whether or not dysplastic change is present.5

Cytokeratin Expression of K1 And K10 In Oral Dysplasia.

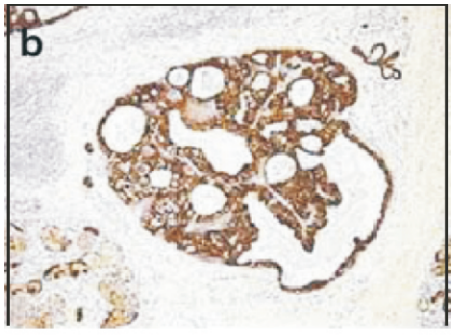


Oral Cancers

Virtually all carcinomas, regardless of their tissue origin, are immunoreactive to keratin antibodies. However, keratin expression patterns in carcinomas from stratified epithelia and from simple epithelia are different6. In oral squamous cell carcinoma (SCC), two main trends have been reported with respect to keratin expression. The first is a reduction in secondary keratins (CK1 & 10; CK4 & 13). In well-differentiated SCCs, these

keratins are generally conserved being localized to prickle cells and at the center of some tumor islands. Such central cells correspond to the cornified and non – cornified counterparts of normal stratified epithelia. Unlike normal oral epithelia, neoplastic cells often co-express both sets of differentiation keratins (CK1 & CK10; CK4 & CK13). In less well-differentiated SCCs fewer clusters of epithelial cells contain these keratins. A second feature of altered keratin expression in SCC is the anomalous expression of simple epithelial keratins i.e. CK8 & CK18 have been detected to an extent, in both frequency and intensity, which appears to vary inversely with the degree of differentiation.

Cytokeratin Expression of Ck7 In Oral Squamous Cell Carcinoma



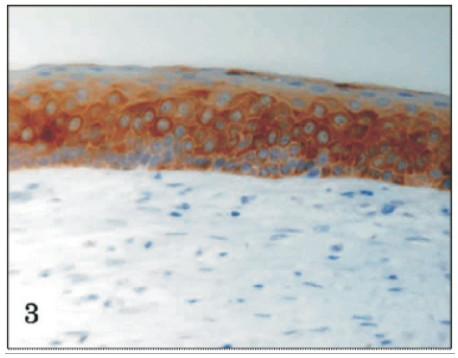
Positivity for CK7 in squamous cell carcinoma

Odontogenic Cysts

The simplest pattern of keratin expression in odontogenic epithelium shows strong expression of CK5 & CK19 and very weak expression of simple epithelia (CK7, CK8 & CK18) and of non – cornifying stratified epithelium (CK4 & CK13). Although keratin 19 appears as a major component in many simple epithelia, it is not detectable in all epithelia. CK19 appears to be a common component of various odontogenig epithelia but its pattern of expression appears to be unusual i.e. in normal oral epithelium CK19 is found in basal region, in proliferating epithelium it appears to be suprabasal but in cyst it is restricted to most superficial cell layers.

Further epithelial change to form a cyst lining was associated with a differentiated phenotype of stratified non – cornifying epithelium but still some simple epithelial keratins were coexpressed. Expression of CK19 & CK5 & CK14 is found in some non – cornifying oral mucosae, but coexpression of these keratins with keratin 8 & 18, does not appear to be a common finding for normal stratified epithelia.

Expression of Ck17 In Odontogenic Cyst



Odontogenic Tumors

Odontogenic epithelium may present as a classically stratified, squamous or simple epithelium, as in the early tooth germ and the various neoplastic and non – neoplastic lesions in which odontogenic epithelial strands and rests are present. Odontogenic tumors, exhibit a histological appearance which is to some extent intermediate between simple and stratified.[4]

CK19, a keratin present in some basal cells of stratified mucosal epithelial as well as most simple epithelia, appears to be an obligatory constituent of all normal and pathological odontogenic epithelia. CK19 as well as CK8 were appeared in all cells of the dental lamina, enamel organ and also heterogeneously in ameloblastoma.[9]

Allthe tumors derived from odontogenic epithelium express basal cell keratins CK5, CK10, CK13 and CK14 and shows no staining to the simple epithelial keratins CK7, CK8, CK18 and CK20.[6]

Cytokeratin Expression of Ck19 & Ck13 In Plexiform Ameloblastoma

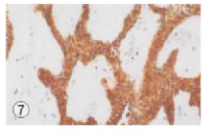


Fig. 7 CK19 immunohistochemical findings in plexiform type (×251). CK19 was positive uniformly all over the cytoplasm.

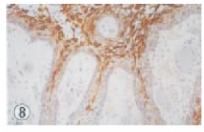


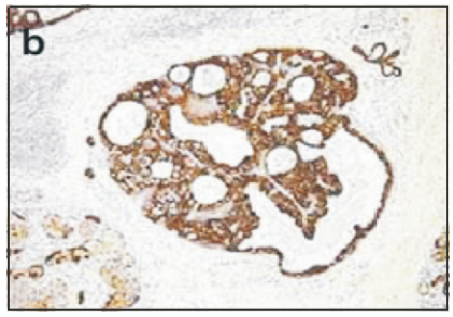
Fig. 8 CK13 immunohistochemical findings in plexiform type (×187). CK13 was negative in BL but serie positive in the cytoplasm in SRL.

Salivary Gland Tumors

Keratin proteins are one type of intermediate – size protein that is generally distributed in epithelial cells or epithelium – derieved structures. In salivary glands, keratins have been distributed in ductal segments.[10]

Salivary gland tumors arising from stratified epithelia (pleomorphic adenoma, myoepithelioma, basaloid squamous cell carcinoma, adenoid cystic carcinoma and mucoepidermoid carcinoma) express CK5, CK6, CK14, CK17 and CK19, whereas the tumors arising from simple epithelia like, adenocarcinoma, monomorphic adenocarcinoma, acinic cell carcinoma, express CK7, CK8 and CK18. Tumors of stratifying epithelium may contain simple epithelial components and frequently shows focally positive CK7 & CK8 areas.[6]

Cytokeratin Expression of Ck7 In Mucoepidermoid Carcinoma



(b) *EXPRESSION OF CK7 SHOWS PSITIVITY*

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