Review Article

Cytokeratin: A Review on Current Concepts

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Abstract

Cytokeratins are proteins which form the intermediate filaments and form the major cytoskeleton of the epithelial cells. It has an enormous role in providing mechanical support to the cell. There are various kinds of cytokeratins each showing varied expression in the epithelium. The cytokeratins can be broadly classified into type I or acidic and type II or basic proteins. The expression of these plays a role in differentiating different types of epithelial cells, thus enabling us to classify tumors. They help in diagnosing different types of tumors and thus have a vital role in diagnostic pathology.

Keywords: *Epithelium, intermediate filaments, keratin*

Introduction

Cytokeratins are intermediate filaments containing keratin usually found in the intracytoplasmic cytoskeleton of epithelial tissue. The term cytokeratin was derived in the 1970s when the proteins in the intermediate filament were identified.[1] However, the terminology was modified as keratins in the new systemic nomenclature in 2006.[2]

Types of Cytokeratin

There are two types of cytokeratins: the low weight or the acidic type I cytokeratins and the high weight or basic or neutral type II cytokeratins. The high-molecular-weight cytokeratins or basic or neutral cytokeratins comprise numerous subtypes, namely CK1, CK2, CK3, CK4, CK5, CK6, CK7, CK8, and CK9. The low-molecular-weight cytokeratins or acidic cytokeratins comprise CK10, CK12, CK13, CK14, CK16, CK17, CK18, CK19, and CK20. The expression of these cytokeratins varies in different organs and is thus organ specific. The molecular weight decreases as the number advances and thus cytokeratin 1 has the highest molecular weight, while cytokeratin 19 has the lowest molecular weight. The subsets of cytokeratins which an epithelial cell expresses depend on the type of epithelium and the differentiation pattern.[3]

Molecular Biology

Cytokeratins are encoded by a family encompassing 30 genes. Among them, 20 are epithelial genes and 10 are specific for trichocytes. Based on the remarkable conservation of protein chain structures and genes of keratin, it has been suggested that a primordial gene was assembled from smaller units encoding multiple heptad repeats (28 residues or 84 base pairs) separated by intervening introns. The number of positions of the introns, but not the intron sequences or length, is generally well preserved. However, the location of introns in keratin genes varies slightly. The smaller and acidic type I keratins (k9–k20) are encoded on chromosome17q, while the larger and more basic type II keratins (k1–k8) are encoded on chromosome 12q. Most of the human keratin genes characterized so far appear to exist as a single copy per haploid genome, with exception of k6. A pseudo gene has been reported for human keratin 14. Keratins have a highly homologous central helical rod domain flanked by variably sized amino and carboxyterminal domain DNAs of members of the subfamily which cross hybridize with one another. K20 was the last keratin to be characterized.[4,5]

Certain general principles of keratin gene expression have been established, of which the most striking is that at least one member of each subfamily is always co-expressed in any given epithelial tissue. Keratin gene expression is developmentally regulated and is not universally expressed during embryonic development;

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rather, different keratin genes are expressed during different stages of epithelial cell development during embryogenesis. All cytokeratin chains are composed of a central α-helix-rich domain (with a 50%–90% sequence identity among cytokeratins of the same type and around 30% between cytokeratins of different type) with non-α-helical N- and C-terminal domains. The α-helical domain has 310–150 amino acids and comprises four segments, in which a seven-residue pattern repeats. Into this repeated pattern, the first and fourth residues are hydrophobic and the charged residues show alternate positive and negative polarities, resulting in the polar residues being located on one side of the helix. This central domain of the chain provides the molecular alignment in the keratin structure and makes the chains form coiled dimers in solution. The end-domain sequences of type I and II cytokeratin chains contain the subdomains V1 and V2 in both sides of the rod domain, which have variable size and sequence. The type II also presents the conserved subdomains H1 and H2, encompassing 36 and 20 residues, respectively. The subdomains V1 and V2 contain residues enriched by glycines and/or serines, the former providing the cytokeratin chain a strong insoluble character and facilitating the interaction with other molecules. These terminal domains are also important in defining the function of the cytokeratin chain characteristic of a particular epithelial cell type. Two dimers of cytokeratin group into a keratin tetramer by anti-parallel binding. This cytokeratin tetramer is considered to be the main building block of the cytokeratin chain. By head-to-tail linking of the cytokeratin tetramers, the protofilaments are originated, which in turn intertwine in pairs to form protofibrils. Four protofibrils give place to one cytokeratin filament.[6]

Cell Biology

The classification and numbering system of the keratins (except those of hair and nail) are based on the catalog of Moll *et al*.; in contrast to homopolymeric vimentin and desmin, keratin filaments contain at least one member from type II subfamily. Pairs of keratins seem to be consistently co-expressed in different types of epithelial cells so that certain keratin pairs are found only to simple epithelia (type I, 18, 19, and type II K8), while others are found in stratified epithelia (type I k14 and type II k4).[7]

The basic member of each keratin pair is always larger than the acidic member by approximately 8 kDa. All keratin protein chains share a common structural plan consisting of a central α-helix-rich domain encompassed by largely nonhelical N- and C-terminal domains of variable size. The α-helical region of human keratin contains 310–350 aminoacids, flanked by nonhelical head-and-tail domains whose length and composition vary extensively. The α–helical domain is about 47 nm and comprises four segments containing a seven-residue repeat pattern (a–g) n, in which the a and d positions are primarily hydrophobic residues, along with a periodic distribution of charged residues with alternate positive and negative charges. Due to hepta D repeat and the resulting polar residues on one side of the helix, keratins spontaneously form coiled dimers in the solution.[8] Chemical, biophysical, and electron microscopy data have established that monomeric chains associate in parallel and axial register to form a 40–50 nm rod-shaped dimer. The dimer associates in an antiparallel fashion to form keratin tetramer. The full width of a keratin filament generally contains 24–40 monomers in cross section. The major keratin building block is the tetramer, and these subunits are linked in a head-to-tail fashion to yield linear chains, or protofilaments. Two protofilaments intertwine to form protofibrils and groups of four protofibrils intertwine to produce 10-nm filaments *in vivo*. These filaments are organized into a complex supramolecular network that extends from surface of the nucleus to the peripheral most portion of the cell. The genesis and maintenance of such a network involves numerous accessory proteins.[8-10]

Human Keratins' Biology and Pathology

The different human keratins and keratin pairs distributed in cells are summarized below:

Simple epithelia

K8/K18: Primary keratins of simple epithelial cells

The keratins K8 and K18 are co-expressed and constitute the primary keratin pair of simple epithelial cells, including various parenchymatous epithelia. They are the first keratins to appear in embryogenesis, as early as in preimplantation embryos, and also seem to be the oldest keratins during phylogenesis. In some epithelial cell types, K8 and K18 are the sole keratins present. Ultrastructurally, keratin filaments are loosely distributed within the cytoplasm and show little bundling. In other words, simple, one-layered epithelia such as duct-lining cells, intestinal cells, mesothelial cells, and additional simple-epithelial keratins (K7, K19, and/or K20) are present in addition to the primary pair K8/K18. K8 and K18 are widely distributed among normal epithelial tissues although they are absent in differentiating keratinocytes.

In regard to malignant tumors, K8 and K18 are expressed in most carcinomas except for some differentiated

squamous cell carcinomas. Therefore, K8 and K18 antibodies strongly stain most adenocarcinomas and hepatocellular carcinomas. Another clinical application of K8/K18 is the detection of these fragments in the serum of cancer patients. These are used to monitor tumour load and disease progression. More recently, an apoptosis-specific fragment of K18 was detected by monoclonal antibody M30 tumor markers to monitor cancer load, cancer progression, and response to therapy.[10]

K7/K19: Secondary keratins of simple epithelial cells

K7 and K19 are "additional" (secondary) and also widely distributed simple-epithelial keratins. They typically occur as a keratin pair in simple ductal epithelia. The type I keratin K19 is the smallest keratin and is exceptional since it widely lacks the nonhelical tail domain typical for all other keratins. It may have evolved from keratinocyte keratins. The expression of K19 may be induced in certain epithelia that normally lack this keratin by pathological alterations. K19 induction is also observed in suprabasal stratified squamous epithelial cells of oral mucosa with epithelial dysplasia, but also with inflammation so that K19 cannot be used as a specific marker for dysplasia in oral mucosa. In carcinomas, K19 is widely expressed in both adenocarcinomas and squamous cell carcinomas and therefore is not extensively used as an immunohistochemical marker for carcinoma subtyping. The type II keratin K7, another "ductal-type" keratin, has a basically similar but comparatively more restricted tissue distribution as compared to K19. Like K19, it is expressed in several simple ductal epithelia, mesothelium, and pseudostratified epithelia.[11]

K20: Keratin of gastrointestinal epithelium, urothelium, and Merkel cells

K20 is the simple-epithelial keratin with the most restricted expression pattern. Although K20 is a keratin typically expressed in simple epithelia, it is also found in the lone basally located Merkel cells of the epidermis and outer root sheath of hair follicle. K20 is a potent immunohistochemical marker in tumor pathology since its peculiar expression spectrum is essentially maintained in the corresponding primary and metastatic carcinomas. It should be noted that the diagnostic value is increased when the markers K20 and K7 are applied in combination. For example, a K7/K20+ phenotype of an adenocarcinoma metastasis strongly favors a colorectal origin.[12]

Stratified epithelia

K5/K14: Major keratins of basal keratinocytes

The type II keratin K5 and the type I keratin K14 form the primary keratin pair of the keratinocytes of stratified squamous epithelia, including the epidermis as well as mucosal nonkeratinizing stratified squamous epithelia They are strongly expressed in the undifferentiated basal cell layer containing the stem cells and are downregulated in the differentiating suprabasal cell layers K5 and K14 which are uniformly expressed throughout all layers. Ultrastructurally, K5/K14 keratin filaments are bundled as tonofilaments and attached to desmosomes and hemidesmosomes. The functional importance of K5 and K14 for the physical stability of the epidermis has become clearly evident by the recognition that dominant-negative mutations of the K5 or the K14 gene cause the hereditary blistering skin disease epidermolysis bullosa simplex. The presence of mutated K5 or K14 results in increased fragility of the basal keratinocytes so that even mild physical trauma leads to intraepidermal cytolysis of basal cells and the formation of fluid-filled blisters. The expression spectrum of K5 and K14 in tumors corresponds well to the patterns in normal epithelia. Thus, most squamous cell carcinomas as well as malignant mesotheliomas strongly express these keratins, whereas little, focal, or no expression is found in adenocarcinomas. In well-differentiated and moderately differentiated squamous cell carcinomas, K5 is preferentially localized in the peripheral layers of the tumor cell formations corresponding to the K5 expression in the basal cell layer of normal stratified squamous epithelia. Focal K5 expression may be observed in certain adenocarcinoma types.[13]

K15: Basal keratinocyte keratin and stem cell "marker" of hair follicle

K15 was first identified as a minor keratin of human epidermis by gel electrophoresis of cytoskeletal preparations. K15 is a specific basal cell component of the epidermis. Frequently, K5 and K14 can also be detected in the lower suprabasal cell layers.

Whereas the mRNA synthesis of these keratins is restricted to the basal layer, the K5 and K14 proteins remain integrated in the complex keratin cytoskeleton for some time when cells leave the basal compartment. Thus, they may be stained by immunohistochemistry in more or less suprabasal layers depending on the epitope of the antibody used. In comparison, K15 seems completely restricted to the basal cell layer of stratified squamous epithelia where it can form heteropolymeric filaments with K5.[14]

K6/K16: Keratins of hyperproliferative keratinocytes inducible in "activated" epidermis

Molecular genetic studies have revealed that in humans three isoforms of K6 exist, namely K6a, K6b, and K6c, encoded by distinct genes. MAb KA12 is an antibody which most probably stains at least keratin K6a isoform

and reacts well with paraffin sections. In humans, mutations in K6a or K16 have been proven to give rise to the hereditary disorder pachyonychia congenita type I (Jadassohn–Lewandowsky form) that manifests with thickened nails, palmoplantar hyperkeratosis, and oral leukoplakias. Thus, K6/K16 is a constitutive keratin of stratified epithelia built up by keratinocytes of relatively high proliferative state such as mucosal tissues, palmoplantar epidermis, and certain skin appendages. Expression of these keratins is not restricted to stratified squamous epithelia but may also be observed in certain glandular structures. K6 as detected by MAb KA12 may be suitable as an immunohistochemical marker of squamous differentiation in poorly differentiated squamous cell carcinomas in addition to K5.[15]

K17: Keratin of basal/myoepithelial cells and inducible in "activated" keratinocytes

The type I keratin K17 was identified in our early gel electrophoretic studies as a major keratin of basal cell carcinomas of the skin. Further protein analyses showed its presence in squamous cell carcinomas of various origins as well as in normal glandular tissues and its apparent absence also from nonkeratinizing stratified squamous cell epithelia. Broad-tissue screening revealed its selective expression in basal and myoepithelial cells of complex tissues. Thus, K17 may be regarded as a "basal-/myoepithelial cell keratin." K17 has been localized as a prominent component of the suprabasal cell layers of the outer follicular root sheath. Another interesting feature of K17 is its inducibility after skin injury. After K6/K16, K17 is switched on in regenerating and migrating epidermal keratinocytes upon wound healing. Hereditary human diseases due to K17 mutations have been identified, most notably pachyonychia congenita type II (Jackson–Lawler form). The phenotype of this genodermatosis includes thickened nails and pilo-sebaceous cysts. Another condition related to K17 mutations is steatocystoma multiplex, in which patients present with multiple hair follicle-associated cysts. These genodermatoses obviously are related to the expression and functional importance of K17 in pilosebaceous and epithelia. Since in keratinocytes K17 is–like K6 and K16–an inducible keratin upon stress, injury, or inflammation, it is not surprising that squamous cell carcinomas consistently express these three keratins. As most normal stratified squamous epithelia lack K17, its presence in the corresponding tumors may be regarded as neo-expression during tumorigenesis.[3,16]

K1/K10: Major keratins of keratinocyte differentiation and keratinization

In the epidermis, the transition of keratinocytes from the proliferative basal cell layer to the postmitotic suprabasal cell layers in the process of terminal differentiation and keratinization is characterized by a profound change in keratin expression. This involves a switch from expression of the basal cell keratins (K5, K14, and K15) to the suprabasal epidermal keratins, the type II keratin K1, and subsequently the type I keratin K10. This is one of the classical examples for the carefully regulated differentiation-specific expression of keratin proteins. Ultrastructurally, keratin filaments composed of the pair K1/K10 form particularly dense bundles which are so characteristic of suprabasal epidermal keratinocytes. Clearly, this imparts mechanical integrity to the cells, the whole epidermis K10 specifically inhibits proliferation and cell cycle progression of keratinocytes, and loss of K10 leads to increased keratinocyte turnover.Mutations in K1 and K10 are associated with blistering disorders.[17]

K9: Palmoplantar epidermal differentiation keratin

The type I keratin K9 is a highly specific keratin of terminally differentiating keratinocytes of palmoplantar epidermis K9, forming a pair with K1, which appears to reflect a special program of keratinocyte differentiation associated with particular mechanical reinforcement. Immunostaining for K9 has significance for characterization of palmoplantar keratinocyte direction of transplants.[18]

K2: Keratin of highly differentiated, advanced epidermal keratinocytes

K2, formerly K2e, is another keratin specific for the advanced terminal differentiation process of epidermal keratinocytes. Being widely distributed over most body sites, this type II keratin is expressed late, at an advanced stage of differentiation, in the uppermost epidermal layers (upper stratum spinosum and stratum granulosum) to a variable extent. Mutations in K2 have been associated with ichthyosis bullosa of Siemens, a blistering disease showing cytolysis in superficial epidermal layers.[19,20]

K3/K12: Keratins of the corneal epithelium

The K3 (type II)/K12 (type I) pair is the cell type-specific and differentiation-related keratin pair of the corneal epithelium. Mutations in these keratins give rise to Meesmann's corneal dystrophy characterized by intraepithelial microcysts in the corneal epithelium.[21]

K4/K13: Keratins of mucosal stratified squamous epithelial cells

In internal stratified squamous epithelia which are mostly nonkeratinizing, a highly characteristic keratin pair indicates the mucosal path of keratinocyte differentiation, i.e., the type II keratin K4 and the type I keratin K13. Immunohistochemical studies using specific MAbs K4 and K13 revealed the presence of

K4 and K13 in the entire suprabasal compartment of mucosal stratified squamous epithelia, whereas the basal compartment is positive for K5/K14. Interestingly, K4/K13 is completely absent in the epidermis and adnexal structures. Functionally, K4 and K13 appear to be important particularly as components of mucosal stratified squamous epithelia.

Mutations in these keratins, lying in the helix initiation or termination motifs (HIM or HTM, respectively), have been shown to cause the hereditary disorder white sponge nevus of Cannon. This mucosal disorder presents with white plaques mainly on the buccal mucosa, histologically showing thickened spongy epithelium with hydropic swelling of suprabasal epithelial cells. Here again, the clinical manifestation of pathological alterations of keratins well reflects their tissue distribution. Squamous cell carcinomas derived from the epidermis essentially lack K4 and K13.[22]

K76 and K77: Keratins with very special expression sites

K76 (previously designated K2p) is specifically expressed in suprabasal cell layers of oral masticatory epithelium, i.e., the slightly orthokeratinized stratified squamous epithelium lining the gingiva and the hard palate. The high specificity of expression makes this keratin recommendable for use as an "eccrine duct marker" in tumor diagnostics.[23]

K23, K24, K78, K79, and K80: Keratins with still unknown expression pattern

These five very different keratins complete the family of human keratin proteins.

Conclusion

Keratins are important protectors of epithelial structural integrity and are also regulators of motility, signaling, growth, and protein synthesis. Keratins have conventionally been used as diagnostic markers. However, accumulating evidence points to their importance as prognostic markers and active regulators of epithelial tumorigenesis and treatment responsiveness.

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Conflicts of interest

There are no conflicts of interest.

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