CYTOKERATINS IN HEALTH AND DISEASE

Shailja Chatterjee
Assistant Professor, Department of Oral and Maxillofacial Pathology and Microbiology, MMCDSR, M. M. University, Mullana (Ambala), Haryana-133203.

Corresponding Author: Shailja Chatterjee, Department of Oral and Maxillofacial Pathology and Microbiology, MMCDSR, M.M.University, Mullana(Ambala), Haryana-133203, Email: shailjachatterjee@gmail.com, Ph: 08053047175

Abstract

Objective: Cytokeratins are epithelium-specific intermediate filaments. Their altered expression is evident in various benign, hyperplastic and neoplastic lesions. This review provides an in-depth description of cytokeratin expression patterns in odontogenesis; normal development, hyperplastic/benign and malignant lesions or conditions.

Materials and methods: Cytokeratin expression can be studied by numerous techniques like immunohistochemistry, immunofluorescence and in situ hybridization.

Results: The variable expressivity of cytokeratins is a subject of continuous deliberation and ongoing research. This paper gives an in-depth analysis of cytokeratin expression in a variety of epithelial tissues.

Conclusion: Cytokeratins are universal indicators of epithelium specificity. An alteration of expression can be well understood by means of a proper understanding of their normal presence.

Key words: Cytokeratin, benign, malignant, odontogenesis, development.

Introduction

Cytokeratins are epithelium-specific intermediate filaments with diameters measuring between 6 nm (microfilaments) to 25 nm (microtubules). All human epithelial keratins are defined by "Co-expression". It is the existence of keratins in pairs of which, one is acidic (type I; CK 1 to 8) and other, basic (type II; CK 9 to 20). This division is based upon properties of charges, immunoreactivities, mRNA hybridization, peptide-mapping patterns, amino acid sequences and relationship to wool keratin[1].

Keratin pairs exhibit specificity with each pair comprising of a basic and an acidic member[2]. For example, CK1 and CK10 are found mainly in keratinized epithelia. Keratins are markers of differentiation rather than lineages. In stratified squamous epithelium, differentiating epithelial cells synthesize a sequence of different keratins as they migrate towards the upper cell layers. This is evident from the expression of CKs 5 and 14 in the basal layer of both the keratinized and non-keratinized stratified squamous epithelia. The differentiating upper layers contain the keratin pairs, CK1/10 (keratinized) and CK4/13 (nonkeratinized).

Cytokeratin pattern complexity shows great variation in expression in tumors. Epithelium-derived tumors maintain the expression of many cytokeratin polypeptides characteristic of nontransformed cells. However, certain cytokeratins are not expressed at the same time. This can be attributed to selection process during cell transformation and tumor development from a cell type that is not quantitatively predominant in total tissue used for comparison. Thus, explaining the predominance of a particular cytokeratin in a tumor.

Cytokeratin expression in normal oral mucosa

A. Cytokeratins 5 and 14 (CK5/14): This cytokeratin pair is strongly expressed consistently in the basal cell layer of both keratinized and nonkeratinized mucosae. Its expression decreases towards the upper cell layers i.e., stratum spinosum[3].

B. Cytokeratin 1 and 10 (CK 1/10): CK 1 and 10 are early markers of keratin differentiation. They are strongly expressed in stratum spinosum which forms the first layer of differentiation compartment. Their expressivity declines in stratum granulosum

and upper cell layers. This cytokeratin pair is absent in parabasal cell layer. However, its presence is detectable as mRNAs i.e., it is regulated at post-transcriptional level as evident by its strong expression in parabasal cells in pathologically altered mucosa. It is expressed strongly in hard palate, fungiform papillae and labial epidermis i.e., orthokeratinized epithelia[4,5].

C. Cytokeratin 19: It is found in a wide range of epithelial tissues and appears usually as a major component in simple epithelia and a minor component in stratified squamous epithelium as well as cultured keratinocytes. Expressed in basal cell layer of nonkeratinized mucosa with variable suprabasal expression and sometimes, full thickness staining of nonkeratinizing epithelia[3].

D. Cytokeratins 4 and 13 (CK4/13): This cytokeratin pair forms a component of nonkeratinized stratified squamous epithelium and is associated with differentiating suprabasal cells. For example, tongue mucosa (anterior compartment) and buccal mucosa[3].

E. K2p: Suprabasal epithelial cells of the hard palate and gingiva express K2p.

Cytokeratin expression in odontogenesis

Odontogenesis is a complex embryological process characterized by reciprocal epithelial-mesenchymal interactions, culminating in tooth differentiation. The epithelial cells of human dental lamina and enamel organ express CKs 7, 13, 14 and 19 with slight changes in pattern during the differentiation phases of odontogenesis.

CK14 is strongly expressed in inner epithelial cells at early bell stage, but is downregulated and replaced by CK19 at late bell stage when complete differentiation of ameloblasts has taken place. CK14 has a binding affinity with amelogenin. The CK 14 molecule acts as a chaperon for nascent amelogenic polypeptide during amelogenesis[6].

The stellate reticulum stains for CK7 at early bell stages along with CK14 which is strongly present at early bell stage. The outer enamel epithelium expresses CK7, CK14 and CK19. The cells of dental lamina remnants are immunopositive for CK7; with sporadic CK 13 expression. However, CK14 is the most strongly labeled cytokeratin in these cells[7].

Cytokeratin expression patterns in pathological states

I. Squamous cell carcinoma:

A. CK19 (CYFRA 21.1): Cyfra 21.1 is a solubilized fragment of cytokeratin 19 generated by necrosis of tumor cells. It is found in serum and shows 50% to 60% sensitivity in head and neck SCC[8]. CK19 expression is normally seen in basal cells of nonkeratinized epithelium and is considered to be a marker of premalignancy when appearing in suprabasal cells.

B. K13/14/5: Reduced expression of CK13 and suprabasal CK14 (which is ubiquitous) is seen along with expression in infiltrating tumor islands. CK5 is not expressed with increasing grades of dysplasia. The expression of CK 5 is thus, an early event in tobacco-associated pathological changes[9].

C. Well and moderately differentiated SCCs: In well-differentiated SCCs, CK4 and 13 are co-expressed with CK1/10 in the same group of cells, including the cells at periphery of tumor islands. Moderately-differentiated SCCs show a substitution of CK4/13 by CK1/10[4,5].

D. Poorly differentiated SCCs: In poorly differentiated SCCs, differentiation keratins are absent. This can be attributed to the impaired epithelial maturation or loss of pattern of keratin gene expression[4,5].

E. CK18: Aberrantly expressed in normal oral tissues, without good hygiene status. Therefore, it is indicative of initiation of abnormal cell differentiation[10].

II. Disease progression from oral submucous fibrosis to oral cancer: Higher molecular weight cytokeratins (CK1/10 and CK5/14) show an increase in staining intensity in OSMF and OSCC. Pancytokeratin cocktail comprising of CK1/10; 4/13; 5/14; 6/16; 7/19; 2/3; 8/15, also show a significant[1].

increase in staining intensity from normal tissue to OSMF to OSCC[11].

III. Epithelial dysplasia: The variable expression of differentiation keratins in mild and moderate oral epithelial dysplasia is partly dependent on altered epithelial differentiation. Disturbed differentiation is a major feature of dysplasia. In mild lesions derived from non-keratinized epithelium, K1/10 synthesis is enhanced and K4/13 is retained. This can be explained by the presence of K1/10 mRNAs, synthesis of which is increased in dysplastic state[9].

In moderate dysplasia arising from non-keratinizing epithelia, K1/10 filaments completely replace the K4/13 complex. In severe dysplasia, both K4/13 and K1/10 complete are absent. CK5/14 keratins that are normally expressed in basal cells, are also expressed in parabasal and spinous cell layers in dysplastic epithelia. This expression pattern probably reflects upon the basilar hyperplasia in dysplastic epithelium. Furthermore, the keratins (K4/13 or K1/10) that are characteristically present in suprabasal cell layers show reduced expression or loss in epithelial dysplasia. Thus, in severe dysplasia, K4/13 is completely lost. In normal epithelium, keratins 8 and 18 are present as mRNA transcripts in basal and lower spinous cell layers. However, in oral epithelial dysplasias, these keratins are detectable by routine IHC in most of the cases[9]. CK19 immunopositivity is found in basal and parabasal cell layers in moderately to severely dysplastic epithelia.

IV. Odontogenic cysts (OKCs/Dentigerous/Radicular cysts)[12]:

A. CK19: It is completely absent in Odontogenic keratocysts. Positive expression is seen in dentigerous and radicular cysts.

B. CK17: Majority of OKCs are immunopositive for CK17 and negative for dentigerous and radicular cysts.

C. CK5/6: It is expressed in all three Odontogenic cysts in all cell layers.

D. CK7: It shows negative expression in radicular cysts. Only superficial layers are positive in dentigerous cysts.

E. CK10: It is more significantly expressed in Odontogenic keratocysts as compared to other odontogenic cysts.

F. CK13: It is expressed in suprabasal cell layers of all odontogenic cysts.

G. CK20: All odontogenic cysts are negative.

V. Odontogenic tumors:

i. Ameloblastoma:

a. Follicular Ameloblastoma: The central stellate reticulum-like cells and basal ends of columnar cells stain with wide-spectrum keratins. Central stellate cells of follicular ameloblastoma show a high frequency of high molecular weight cytokeratins than Plexiform type, therefore, indicating presence of elements of squamous differentiation[13].

b. Acanthomatous Ameloblastoma: Expression is same as above.

c. Basal cell Ameloblastoma: Cells are partly stained with wide-spectrum keratins.

d. Plexiform Ameloblastoma: Cells are stained with wide-spectrum or HMW cytokeratins.

ii. SquamousOdontogenic tumor: Polyclonal antibodies are expressed in entire epithelial strata. CK1 is expressed in upper spinous and granular layer cells, whereas, LMW keratin expression is confined to basal cell layer[14].

a. Calcifying Epithelial Odontogenic Tumor: The tumor cells of CEOT are morphologically similar to the stratum intermedium cells and are also positive for alkaline phosphatases and adenosine triphosphate. They are strongly positive for cytokeratins (CK1, 5, 6, 8, 13 and 16)[15].

iii. Calcifying cystic odontogenic tumor: The basal cell layers express CK14, whereas, the upper cell layers express CK10/13. CK10/13 expression is seen in squamous and stellate reticulum-type tumoral cells identifying with similarity in expression in upper layers of squamous-type reduced in

enamel epithelium and some dental lamina cells. CK19 is expressed in the basal cell layer of COC which is similar to oral mucosa. Therefore, it can be hypothesized that CCOT epithelium differentiates towards stratified squamous type and has an apparent relationship to reduced enamel epithelium. These CK10/13 positive cells are the squamous transitory elements towards ghost cell development and are placed towards the plasma membrane. These cells accumulate certain other substance during the differentiation process, and gradually repel the cytoskeletal system to the periphery, until becoming CK10/13 negative ghost cells[16].

VI. Cell Rests of Malassez and periapical lesions[17]:

i. CKs4/13: These are not expressed in cell rests but in altered epithelium of periapical granuloma with increasing intensity of expression with transition to periapical cysts.

ii. CK19: CK19 is coexpressed with CK5 in cell rests of various odontogenic epithelia. In proliferated epithelia, CK19 expression is suprabasal and in cyst lining, often restricted to most superficial cell layers.

VII. Salivary gland morphogenesis: Numerous studies have indicated that cytokeratins can serve as the most useful marker in epithelial morphogenesis in a variety of systems including salivary glands. Greiger et al, 1987 reported the localization of specific cytokeratin polypeptide in different epithelial elements of human submandibular salivary gland[18]. All epithelial elements of human salivary glands contain CK18 whereas, CK19 is present throughout the acini and myoepithelial cells. The basal or reserve cells express CK13 and 16[19,20,21]. Keratin is expressed in all true luminal and absent in abluminal cells. In normal salivary glands, intercalated ductal nonluminal cells are positive for CK14. CK7 positivity is see in ductal luminal cells[22].

VIII. Salivary gland neoplasms:

Role of cytokeratin 5 in development of salivary gland carcinomas

The inducible expression of a mutated K-ras under the influence of CK5 promoter leads to the development of hyperplastic and dysplastic epithelial lesions and carcinomas. These tumors appear to arise from CK 5 positive basal cell compartment. Thus, the ras oncogene when targeted to a specifically sensitive cell compartment within the salivary glands can trigger a series of events that are sufficient for causing carcinogenesis[23].

IX. Keratin2e: K2e expression in skin is resultant of keratinocytes activation, however, it is up-regulated in oral lesions as a reflection of degree of ortho keratinization. For example, benign keratoses of lingual mucosa where it is coexpressed with CK1 and CK10. In mild to moderate dysplasia with orthokeratinization, it is highly expressed compared with parakeratinized as mRNA transcripts[4,5].

Conclusion

Cytokeratins are an important tool in molecular progression of certain diseases, their embryological development and lineage. Use of techniques like immunohistochemistry and polymerase chain reaction, aid in establishing the true nature and diagnosis of lesions with suspected nature. Thus, an in-depth knowledge of these profile markers is essential for a pathologist.

References

1. *Cooper D., Schermer A. and Sun T.T.: Biology of disease. Classification of human epithelia and their neoplasms using monoclonal antibodies to keratins: Strategies, Applications and Limitations. Lab Inv 52(3):243-256,1985.*
2. *Eichner R., Sun T.T. and Aebi U.: The role of*

keratin subfamilies and keratin pairs in the formation of human epidermal intermediate filaments. The J Cell Biol 102:1767-1772,1986.

3. *Barrett A.W., Morgan M., Nwaeze G., Kramer G. and Berkovitz B.K.B.: The differentiation profile of the epithelium of the human lip. Arch of Oral Biol 8:431-438,2004.*
4. *Bloor B.K., Tibman N., Leigh I.M., Odell E., Dogan B., Wollina V., Ghali L. and Waseem A.: Expression of keratin 2e in cutaneous and oral lesions. Association with keratinocytes activation, proliferation and keratinization. Am J of Pathol 162(3):963-975,2003.*
5. *Bloor B.K., Seddon S.V. and Morgan P.R.: Gene expression of differentiation-specific keratins in oral epithelial dysplasia and squamous cell carcinoma. Oral Oncol 37:251-261,2001.*
6. *Ravindranath R.M.H., Tam W.Y., Bungas P., Santos V. and Finchan A.G.: Amelogenin-cytokeratin 14 interactions in ameloblasts during enamel formation. The J of Biol Chem 2761(39):36586-36597,2001.*
7. *Domingos M.G., Jaeger M.M.M., Araryo V.C., Araryo N.S.: Expression of cytokeratins in enamel organ. Eur J Oral Sci 108:43-47,2000.*
8. *Hamakawa H., Bao Y., Takarada M., Fukuzumi M. and Tanioka H.: Cytokeratin expression in squamous cell carcinoma of the lung and oral cavity. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 85:438-443,1998.*
9. *Vaidya M.M., Sawant S.S., Borges A.M., Ogale S.B. and Bhisey A.N.: Cytokeratin expression in precancerous lesions of the human oral cavity. Oral Oncol 34:261-264,1998.*
10. *Su L., Morgan P.R. and Lane E.B.: Keratin 14 and 19 expression in normal, dysplastic and malignant oral epithelia. A study using in situ hybridization and Immunohistochemistry. J Oral Pathol Med 25:293-301,1996.*
11. *Ranganathan K., Kavitha R., Sawant S.S. and Vaidya M.M.: Cytokeratin expression in oral submucous fibrosis – an immunohistochemical study. J Oral Pathol Med 35:25-32,2006.*
12. *Stoll C., Stollenweck C., Riediger D., Mittermayer C. and Alfer J.: Cytokeratin expression patterns for distinction of odontogenic keratocysts from dentigerous and radicular cysts. J Oral Pathol Med 34:558-564,2005.*
13. *Matsuo A. and Ueno S.: Immunohistochemical demonstration of keratin in ameloblastoma as an indicator of tumor differentiation. J Oral Maxillofac Surg 49:282-288,1991.*
14. *Tatemoto Y., Okada Y. and Mori M.: Squamous odontogenic tumor: Immunohistochemical identification of keratins. Oral Surg Oral Med Oral Pathol 67:63-67,1989.*
15. *Ronen S.A., Liokumovich P., Rahima D., Charcon S.P., Goldberg I. and Horowitz A.: The amyloid deposit in calcifying epithelial odontogenic tumor is immunoreactive for cytokeratins. Arch Pathol Lab Med 124:872-876,2000.*
16. *Crivelini M.M., Felipini R.C., Coclete G.A. and Soubhia A.M.P.: Immunoexpression of keratins in the calcifying cystic odontogenic tumor epithelium. J Oral Pathol Med 38:393-396,2009.*
17. *Gao Z., Mackenzie I.C., Williams D.M., Guchley A.T., Leigh J. and Lane E.B.: Patterns of keratin-expression in rests of Malassez and periapical lesions. J Oral Pathol 17:178-185,1998.*
18. *Geiger S., Geiger B., Leitner O. and Marshak G.: Cytokeratin polypeptide expression in different epithelial elements of human salivary glands. Virchows Arch (Path. Anat. Physiol) 410:403-414,1987.*
19. *Mershak G., Leitner O. and Geiger B.: Cytokeratin polypeptide expression during the histogenesis of guinea pig submandibular salivary gland. Development 100:699-711,1987.*
20. *Batsakis J.G.: Salivary gland neoplasia: An outcome of modified morphogenesis and cytodifferentiation. Oral Surg 49:229-232,1980.*
21. *Cutler L.S. and Chaudhary A.P.: Intercellular contacts at the epithelial-mesenchymal interface during the prenatal development of the rat submandibular gland. Dev Biol 4:31-41,1974.*
22. *Vasconcellos L.M.R., Silveira V.A.S., Rosa L.E.B., Cavalvante A.S.R., Carvalho Y.R.: Immunohistochemistry as a fundamental tool for the differential diagnosis of polymorphous low-grade adenocarcinoma. Quintessance Int 37:565-573,2006.*
23. *Chen J.C., Gnepp D.R. and Bedrossian L.W.M.: Adenoid cystic carcinoma of the salivary glands: An immunohistochemical analysis. Oral Surg Oral Med Oral Pathol 65:316-326,1988.*

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